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IMEDEX BV
Bruilensingel 360
P.O. Box 3283
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The Netherlands
Tel: +31 (0)73 646 2929
Fax: + 31 (0)73 641 4766
E-mail: congress@imedex.nl
Website: http://www.haematology.nl
### Thursday, June 10, 1999

#### Presidential Session

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Saturday, June 12, 1999

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**PS-0003** Turnover of hematopoietic stem cells and lymphocytes estimated from telomere fluorescence measurements


* Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, BC, Canada; ° University of Aarhus, and # Odense University Medical School, Denmark; † IBC Children's Hospital, and Depts. of * Statistics and § Medicine, University of British Columbia, Vancouver, BC, Canada

In several previous studies the length of telomeres in blood cells as a function of age has been analyzed. Most of these studies, however, have been focused on the decline that begins in early adult life and progresses gradually with advancing age and very little information is available on telomere length dynamics in early childhood. Furthermore, none of these previous studies have adequately addressed questions about the length of telomeres in subpopulations of leukocytes from individual donors, (e.g., granulocytes and T lymphocyte subpopulations). We recently described a novel flow cytometry-based method (Flow-FISH) to measure the content of telomeres repeats in cells using quantitative fluorescence in situ hybridisation with directly labeled (CCCTAA)3 Peptide Nucleic Acid probes (Rufer et al., Nature Biotechnology 16:743-47). Here we report results of flow FISH measurements in granulocytes and lymphocytes as well as T lymphocyte subsets from peripheral blood (PB) samples of > 500 individuals from 0 to 90 years including 36 pairs of MZ and DZ twins. Telomere fluorescence showed marked variation that was to a large extent genetically determined. Granulocytes and naive T lymphocytes showed parallel declines in telomere fluorescence with age ranging from over a thousand base pairs per year in early childhood to less than 50 bp/yr after four years. Memory T cells showed higher rates of telomere attrition and, as a result, the telomere fluorescence in lymphocytes from individuals over the age of fifty was typically less than in granulocytes. Our findings support a direct linkage between telomere length and replicative history in granulocytes, T cells and their precursors in vivo. The previously unrecognized physiological change in the turnover of hematopoietic stem cells in early childhood is compatible with developmental control of stem cell numbers and properties.

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**PS-0002** Lessons learnt from targeted inactivation of the coagulation and fibrinolytic system

Clevers H

Department of Immunology, University Hospital Utrecht, The Netherlands

Targeted gene inactivation in embryonic stem cells has revealed that loss of several coagulation factors (factors V, VII, VIII, IX, X, XI, prothrombin, fibrinogen) impairs haemostasis, frequently resulting in fatal bleeding, similar to the clinical symptoms in patients. Instead, loss of anticoagulant factors (protein C) results in uncontrolled thrombosis, like in humans. However, these studies also unveiled that other coagulation factors (tissue factor, factor V, X, prothrombin) appear to play roles in morphogenetic processes, including vascular development. Gene targeting and gene transfer studies of the plasminogen (Pig) system with its two principal activators tissue-type (t-PA) and urokinase-type plasminogen activator (u-PA), and its inhibitor plasminogen activator inhibitor-1 (PAI-1) have revealed that it is not essential for embryogenesis, but that deficiency of plasmin predisposes to thrombosis. In addition, uronuclease (u-PA)-mediated plasmin mediates migration of smooth muscle cells during neointima formation following arterial injury or allograft transplantation, or of cardiac fibroblasts during scar formation after myocardial infarction. In addition, excess u-PA contributes to aneurysmal dilatation of atherosclerotic aorta or predisposes to cardiac rupture after myocardial infarction. Surprisingly, loss of PAI-1 prevents tumour vascularization, whereas deficiency of t-PA protects against neurotoxic insults. Taken together, these studies define a role for the plasminogen system in several cardiovascular diseases.

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**PS-0001** Transcriptional regulation of haemopoietic stem cells

Green AR

University of Cambridge, Department of Haematology, MRC Centre, Hills Road, Cambridge, UK

One of the central issues of haemopoiesis concerns the molecular mechanisms whereby a pluripotent haemopoietic stem cell gives rise to multiple distinct cell types. Transcription factors play a pivotal role in this process, the importance of which is reflected by the frequency with which transcription factor genes are targeted by leukaemogenic alteration. The SCL gene encodes a BH3 transcription factor which is highly conserved throughout vertebrate evolution and which plays a critical role in the regulation of haemopoiesis. Knock out studies in mice have shown that SCL is essential for the development of all haemopoietic lineages and a subset of endothelial cells. SCL is also capable of specifying the development of haemangioblasts, the common precursor of blood and endothelial lineages, from early mesoderm during zebrafish development. These data underline the striking similarities between the role of SCL in haemopoiesis/vasculogenesis and the function of other BH3 proteins in muscle and neural development. The biological functions of transcription factors such as SCL depend on their spatial and/or temporal patterns of expression. We have therefore also characterised a number of enhancers responsible for the specific pattern of SCL expression. A 3′ enhancer has been identified which directs lacZ expression to haemopoietic and endothelial cells throughout development. Moreover, lacZ+ cells were highly enriched for haemopoietic progenitors and CFU-S. Further characterisation of this enhancer will shed light on the transcriptional programs of haemopoietic stem cells and may also provide an important tool for experimental and therapeutic manipulation of haemopoietic stem cells.

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**PS-0004** TCF/ LEF factors earn their wings

Clevers H

Center of Transgene Technology and Gene transfer, Flanders Interuniversity Institute of Biotechnology, Leuven, Belgium

The founding members of the TCF/LEF family are T cell factor 1 (TCF1) and lymphoid enhancer factor 1 (LEF1). In adult mammals, TCF1 is uniquely expressed in T lymphocytes, while LEF1 is expressed in T cells and early B cells. During murine development, however, expression of TCF1 and LEF1 occurs in complex overlapping patterns in many tissues. The unique in vivo functions of TCF1 and LEF1 have been explored by gene disruption experiments. LEF1−/− knockout mice are severely impaired in the generation of T cells, but are otherwise normal. LEF1−/− mice lack hair, teeth, mammary glands and trigeminal nuclei and as a consequence die around birth. As deduced from direct analyses and from transplantation experiments, the LEF1 mutation has no major effects on the immune system. In TCF1/LEF1 double knockout mice, development of T cells is completely abrogated, indicating that LEF1 can substitute for TCF1 in T cell differentiation. Factors of the TCF/LEF HMG domain family (TCFs) exist throughout the animal kingdom. It has very recently become evident that TCFs interact with the vertebrate WNT effector β-catenin to mediate axis formation in Xenopus. Likewise, Armadillo (the Drosophila ortholog of β-catenin) is genetically upstream of a Drosophila TCF in the Wingless pathway. Upon Wingless/ Wnt signaling, Armadillo/β-catenin associate with nuclear TCFs and contribute a trans-activation domain to the resulting bipartite transcription factor. The cytoplasmic tumour suppressor protein APC binds to β-catenin causing its destruction. In APC-deficient colon carcinoma cells, β-catenin accumulates and is constitutively complexed with TCFs. In APC-positive colon carcinomas and melanomas, dominant mutations in β-catenin render it indestructible, providing an alternative mechanism to inappropriately activate transcription of TCF target genes. So, transcriptional activation of TCF target genes by β-catenin appears to be a central event in development and cellular transformation.
Acute lymphoblastic leukaemia

PO-0005 Glutathione and glutathione-dependent enzymes in haematological diseases in children

Asseem H, Abdel Halim N
Department of Paediatrics, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Objective. To study antioxidant status in children with some haematological diseases and to relate the results to metabolic alterations and complications of these diseases. Design and Methods. We studied red blood cells glutathione content (GSH), glutathione-related enzymes, catalase, and superoxide dismutase (SOD), in addition to plasma total radical trapping antioxidant capacity (TRAP), and vitamins E and C in blood samples from 20 children with acute G-6-PD haemolytic anaemia (favour before transfusion), 18 with homoygous 3-thalassaemia and 15 children with acute lymphoblastic leukaemia (ALL), in addition to 20 healthy children matched for age and sex as controls. Results. The concentration of glutathione was highest in youngest children and declined with age. Children with favism showed significantly decreased TRAP activity (28% lower than in the controls), in addition to decreased GSH and catalase (p<0.05) which may indicate the active role played by catalase under oxidative stress and that this reduction in catalase activity may exaggerate haemolysis. Thalassaemic cases showed reduced TRAP activity (18.6% lower than in the controls) in addition to reduced levels of vitamins E and C and GSH content with positive correlations between ferritin levels and markers of oxidative stress which may indicate that under conditions of iron overload, more peroxidative damage to the tissues is expected. On the other hand, leukaemic cases showed TRAP activity that was significantly different from the controls and increased activity of glutathione peroxidase enzyme which may reflect an adaptive response to free radicals, or leakage from fragile lymphocytes. Conclusions. This study indicates that antioxidant defenses are not defective in ALL, however, a more pronounced or leakage from fragile lymphocytes.

Conclusions. This study indicates that antioxidant defenses are not defective in ALL, however, a more pronounced or leakage from fragile lymphocytes.

PO-0006 Investigation of the endocrine system in childhood survivors of acute lymphoblastic leukaemia. A pilot study.

Papelianė L, Matulevičius V, Savinas A, Savina J
Vilnius University Pediatric Centre, Vilnius, Kaunas University Institute of Endocrinology, Kaunas, Lithuania

The aim of this study was to evaluate the influence of applied therapy on the endocrine system in long-term survivors of ALL. This pilot study was important search for most efficient protocol of investigating the endocrine system, adapted to local possibilities. We investigated 26 ALL survivors: 13 girls and 13 boys, aged 10-20, 5-11 years after initial diagnosis of ALL. They received full chemotherapy according BFM ALL protocols and 18-12 Gy irradiation for neuroleukaemia prophylaxis. Endocrine examination included: TSH, TSH, T4, T3, TSH, prolactin, testosterone, DHEAS, E2, progesterone, cortisol. The number of hormones investigated for each patient varied according to laboratory possibilities. There was no evidence of precocious or late puberty nor significant retardation of growth in this group of patients. Seven boys of prepubertal age had low testosterone and gonadotropin concentrations. Three boys of 18, 14 and 13 years had suffered unilateral orchidectomy because of extramammary spread of ALL. The oldest of them had normal testosterone (22.1 mmol/L) with FSH of 8.21 mIU/L and no signs of hypogonadism. Two others had low testosterone and high FSH, indicating hypogonadism with important damage of the remaining testis. In a patient of 10 years old, chronic thyroïd with euthyroidism was diagnosed. Twelve girls out of 13 had passed menarche. Ten of them had apparently normal menstrual cycles with hormone values corresponding to the cycle day. In 2 girls dysmenorrhea with clear signs of polycystic ovary was detected. In 2 girls gonadotropic changes of the thyroïd indicated fine-needle biopsy. This investigation revealed malignancy in both cases, and thyroid papillary carcinomas were detected at surgery and confirmed morphologically. Conclusions. The study is not finished, but results of the endocrine system is very often affected in ALL long-term survivors very often. These patients should be carefully monitored for gonadal or thyroid dysfunction for a long time period.

PO-0007 Involvement of CD95- and bax-dependent apoptotic pathways in doxorubicin-induced apoptosis in T-lineage acute leukaemia

Dept. of Haematology, Oncology, and Tumour Immunology, Robert-Röside-Clinic, Charité, Humboldt-University of Berlin, Berlin, Germany

The anthracycline doxorubicin exerts its cytotoxic effects by activation of different apoptotic pathways, including CD95-receptor/ligand system and p53-dependent expression of the apoptosis-promoting protein Bax. In leukaemia, the role of these factors is controversial. Here, we investigated the role of these apoptotic pathways in vitro doxorubicin-induced apoptosis in freshly isolated T-ALL samples. Dose-dependent induction of apoptosis by doxorubicin (20 h, 37°C) in 49 T-ALL samples was studied by flow cytometry (Annexin-V propidium iodine staining). T-ALL cells revealed heterogeneous sensitivity to doxorubicin. In 13 T-ALLs, doxorubicin induced high levels of apoptosis (+50%) at concentrations lower than 1 µM, whereas in 26 samples the same extent of apoptosis was observed at concentrations of 1 µM and higher. In several samples (n=10), no apoptosis could be induced. Treatment of cells with agonistic CD95 antibody (clone CH11) resulted in an induction of apoptosis in 20 of 58 (35%) T-ALL samples (threshold level of CD95-specific apoptosis >10%). However, no correlation between sensitivities to doxorubicin and CD95-crosslinking was found. Interestingly, combined treatment of leukaemic cells with CH11 and doxorubicin resulted in an increased extent of apoptosis in 9 of 45 cases (20%). Most of these cases (8 of 9) were totally resistant to CD95-mediated apoptosis in the absence of doxorubicin. To study a possible induction of CD95-ligand expression due doxorubicin treatment, CD95-ligand-neutralizing antibodies (clones NOK1, NOK2) were used (n=2). Only in 2 T-ALLs, was an inhibition of apoptosis by NOK antibodies found. As to the bax expression, a doxorubicin-induced upregulation of bax was observed in 4 of 11 T-ALL cases (calculated as ratios of MESFdoxo/MESFmedium; MESF: units of mol-ﬁctior, a doxorubicin-induced upregulation of bax was observed in 4 of 11 T-ALL cases (calculated as ratios of MESFdoxo/MESFmedium; MESF: units of mol-
ubile L-selectin retains bioactivity, and at high concentrations can inhibit binding of lymphocytes to endothelium. Little information is yet available on the diagnostic and physiologic significance of the selectin levels during the clinical course of patients with acute leukaemia. Design and methods. Serum samples were obtained from 59 patients with newly diagnosed acute leukaemia, including 37 patients with AML and 22 patients with ALL. The diagnosis and classification of AML or ALL were based on the criteria of the French-American-British Cooperative Group and immunophenotyping. Additional samples were obtained from patients in complete remission and patients after relapse. Control serum samples were obtained from 15 healthy blood donors. Serum samples were separated immediately and kept frozen at -20°C until assay. Leukemic patients were treated according to standard protocols for AML or ALL. Immunophenotyping was done on mononuclear cells separated from peripheral blood. Cell-sorted subsets were detected by standard immunofluorescence methods using a flow cytometer. Shed L-selectin assay was done using a sandwich immunoenzymometric technique. Results. This study shows high levels of sL-selectin in serum of patients with acute leukaemia. The mean value of sL-selectin among healthy individuals was 1120±178 ng/mL. This value was increased in 17 of 22 patients with ALL (77%) and 25 of 37 patients with AML (65.5%). Repeated measurements in 24 patients showed a normal range in 19 patients with complete remission and high levels in 5 patients with therapy resistant acute leukaemia. There are also increased levels in patients with relapse. Conclusions. High levels of serum sL-selectin in patients with acute leukaemia may have an important role in regulating the initiation of blast cell adhesion to endothelium. In addition, measuring sL-selectin may be useful in detection of leukaemia relapse. However, further in vivo studies are needed to establish definitely the role of sL-selectin in the regulation of blast cell migration into tissues.

PO-0010 CD66 expression in adult acute lymphoblastic leukaemia

Muñoz L, Carrasco M, Bernat S, Bellido M, Ubeda J, Sierra R, Nomdedeu JF
Laboratori d’Hematologia i *Servei d’Hematologia Clinica, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

Introduction. Antibodies against CD66 identify antigens from the CEA family of proteins which belong to the immunoglobulin gene superfamily. Antibodies were used to investigate MRD in adults suffering from ALL. CD66 antigenic combinations could be used to investigate MRD in adults suffering from ALL. CD66 was associated with particularly aggressive courses of CML. In one case cytogenetic study was not available. CD66 expression was used to monitor the persistence of minimal residual disease (MRD) in patients with relapse. Control serum samples were obtained from patients in complete remission and patients after relapse. Control serum samples were obtained from 15 healthy blood donors. Serum samples were separated immediately and kept frozen at -20°C until assay. Leukemic patients were treated according to standard protocols for AML or ALL. Immunophenotyping was done on mononuclear cells separated from peripheral blood. Cell-sorted subsets were detected by standard immunofluorescence methods using a flow cytometer. Shed L-selectin assay was done using a sandwich immunoenzymometric technique. Results. This study shows high levels of sL-selectin in serum of patients with acute leukaemia. The mean value of sL-selectin among healthy individuals was 1120±178 ng/mL. This value was increased in 17 of 22 patients with ALL (77%) and 25 of 37 patients with AML (65.5%). Repeated measurements in 24 patients showed a normal range in 19 patients with complete remission and high levels in 5 patients with therapy resistant acute leukaemia. There are also increased levels in patients with relapse. Conclusions. High levels of serum sL-selectin in patients with acute leukaemia may have an important role in regulating the initiation of blast cell adhesion to endothelium. In addition, measuring sL-selectin may be useful in detection of leukaemia relapse. However, further in vivo studies are needed to establish definitely the role of sL-selectin in the regulation of blast cell migration into tissues.

PO-0012 Blast cells in peripheral blood after one week of corticosteroids in childhood acute lymphoblastic leukaemia

Laatiri M.A, Chehata S, Ennabli S
Service d’Hematologie clinique, CHU Farhat Hached, Sousse, Tunisia

Objective. Early response to therapy is typically assessed by bone marrow status and is predictive of outcome in childhood acute lymphoblastic leukaemia (ALL). The significance of early clearance of blast cells in peripheral blood after one week of corticosteroids is less known. Design and methods. We reviewed medical records of all children with ALL referred to the European Organisation for Research and Treatment of Cancer (EORTC) protocol to determine the presence of blast cells in peripheral blood at diagnosis, and after 1 week of corticosteroids. Results. The prognostic significance of persistent circulating blast cells in the 70 patients was assessed in a multivariate analysis that included known adverse prognostic factors. Persistent circulating leukemia blasts in 17 of 20 children (85%) were present at day 8 in 14 patients (20%). Compared with the blast-negative group, these patients had a significantly higher frequency of several adverse clinical features (leukocyte count > 50×10⁹/L, mediastinal mass, central nervous system leukaemia, T-cell phenotype and a 2 and 4 morphologic and a significantly poorer prognosis. By multivariate analysis, blast cell persistence at week 1 was the most significant adverse feature in the overall cohort. Conclusions. We concluded that the persistence of blasts one week after one course of corticosteroids confers a poor prognosis in childhood ALL and these patients should benefit from early intensification of therapy.

PO-0013 Acute biphenotypic leukaemia in children

Günsel T, Kocak U, Özturk G, Ezer U
Department of Paediatric Haematology, Medical School of Gazi University and Social Security Children’s Hospital, Ankara, Turkey

Acute leukemias with the immunologic characteristics of both lymphoid and myeloid blasts are classified as biphenotypic (ABIL) or mixed lineage leukemias. ABIL is associated with poor prognosis in adults but controversy exists on the outcome of children with this rare subtype of acute leukemias. We report here on the clinical characteristics and treatment outcome in 7 children who fulfilled the diagnostic criteria of ABIL, i.e. co-expression of 2 myeloid and lymphoid associated antigens on blast cells. These cases included 6 children with lymphoblastic leukaemia (age < 18 yr) and 1 child with lymphoid leukaemia (age > 18 yr) without evidence of ALL or AML. In 6 of 7 patients, normal karyotype was found. In 3 patients, cytogenetic studies showed clonal abnormalities. In 2 patients, chromosomal abnormalities were present. In 1 patient, hyperleukocytosis (>50×10⁹/L) was noted in 3 patients (2 with T-ALL and 1 with B-ALL). Other morphologic abnormalities included hand-mirror morphology in 2, dimorphism in 2 and cytoplasmic blebs in 1 patient. Cytogenetic analysis in 3 patients revealed rearrangement of 11q23 in the case of myeloid antigen (+) T-ALL. Six patients with ALL received induction chemotherapy (COG) and all patients relapsed during the first 12 months. Five of 6 patients died of leukemia. Only one of these three relapsed children obtained second remission. The patient with lymphoid antigen (+) ALL also relapsed after complete remission had been obtained with 2 courses of DAT and died. Only a half of our patients survived despite successful administration of intensive chemotherapy. Our findings indicate that ALL is associated with a poor outcome. The discrepancy among results with regard to the prognostic significance of ABIL may be due to the biological and clinical heterogeneity of ABIL.
Background. The TEL AML1 fusion resulting from a cryptic t(12;21) (p13;q22) is a very common genetic rearrangement in childhood B-lineage leukaemia and confers a good prognosis. Recent findings also showed that the percentages of CD13/CD33 expression were higher in TEL AML1 + than TEL AML1 - cases. However, data on Chinese are rare. Objective. To study the incidence and the relationship of this fusion transcript with the karyotypes, the presence of aberrant myeloid antigen expression and the clinical behaviour in childhood B-lineage ALL. Methods. Forty-one cases of paediatric B-lineage ALL diagnosed and managed at the Children Cancer Centre in the Prince of Wales Hospital were analysed using nested RT-PCR. Immunophenotyping of the leukaemic cells was performed using flow cytometry or APAAP with a panel of markers including HLA-DR, CD10, CD19, CD20, CD22, CD23, anti-Cy and anti-MPO. Results. This patient cohort included 18 males and 23 females with a median age of 4.0 y.o. (range: 0.4-15.0 y.o.). TEL AML1 were detected in eight cases (19.5%), 6 B-progenitor, 1 pre-B and 1 biphenotypic ALL, all of CD10+ phenotype. Four (50%) of these cases demonstrated aberrant myeloid (CD13 and/or CD33) antigen expression in 30-90% of the leukaemic cells and in the biphenotypic ALL, anti-MPO was also positive. However, only 3/33 the TEL AML1 cases showed this myeloid antigen aberrancy (p=0.018). Four cases were of a normal karyotype. One had isolated trisomy 16. One had trisomy 21 with structural changes. One demonstrated hypodiploidy with 46- and one showed structural aberrations including changes at 12p12. In the seven cases in which one or more subsequent bone marrow samples were available for molecular analysis, initial molecular remission was achieved in five cases within 28-35 days after induction chemotherapy. However, TEL AML1 were still detectable in two with samples showing morphological remission on days 27 and 33. All cases are in continuous complete remission a median follow-up of 17 months after presentation. Conclusions. Our findings are very similar to those reported in western literature. However, older children were also studied. The presence of TEL AML1 was found to be associated with a high continuous remission rate.

Objective. There are few trials analysing the use of HGF after chemotherapy in patients with relapsed acute leukaemia. These trials, although showing reductions in neutropenia period, do not show reductions in death or remission rates. Our main objective is to review the effect regarding the mortality and remission rates of HGF after high-dose chemotherapy in patients treated for relapsed acute leukaemia. Design and Methods. Among 70 patients with acute relapsed leukaemia, 50 received high dose chemo-therapy and presented bone marrow relapse were included in this retrospective investigation. Mean age was 17 years and 60% were men. Sixty-two percent had acute lymphoblastic leukaemia. High-dose Ara-C and VP16 was the chemotherapy regimen used by 26 patients. Fifty-two percent were treated with HGF. F-test and Chi-square test were employed in the bivari-ate and logistic regression models for multivariate analysis. Results. The mortality rate was not significantly different between the two groups, corresponding to 3 deaths among patients treated without growth factors and 8 in patients treated with (p=0.12). The remission rate was also statistically not different between the two groups: 45.8% in those treated without growth factors and 23% in patients treated with HGF (p=0.09). There was no difference in the incidence of infections, sites of infections and germs identified in culture tests. The duration of neutropenia period in the group treated with HGF (15.1 ± 20.7 days) and in the control group (15.1 ± 20.7 days) was not statistically different (p=0.22). The incidence of infections was not different in the two groups (p=0.26). Mortality and absence of remission were not associated with age, treatment with HGF, clinical diagnosis or chemotherapy regimen in multivariate analysis. Conclusions. The use of hematopoietic growth factors in patients with relapsed acute leukaemia treated in the haematological clinics of the Hospital de Clinicas de Porto Alegre did not modify the mortality rate, or frequency of remission or other clinical parameters.

Flow cytometric immunophenotyping not only has improved the diagnosis and classification of acute lymphoblastic leukaemia (ALL) but it has proven its value for minimal residual disease (MRD) detection by identifying the leukaemia-associated phenotype (i.e. ectopic antigen expression or the presence of aberrant myeloid antigen expression, asynchronous antigen expression or antigen overexpression). The reported inci- dence of this phenotype in ALL is extremely variable. In this study we reviewed the immunophenotype of the cells prefurshed from 119 consecutive patients with ALL, 59 males and 60 females, aged 4 months-68 years (mean 17.085±16.9), admitted in our department between 1995-1998. Immunophenotyping was performed on isolated mononuclear cells was evaluated using two-color flow cytometry (Becton Dickinson Micro Diff II counter. The expression of lymphocyte surface antigens (CD3, CD8, CD4, TCRβ, CD19, CD20, CD13, CD33, anti-Cy and anti-MPO). Results. The patient cohort included 18 males and 23 females with a median age of 4 y.o. (range: 0.4-15 y.o.). TEL AML1 were detected in eight cases (19.5%), 6 B-progenitor, 1 pre-B and 1 biphenotypic ALL, all of CD10+ phenotype. Four (50%) of these cases demonstrated aberrant myeloid (CD13 and/or CD33) antigen expression in 30-90% of the leukaemic cells and in the biphenotypic ALL, anti-MPO was also positive. However, only 3/33 the TEL AML1 cases showed this myeloid antigen aberrancy (p=0.018). Four cases were of a normal karyotype. One had isolated trisomy 16. One had trisomy 21 with structural changes. One demonstrated hypodiploidy with 46- and one showed structural aberrations including changes at 12p12. In the seven cases in which one or more subsequent bone marrow samples were available for molecular analysis, initial molecular remission was achieved in five cases within 28-35 days after induction chemotherapy. However, TEL AML1 were still detectable in two with samples showing morphological remission on days 27 and 33. All cases are in continuous complete remission a median follow-up of 17 months after presentation. Conclusions. Our findings are very similar to those reported in western literature. However, older children were also studied. The presence of TEL AML1 was found to be associated with a high continuous remission rate.

Incidence of leukaemia-associated phenotype in acute lymphoblastic leukaemia patients at diagnosis

Dumitrescu AM
Fundeni Clinical Hospital, Bucharest, Romania

Clinical analysis

Maurz B, Wylezol I, Szczepanski T, Ojekaj L, Soba-Jakimczyk D
Dept. of Paediatric Haematology and Chemotherapy, Silesian Medical Academy, Zabrze, Poland; Dept. of Immunology, Erasmus University Medical Center, Rotterdam, The Netherlands

Peripheral blood lymphocyte sub-sets after treatment of childhood acute lymphoblastic leukaemia

Long-term cytotoxic treatment for childhood acute lymphoblastic leukaemia (ALL) is associated with immunosuppression of variable duration after treatment. Preliminary reports have suggested persistent abnormal fre- qualities of different peripheral blood (PB) lymphocyte sub-sets. To determine long-term effects of chemotherapy on PB lymphocyte sub-sets, we investigated the group of 20 ALL patients one year after treatment endpoint. Control group consisted of 20 sex- and age-matched healthy children. Absolute leucocytes and lymphocyte counts were determined in Coulter Micro Diff ii counter. The expression of lymphocyte surface antigens (CD3, CD4, CD8, CD19, CD5, CD20, CD23, CD14, CD15) on PB mononuclear cells was evaluated using two-color flow cytometry (Becton Dickinson, Heidelberg, Germany). Mean absolute numbers of different lym-
phocyte subsets were compared between the groups using Cochran's C test. The relative as well as absolute numbers of major lymphocyte subsets were within normal range in ALL patients one year after completing the treatment and did not differ from values determined for normal healthy children. Further studies should determine if the function of different lymphocyte subsets in ALL survivors is normal as well.

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PO-0019 Acute lymphoblastic leukaemia (ALL) in the elderly: results of treatments in cohort of 102 patients

Dept. of Cellular Biotechnology and Haematology, University "La Sapienza" of Rome, *Haematology, Catholic University "Sacro Cuore", Rome; *Haematology, University "Federico II", Naples, Italy

In the past yrs ALL in the elderly was considered a rare disease, characterized by a poor prognosis, since in itself it represents one of the most important prognostic factors negatively influencing both the CR rate and disease outcome. In this retrospective study the clinical characteristics, the types of treatment and disease outcome were compared between 2 groups - patients aged 60 yrs old and younger and patients aged >60 yrs old.

Results. There were 68 CR (78.2% of evaluated pts, 66.2% from all) and 14 ID; median CR length was 8 mos, median survival 9.5 mos. During follow-up 78% and 66% of pts treated with DA and CD20- the best impact to survival.

PO-0020 Prognostic factors in adult acute lymphoblastic leukaemia

Genadijeva-Stavririć, S., Cvececa L., Georgievski B., Arnova F., Stankovski V., Efremov D., Stojanovski Z.
Department of Hematology, Medical Faculty, Skopje, Macedonia

Adult acute lymphoblastic leukaemia (ALL) is characterised by considerable variability in course and prognosis. A number of prognostic factors for predicting prognosis is increasingly reported. To investigate whether the simple haematological, biochemical and clinical parameters routinely taken at diagnosis and immunophenotyping (immunofluorescent method and flow cytometry) are useful in predicting prognosis are increasingly reported. To investigate whether the simple haematological, biochemical and clinical parameters routinely taken at diagnosis and immunophenotyping (immunofluorescent method and flow cytometry) are useful in predicting treatment and disease outcome of 102 ALL (>60 yrs) pts, observed from 1989-1996.

Results. There were 68 CR (78.2% of evaluated pts, 66.2% from all) and 14 ID; median CR length was 8 mos, median survival 9.5 mos. During follow-up 78% and 66% of pts treated with palliative 2 drug (VCR+PDN) induction, group B 44 pts – median age 71 yrs – 58% of pts were Crs, 22% resistant, 20% ID; overall median CR length was 6.2 mos, median OS 7.5 mos. In group A 32 (55%) pts were Crs, 12 resistant, 20% ID; median CR length was 6.2 mos, median OS 7.5 mos. In group B 36 (80%) achieved CR, 2 were resistant, 6 ID: median CR length was 8 mos, median OS 7.5 mos. In group A 14 pts were not classifiable but were B-ALL. Positivity of each marker was as shown in the table (in brackets percent of positive cases to N° of pts, *p<0.05, ANOVA, U-test):

PO-0021 Prognostic significance of immunophenotype markers in B-type adult acute lymphoblastic leukaemia

Donfrid M, Kraguljac N, Boskovic B
Dept. of Cellular Biotechnology and Haematology, University "La Sapienza" of Rome; *Haematology, Catholic University "Sacro Cuore", Rome; *Haematology, University "Federico II", Naples, Italy

Objective. To evaluate prognostic influence on immunophenotype markers in B-ALL. Patients were analysed 103 patients with B-ALL treated with LALBA (50 pts), YAUALLA (34) and LALABA (17) protocol. There were 60/M:43/F with age 36.5±15.8 yrs. FAB was L2: 53, L1: 8 yrs. Patients were classified according to MIC classification for B-ALL: as early B-ALL 12 pts, B-ALL 63 pts, pre B-ALL 10 pts and B-cell ALL 8 pts. Ten pts were not classifiable but were B-ALL. Positivity of each marker was as shown in the table (in brackets percent of positive cases to N° of pts, *p<0.05, ANOVA, U-test):

PO-0022 Immunological characterisation of adult T-cell acute lymphoblastic leukaemia: a single institution study

Kraguljac M, Bosgadorovic A, Jankovic G, Donfrid M, Tomicin D, Miletić N, Colovic M
Clinical Center of Serbia, Institute of Haematology, Belgrade, Yugoslavia

The present study reports immunophenotypic data and analyses their relationship with clinical characteristics and outcomes in a group of 48 adult patients (pts) (48/155, 31%) at presentation, in the period 1989-1999. Accurate diagnosis of T-ALL was made using standard FAB classification and immunophenotyping (immunofluorescent method and flow cytometry). Immunological classification was made according to thymus maturation stages: group I (prothymocyte), group II (common thymocyte) and group III (mature thymocyte). The group characterised male gender (71%) and Ly1+ (56%) predomination, with a mean age of 36 years (range 14-66) and mean WBC of 80 x10^9/L. Membrane antigens (Ag) were expressed in two patterns in the whole group. Only CD7 Ag showed permanent expression. Of all the other studied Ags showed variable expression: CD34, HLA-DR, CD4, CD5, CD2, CD5, CD3, CD1a, CD4, CD8, CD10. Distribution of pts according to immunological class was: group i (69%), group ii (31%), group iii (0%). Group i was characterised by relatively low frequency of expression of Ags: CD10 (100%), CD4 (83%), CD5 (58%) and CD2 (57%) and relatively low frequency of Ags: CD3 (21%), CD25 (25%), CD14 (14%) and CD1a (0%). Group ii was characterised by permanent expression of Ags: CD2, CD7, CD25, CD1a, a relatively high frequency of expression of Ags: CD8 (42%), CD3 (73%), CD2 (55%), and a relatively low frequency for CD34 Ag (36%). Correlation frequencies between groups i and ii disclosed a significant difference (p<0.01), but the correlation mean Ag expression between group i and ii disclosed a significant difference in cases of: CD34 (43% vs 10%, p<0.01), CD5 (47% vs 81%, p<0.01), CD14 (21% vs 59%, p<0.01), CD14 (14% vs 38%, p<0.01) and CD1a (5% vs 62%, p<0.01). Correlation frequency of Ag expression to clinical characteristics, disclosed significantly higher WBC in pts with expression of CD3 and CD4 Ags (p<0.05). Correlations differences in CR, DFS and OS between groups i and ii were not significant. CD2 Ag was predictive for OS (p<0.04, p<0.04, p<0.04, p<0.04). Our results suggest that the frequency of adult T-ALL as well as the frequency of group I, are higher in our group according to literature...
During acute lymphoblastic leukaemia (ALL) treatment the cardiovascular disturbances are caused by different factors, including the anthracynides (Atc). Cardioprotective agents, e.g. Dextrazoxane have been added to the treatment regimen to prevent the development of heart injury. The objective of our study was to evaluate, using echocardiography, the hearts of children during the ALL treatment. Design and Methods, the study group consisted of 20 children, 10 boys and 10 girls, aged 1-16 years (mean 9.4 y) with diagnosed ALL and risk factors (RF) differing from 0.3 to 1.7. Among them 5 presented with high RF, 15 with standard RF. In all the children Atc were given to cumulative doses of 164-368 mg/sq m (mean 274.4) at the end of doxorubicin 20 times higher. During the study echocardiographic examination (M-mode, 2D and Doppler flow study) was performed before the treatment initiation, after 1 month, 3 months, 6 and 12 months. Standard measurements and left ventricle (LV) systolic and diastolic diameters were taken. The change of the parameters and correlation with study time was analysed. Results, over 12 months LV systolic and diastolic diameters, wall and septum thicknesses, left atrial dimension, LV mass index, as well as Doppler diastolic indices-A and E wave of mitral flow, isovolumetric relaxation time to ejection ratio and systolic indices: fractional shortening, ejection fraction, cardiac index, mean velocity of circumferential fibres shortening did not show significant changes and did not correlate with Atc doses. Conclusions, in the children of ALL during the 12-month treatment neither the heart dimensions nor systolic and diastolic left ventricle indices assessed by echocardiography changed significantly.

PO-0024 Infrared spectroscopic study of human Tymphoblastic leukaemia and its vinblastine-resistant CEM cell lines

Liu KZ, Jia L*, Kelsey SM*, Schultz CP, Newland AC*, Mantsch HH

It has been reported that solid tumour cells rely on glycolysis for energy and that the increased rate of glycolysis in MDR-1 positive cells is associated with increased malignancy of tumours. We have previously shown that the MDR-1 positive vinblastine-resistant CEM/VLB100 cell line has an increased mitochondrial electron transport chain activity compared to that of the parental CEM cell line. In the present study, we found that the mitochondrial DNA (mtDNA) content in the CEM/VLB100 cell line was significantly higher compared to that of the parental CEM cells. The increased mtDNA was not, however, accompanied by an increase in mitochondrial protein. Synthesis of mitochondrial protein was diminished by a significant inhibitor of mitochondrial F1-F0-ATPase, as measured by intercellular idurianidin accumulation. We also tested whether the increased oxidative phosphorylation in the leukaemic MDR-1 positive cells enhances their malignancy by assessing the cell proliferation rate using a CyQUANT dye. However, the CEM/VLB100 cell line showed a lower proliferation rate than the parental CEM cell line. We therefore conclude: (i) Leukemic cells rely on glycolysis for energy; (ii) The increased mitochondrial respiration in the drug-resistant cells does not increase the malignancy in leukaemic cells; (iii) Infrared spectroscopy is a potentially powerful technique for detecting mitochondrial DNA, protein and lipid contents simultaneously.

PO-0025 Single high dose-idarubicin (SHD-IDA) and cytarabine in the treatment of refractory and relapsed adult ALL

Tedeschi A, Montillo M, Nosari A, Dracisi M, Santorini L, Gargantini L, Morra E

Department of Haematology, Ospedale Niguarda, Milan, Italy

Objective, weiss et al. concluded a phase 1 dose escalation study of a SHD-IDA combined with HD Ara-C plus G-CSF in the treatment of relapsed and refractory ALL: IDA 40 mg/sq m was indicated as the maximally tolerable dose when combined with HD Ara-C; we conducted a study to evaluate the efficacy and the safety of this combination therapy in the treatment of relapsed and refractory ALL pts. Design and Methods, Ten adult pts, 4 refractory and 6 relapsed ALL were treated with a salvage therapy consisting of Ara-C 3-0.9 g/m²/day iv over 3-5 days, 1-5 and shD-IDA 40 mg/sq m iv on day 3, G-CSF was started on day 7 and continued until PMN recovery. Results, There were 6 males and 4 females, the median age was 27 (range 19-46); immunophenotyping demonstrated 8 lineage markers in 7 cases and lineage markers in 2 cases. Two pts were Ph+ and one pt showed the TEL/AML1 rearrangement. All pts had been previously treated with an induction regimen including prednisone, vincristine, asparaginase and daunorubicin (DNR). In relapsed pts the median time to relapse was 4.5 (range 4-24) months. The total dose of DNR administered was 270 mg/sq m in 8 cases and 120 mg/sq m in the remaining two. Ejection fraction was >50% in all pts before salvage therapy. All pts were evaluable for response. CR was obtained in 4 cases (40%) while 6 showed a resistant disease. Two pts in CR were submitted to allo-BMT and relapsed after 9 and 12 months respectively; one pt received HD Ara-C as consolidation therapy relapsing after 2 months. A sudden death occurred in the fourth pt. The autopsy revealed a myocardial perforation due to Aspergillus infection. The overall toxicity was acceptable, median day of PMN and PLT recovery was 18 and 20, respectively. We did not observe extrahepatic toxicity of grade 3 or 4, ejection fraction evaluated after treatment was >50% in all pts. All but one pts presented fever >38°C, median days 5 (range 3-12); infections were documented in 7 cases (in one only post-mortem examination detected a myocardial Aspergillus infection). Conclusions, We conclude that the association of HD Ara-C and IDA 40 mg/sq m as a single dose appears to be active in a cohort of poor prognosis ALL. This HD regimen may be administered safely as the overall toxicity observed was acceptable and none of the pts developed cardiotoxicity even they had been treated with high doses of anthracyclines.

PO-0026 Heterogeneity of lymphoblasts cells via a CD45-DNA flow cytometric analysis in children's acute lymphoid leukemia

Pelier L*, Chassevant A*, Riallant X*, Baranger L*, Hfrin M*, Hurnault M*, Mantsch HH*

A simple DNA flow cytometric analysis of acute lymphoid leukemia (ALL) bone marrow aspirates accurately identifies lymphoblasts (LB) DNA-ploidy, but does not allow a specific measurement of their proliferation because only a mean S-phase (%S) of the different cell populations included in such samples can be calculated. Lymphoblasts can be identified with a CD45-DNA double staining. But sometimes, several LB populations can also be detected when they show different CD45 expressions. When applied to bone marrow aspirations (previously prepared with Ficoll and frozen in liquid nitrogen), this double staining demonstrates a significant difference between mean %S of sample cells and specific lymphoblasts (%S). To date, 284 bone marrow aspirates of 229 children with ALL have been analyzed. A simple DNA flow cytometric analysis in childhood ALL, and analyse their correlation with Atc doses. Conclusions, In the children with ALL during the 12-month treatment neither the heart dimensions nor systolic and diastolic left ventricle indices assessed by echocardiography changed significantly.

PO-0027 Detection of minimal residual disease by IgH gene rearrangement-PCR with V6 family specific primers in childhood ALL

Park CJ, Kim MC, Seo EJ, Kim SR, Chi HS, Seo J, Kim TH, Moon HN

Dept. of Clinical Pathology and Pediatrics, University of Ulsan, College of Medicine and Asan Medical Center, Seoul, Korea

Objective, We wanted to know clonality number and clonal evolution at diagnosis and during chemotherapy in childhood ALL, and analyse their correlation with the morphologic evaluation and the clinical course. Design and Methods, In 30 cases of childhood ALL the DNA was extracted from the bone marrow aspirates at the diagnosis and during the chemotherapy. Forty-cycle PCR was performed with seven each VH family specific primers and the common JH primer of immunoglobulin heavy chain (IgH). The PCR products were electrophoresed on agarose gel, and those showing specific bands were electrophoresed on 6% urea 6% polyacrylamide DNA sequencing gel. We compared and analysed the IgH gene rearrangement (GR-PCR) results, the morphologic diagnosis of the bone marrow and the clinical course.

Acute lymphoblastic leukaemia
Results. IgH GR was detected in 93.3% (28/30) at diagnosis and the other two cases showed IgH GR during therapy. IgH GR was detected in all specimens diagnosed as persistence, partial remission and relapse. There was 86.8% of hypercellular marrow with persistence of blasts, 72.7% of the hypercellular marrow, and 59.2% of complete remission. In the complete remission states the patients with IgH GR showed a significantly higher relapse rate (26.2%) than those without IgH GR (17.3%) (p=0.019). Number of clones of IgH GR was from one to five. The more number of clones was associated with a shorter mean survival time (p=0.1172). VJ3 was most frequent (70%). IgH GR had been detected on average 3.5 months (range 1-12 months) earlier than the morphologic relapse appeared. During the chemotherapy the evolution of IgH GR was observed in seven cases (23.3%). Conclusions. The IgH GR-PCR with VH family specific primers will help the understanding of biological characteristics of leukemic cells, the interpretation of the bone marrow studies after chemotherapy and the plans for further therapy can be used as a prognostic indicator in the morphologic complete remission state.

PO-0028 Infant acute lymphoblastic leukaemia: a retrospective evaluation of eleven patients
Luciani M,* Baroni C,* Ciaffi P,* Pinto RM,* Rana I,* Russo LA,* Cimino G,*
*Haematology Division, “Cytogenetic and Immunohaematology Lab-Children’s Hospital “Bambino Gesù”, Vatican City State;
°Department of Cellular Biotechnology and Haematology, Haematology Division, University of Rome “La Sapienza”, Italy
Acute leukaemia with onset in the first year of life (Infant ALL), represents a distinct subset with its own biological and clinical features. Here we report a retrospective evaluation of the biological characteristics and clinical features and therapeutic results observed in a group of 11 infant acute lymphoblastic leukaemia (ALL) cases observed between August 1986 and December 1998 in the Children Hospital “Bambino Gesù” of Vatican City State. All the patients were intensively treated according to current treatment protocols for ALL of the Associazione Italiana di Ematologia ed Oncologia Pediatrica (AIEOP). Among the four patients treated with LAB 4 INF, two are in first complete remission (CR) at 95 and 120 months from diagnosis, and two are in second CR after extra-haematological relapses (tissue, CNS) which occurred at 110 and 70 months from diagnosis. All six patients treated with ACVBP, 9502 and Interfant protocol, achieved a clinical CR. One out of six of these patients is still in first CR at 29 months; one patient presented a CNS relapse 8 months from CR, and is now in second CR at 47 months after an allogeneic bone marrow transplantation received as consolidation therapy; one patient is still in maintenance treatment in 1st CR, at 8 months; one patient (ALL1 rear.) died in CR of cardiac failure 29 months from diagnosis; one patient (ALL1 rear.) presented a CNS relapse at 22 months from CR and is now in 2nd CR; the remaining patient (ALL1 rear.) had haematological relapse 3 months after achievement of 1st CR, and died of disease 11 months after diagnosis. Finally, one patient with t(1;19) did not respond to the induction treatment with adriamycin, cytarabine and prednisone and died of progressive disease. In this group 11 ALL infant patients, actuarial EFS and OS were 28% and 71% at 120 and 143 months, respectively. These results appear a little better than those usually reported for Infants ALL. The results, although achieved in a small series of eleven ALL infant patients who received different treatment modalities, confirm that also in this leukemic subset there is a group of patients which can be cured by conventional chemotherapeutic program, and stress the importance of a careful evaluation of infant ALL risk factors, in order to perform adequate risk-adapted therapeutic strategies.

PO-0029 Clearance of maternal leukaemic cells in a neonate
Pongers-Willemsen MJ,* Szczeparski T,* Langerak AW,* Wijkhuizen JM,* Harts WA,* van Wering EL,* Mulder M,* van Donen JM,°
°Department of Immunology, University Hospital Rotterdam/Erasmus University Rotterdam, Rotterdam; °Dutch Childhood Leukemia Study Group, The Hague; Dept. of Pediatrics, St. Antonius Hospital, Nieuwegein, The Netherlands
It has become widely accepted that the placenta is not a 100% barrier but small series of eleven ALL infant patients who received different treatment modalities, confirm that also in this leukemic subset there is a group of patients which can be cured by conventional chemotherapeutic program, and stress the importance of a careful evaluation of infant ALL risk factors, in order to perform adequate risk-adapted therapeutic strategies.

Po-0030 Efficacy of BFM 90 protocol in children with 1st relapse of acute lymphoblastic leukaemia (ALL): experience of the Polish Children’s Leukaemia/Lymphoma Study Group
Boszulawska-Lawrowska L,* Chybicka A,* Gorczycka E,* Juszczak K,*
Turkwiecz D,* Amata M,* Balcerska A,* Balwierz W,* Bubala H,* Filiks Litwin B,* Kolecki P,* Kowalczyk J,* Lukowska K,* Małyk M,*
Rocicka Miewska R,* Rola Kurc E,* Stencel D,* Sorfá-Jankiczky D,*
Strozy W,* Achwojki J,* Wloccorzek M,* Wysocki M,* Zelenay E,
Departments of Children Hematology and Oncology in Wroclaw,*
Bydgoszcz,** Gdansk,** Cracow,** Lublin,** Poznan,** Warsaw,**
Zabrze,** Poland
Between 1993 and 1998, 113 children aged from 6 mths-18 years (41 girls and 72 boys) with first relapse of ALL were included in the study. There were 88 (74.08%) cases with early (including 32 children with very early and 25 (25.92%) cases with late relapse (BM-61, CNS-21, testes-8, combined-23). The children were treated according to the BFM 90 relapse protocol. In 16/113 children hematopoietic stem cell transplantations were performed (10 Allo-BMT, 4 auto BMT, 1 autologous blood from a related donor). The overall second complete remission (CR) rate in very early relapse was 72.8%, in early relapse 85.76% and in late relapse 86.71%. The probability of overall event-free survival (EFS) observed in children after 2 years was 39.9%. EFS at 32 months of follow-up in children with late relapse was similar to that in children with early relapse and almost twice as high when compared with children with very early relapses (37.5% v 47.7% v 16.42% p=0.02). The best EFS - 60.3% at 32 months – was achieved in children treated with BMT. The results obtained with BFM chemotherapy in children with first late relapse are acceptable. One must conclude that for children with very early relapse mesenchymotherapy together with BMT in second remission must be designed.

PO-0031 Childhood acute lymphoblastic leukaemia treatment results with CCg-106 protocol regimen A (modified BFM): a nine year experience
Istanbul University, Istanbul School of Medicine, Department of Pediatric Hematology and Oncology Our-Children Leukemia Foundation Health Center, Istanbul, Turkey
In Istanbul University, Istanbul School of Medicine Department of Pediatric Hematology/Oncology and Our-Children Leukemia Foundation Health Center 292 cases of Pediatric Acute Lymphoblastic Leukaemia (117 male, 115 female) were treated with BFM 86 CCg-106 modification. All the cases were grouped by the prognostic criteria of the same protocols into low risk (26%), average risk (31%), or high risk (43%). All the patients received the same chemotherapeutic regimen regardless of risk groups. The 9 years survivals were 85.3%, 72.8% and 65.7% respectively and EFS was 87.7% in low risk, 60.45% in average risk, 52.11% in high risk patients (p<0.007). The overall survival for all the groups was 71.81% (9 years). There was no significant difference in survival according to age, sex, risk groups, hepatomegaly, splenomegaly, Léna, het, plt levels, bleeding symptoms. A highly significant difference was found for the duration of early response to treatment (p<0.0001), FAB type (p<0.0002) and WBC count (p<0.01). EFS was significantly different according to early response to treatment (22% v 77%). In conclusion, the same chemotherapy regimen for pediatric ALL is feasible in a less developed country with satisfactory results except for high risk patients. Our results are comparable with the developed countries and improved outcome may be achieved by risk adapted therapies.

Acute lymphoblastic leukaemia
A human acute lymphoblastic leukaemia (ALL) cell line, BALM-18, was established from the peripheral blood specimen of a 35-year-old male patient with B cell ALL, type Li, at diagnosis by using monolayered bone marrow stroma cells (BST) as feeder cells. The primary leukaemia cells without BST feeder cells did not grow, but rather went into apoptosis in regular culture medium (RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum). As with the primary leukaemia cells, BALM-18 showed an immunophenotype of Burkitt’s lymphoma group 1 (CD10+, CD20+, CD23-, CD38+, CD7+), with the b(8;14)(c12q32;13) chromosomal abnormality which is strongly associated with both ALL-L1 and Burkitt’s lymphoma, and revealed a significantly low level of bcl-2 protein. Strikingly, anti-human IgM antibody did induce apoptosis in in vitro experiments. However, it was inhibited by the addition of anti-CD40 antibody or BST cells, whereas the culture supernatant of the stroma cells did not show any effect on the inhibition of apoptosis. BALM-18 may be useful for analysing both the mechanisms of anti-IgM induced apoptosis and signalling during the inhibition of apoptosis by CD40 or BST cells.

PO-0033 High diversity of splicing-derived interleukin-7 isoforms in acute lymphoblastic leukaemia
Korte A, Seeiger K, Mörckke A, Beyermann B, Köchling J, Taube T, Kebelmann-Betz-Ch, Hense G
Department of Pediatric Oncology/Hematology, Charité Universitätsklinikum, Campus Virchow, Humboldt-University at Berlin, Germany

Interleukin-7 (IL-7) plays a pivotal role in early stages of normal B and T cell development. In addition, IL-7 stimulates the proliferation of both anti-tumour reactive cells and a number of T and B cell malignancies underlining its significance for leukaemogenesis. However, its exact role in the process of pathological maturation of lymphocytes and regulation of the immune response is not completely understood. Objective. Since alternative splicing of pre-mRNA has been shown to be involved in the control of gene expression and splicing-derived protein isoforms with antagonistic activity have been found, we assessed the mRNA-expression of IL-7 and its previously described alternative splice variant lacking exon 4, IL-7ε, in leukemic cells from children with acute lymphoblastic leukaemia (ALL). Methods/Results. PCR of full-length IL-7 cDNA enabling the competitive amplification of both variants led to the amplification of diverse unexpected PCR products. The sequence data demonstrated the existence of three additional in-frame splice variants resulting from exon-skipping of exon 3 and/or 5 in combination with exon 4, which we named IL-763/4, IL-764/5, and IL-763/4-5. Furthermore, three out-of-frame splice variants were identified, IL-7ε3/45/6-2, IL-74-ε3/45/6-2, and IL-7ε63/4-5 (-56bpexon2), in which, in addition to the aforementioned exon-skipping, 56 bp of the 3′ end of exon 2 are omitted. Conclusions. Our results lead us to assume that splicing derived IL-7 isoforms play a potential role in modulating IL-7 mediated biological effects. Further studies are required to clarify the significance of the diverse IL-7 protein isoforms for the regulation of IL-7 function and the pathogenesis of leukaemia.

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PO-0034 Infant leukemias in a Turkish oncology centre
Gözda AO, Yavuz G, Ünal E, Tacyıldız N, Etemen M, Ikinciogullari A, Cevadir AD
Dept. of Paediatrics, Divisions of Paediatric Haematology, Oncology, Immunology and Paediatric Haematology/Oncology Research Centre of Ankara University, Ankara, Turkey

Twenty-two males (67%) and eleven females (33%) totalling 33 infants with acute leukaemia were diagnosed during the 29 years between 1970 and 1998 inclusive. The age range was from 2.5 months to 18 months (median 18 months). FAB criteria revealed 22 patients (pts) with acute lymphoblastic leukaemia (ALL) and 11 pts with acute myeloid leukaemia (AML). Biphenotypic leukaemia was encountered in 3 cases. One patient had Down’s syndrome. All pts had hepatosplenomegaly, two ALL pts had a mediastinal mass, 6 AML and 5 ALL pts had extramedullary involvement (EMI). EMI was significantly higher in the AML group at presentation (p<0.05). Skin was the involved site (15 AML, 5 ALL pts) followed by CRS (2 AML pts). Orbital and skin infiltrates were present in 2 AML pts. At presentation the median haematological laboratory values for AML and ALL consecutively were: Hgb 7.7 g/dL and 7.3 g/dL, WBC 40,200/µL and 58,400/µL, platelets 79,200/µL and 54,500/µL. These differences were not statistically significant. One of four cases cytogenetically examined showed hyperdiploidy. Treatment regimen for ALL during 1970-85 was VCR, prednisone, MTX, and i.M. Since then until 1990-1991 received ADR, L-asparagin and intermittent MTX and the pts during 1990-1996 received a more intensive regimen of CCG-107, CCG-1882, CCG-1883. Treatment for AML before 1985 comprised various protocols but since then CCG-213 (Denver) and regimens containing idarubicin have been used. Twenty-sev- en pts were evaluable (19 ALL, 8 AML): 16 of 19 (84.2%) ALL pts had complete remission (CR) and 5 of 8 (62.5%) AML pts had CR and partial remission (PR). Nine non-responders and PR were lost to follow-up. Seven pts (6 ALL, 1 AML) have survived to date. Our experience shows that acute leukaemia infants younger than 2 years present with a variety of features predicting a poor outcome. CCG-1883 have helped us to achieve a higher remission rate and duration in the ALL group.

Poster discussions - Acute myeloblastic leukaemia I
PO-0035 Clinical relevance of tumour cell distribution in B-CLL
Department of Medicine, University Hospital *Merkur*, Zagreb, Croatia and **Azienda Ospedaliera “Bianchi-Melacrino-Morelli”, Reggio Calabria, Italy

Objective. This study was performed to evaluate clinical relevance of tumour distribution in B-CLL with 3 main objectives: 1) to introduce a quantitative model for tumour distribution assessment, 2) to assess its relationship to other established clinical and laboratory parameters, 3) to evaluate its impact on prognosis. Methods and Design. Tumour mass distribution was evaluated by the TTM score system. In this system T1M is the score from blood and bone marrow, T2M is the score from the lymph node compartment, and T3M is the score from the spleen compartment. Br J Haematol 1981; 49:405. We proposed the following model for tumour mass distribution assessment: X=1.78 (22.9%), 0.5x<1 190 (55.7%), x=0.73 (21.4%) patients. The new parameter was correlated at significant level (p<0.05) with TTM (<56), Rai (<=58), Binet (<=53) stages, spleen (<=77), β2-microglobulin (<=72), and to a lesser extent to erythrocyte sedimentation rate, lymph nodes, liver, haemoglobin and platelets. X was not correlated with age, sex, lymphocytosis, soluble CD23, or bcl/2-1/bax ratio. It showed a stronger relation-ship with prognosis in females (p<0.001) than in males. In a female population with organomegaly, it distinguishes 2 prognostic groups, with median survivals of 68 months for the 0x<1 group and 38 months in the x3 group, but failed to discriminate 2 groups (median survival of 49 and 50 months respectively) in a male population. In multivariate analysis X was the strongest predictor of prognosis (p<0.000) along with TTM size (p<0.001), and age (p<0.023) in females. In the male population it failed to enter the model as an independent prognostic factor at a significant lev-el after Binet stages (p=0.000), age (p=0.000) TTM size (p=0.000), and therapy (p=0.006). Conclusions. New, quantitative and simple clinical parameter easily assessed in all patients, offers reliable tool for evaluation of tumour cell distribution in B-CLL. It may be particularly helpful for investigat-ing the role of adhesion molecules at a clinical level. In the female popula-tion it shows an independent and strong prognostic power.
Objective. ATRA is now being considered as an adjunct to chemotherapy for AML. At Bergamo Hosp, with the ICE+G regimen for adult AML (51, Bartholomew's Hosp. BXIV: idarubicin 10 mg/sqm on d 0 and d 1-3, cytarabine 100 mg/sqm on d 0 and d 1-7, etoposide 100 mg/sqm on d 1-5, G-CSF 5 mg/ci on d from 8), the complete remission (CR) rate was 75% (37/49) but 11 pts. had resistant (RES) AML (22%). We activated the ICE+G+ATRA study in order to assess whether (a) ATRA could positively influence remission results and (b) whether this effect correlated with in vitro study results. Design and Methods. AML pts. received ICE+G plus oral ATRA 45 mg/sqm on d on d 1-14 and then 25 mg/sqm on d 15-28. The in vitro study included a clonogenic assay and apoptosis (annexin V) differentiation (CD11b) tests on patient AML cells challenged with various combinations of ATRA, G-CSF, and idarubicin. Results. Nineteen pts. were treated: age 14-64 yr, M/F 10/9, median blast count 12 400, 3 MDS-AML, FAB-M1 7, M2 5, M4 5, M5 1, M6 2. ATRA induced few toxic side effects (headache 3, lethargy 1, diarrhea 1) and was administered for 5-28 days

Conclusions. The use of ATRA was not associated with better induction results. No clear association with the in vitro study was found, but AML cell growth was stimulated by ATRA in some cases while apoptosis differentiation were uncommon. This study does not support the indiscriminate application of ATRA in the early treatment of AML.

PO-0037 Chemotherapy (CT) and stem-cell transplantation (SCT) in patients with primary acute myeloid leukaemia (AML): current results of the LAM-94 protocol


Objective. To evaluate the results of chemotherapy (CT) followed by SCT, allogeneic (allo-SCT) or autologous (auto-SCT), in 192 patients (pts) ≤ 60 years (yr) (mean age±SD; 61±22 yr, M/F 93/99) with primary AML (M3 excluded). Pts. were diagnosed between May 94 and November 98. Design and Methods. Induction CT consisted of idarubicin, standard dose ara-C and VP16 (ICE). Intensification CT included mitoxantrone and ara-C at 1500 mg/m² on d 2-3, and ara-C at 100 mg/m² on d 15-28. Results. CR, no. (%) = 71/121 (59), 21/21 (100), p=n.s.

Perugini, Italy

Background. An accurate and fast diagnosis of acute promyelocytic leukaemia (APL) is required after the introduction of specific treatment with trans-retinoic acid. APL is characterised by t(15;17) that produces a chimeric protein PML/RARA with an abnormal nuclear pattern of distribution. PG-M3 is a monoclonal antibody (MoAb) against wild type PML protein that could be used in the rapid diagnosis of APL. Objective. To assess the value of PG-M3 MoAb in the diagnosis of APL. Design and Methods. PG-M3 was used in an immunofluorescence (IF) or immunocytochemical techniques (APAAP) on smears of 4 normal controls, 28 AML (11 APL and 16 non-APL), 4 ALL, and 1 multiple myeloma (MM). 1 RAEB and 1 blast crisis of CML. Phenotype was studied by flow cytometry, and cytogenetics by conventional G banding. Results. APL was studied by RT-PCR. Evaluation of immunosignalling was obtained with IF (60 minutes) and double step APAAP (120 minutes). Nuclear pattern was not evaluable in 7 samples stored at -80°C for more than 4 months (2 M2, 4 M3, 1 M5). The normal nuclear staining pattern typical of wild-type PML was observed in normal samples, ALL, MM, RAEB. blasts crisis and 13 AML (2 M0, 2 M1, 4 M2, 1 M4, 4 M5). Abnormal nuclear microgranular pattern was detected in all APL (6 clas- sic and 2 microgranular). All cases with abnormal staining pattern carried t(15;17) detected by cytogenetics in 6 cases and by RT-PCR in all cases. No case with normal nuclear pattern showed the translocation (17 by cyto- genetics and 6 by RT-PCR). PG-M3 was very useful in the diagnosis of two cases: an AML morphologically mimicking APL (finally M2) and an APL appearing as a second neoplasm after chemotherapy for a solid tumour. Conclusion. Immunostaining of PML protein with PG-M3 MoAb using IF or double APAAP is a rapid, simple, and reliable method to detect the chimeric product of t(15;17) and to diagnose both classical and microgranular APL.

PO-0039 Identification of the fusion of partner of ETV6 in 2 patients with AML and a t(4;12)(q11;p13).

Burton S, Fernandez F, Carbuccia N, de Roux C, Mozziocenacchi MJ, Sainty D, Lafage-Pochitaloff M, Birot F, INSERM U119, and Institut Paoli-Calmettes, Marseille, France

The ETV6/TEL gene is the target of several translocations involving the 12p13 band, in both lymphoid and myeloid malignancies. Formation of fusion genes, and expression of the fusion transcripts is generally observed. Alternative breakpoints have been identified in the ETV6 gene. The t(4;12)(q11;p13) is a rare, recurrent chromosomal translocation reported in patients with AML and ALL. To identify the partner genes of this translocation, and to diagnose both classical and microgranular APL.

PO-0040 Clinicobiological features in 29 cases of minimally differentiated acute myeloid leukaemia (AML M0)


AML M0 is a class of AML undetectable by standard morpho-cytochemical analysis. In this study we report 29 AML M0 in order to correlate bio-

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logical findings, therapeutic strategy and outcome. In all cases flow cytometric analysis by CD33, CD14, CD15, CD11b, CD11c, CD4, CD24, CD25, CD34, HLA-DR, CD90, CD117, CD45RA, CD45RO, CD38, CD23, TdT, T and CD10 were performed. Doublet and bic-2 and bins-2 doublet loss studies (IGH-TCR, BCR/ABL, AML1/ETO and CBFB-MYH11 rearrangements) were performed. Of the 29 pts, 27 were treated with GIMEMA intensive protocols. We noted a greater incidence of older (over 60 years) and male pts (52% and 65% respectively, CD33, CD13, aMP0, CD15, CD7 and DT were expressed in 79%, 83%, 28%, 77%, 59% and 43%, respectively. Most of the cases expressed CD34 (93%), HLA-DR (93%), CD117 (80%), CD38 (94%), CD71 (79%), CD45RA (87%). CD45RO and CD20 were always negative. In all the cases we observed up-expression of b-TCR and down-expression of CD59 with an inverse trend (r=-5253; p=0.03). Karyotypic abnormalities were found in 54% of the cases. Of these, 6 cases involved chromosomal breaks (5 and 5q-), 7 (-7) and 8 (+8). (9;22), confirmed by BCR/ABL transcript, was detected in one case. Rearrangements of b-TCR and Ig were observed in 3 and 2 cases, respectively. No AML1/ETO and CBFB-MYH11 were detected. In 3 cases subsequent de novo acute myeloblastic leukaemia (AML) was observed. In the remaining cases the effect of chemotherapy was partial. Complete remission (CR) was achieved in 10 (37%) of the cases with a median CR duration of 23.5 months after onset of mitoxantrone. Half of them (4/8) were acute myeloblastic leukaemia I (AML-M0), one AML-M2, one AML-M4, one AML-M5 and one t-RAEB which very quickly became acute. The four LAM3 were treated by trans retinoic acid and three pts received the same treatment. Of the 5 pts alive, 4 (all underwent autologous or allogeneic bone marrow transplantation) are still in CR. We conclude that AML M0 is endowed with many biological adverse parameters and a bad outcome, but that a more aggressive consolidation treatment, based on transplantation procedures, is able to cancel the negative impact of the prognostic factors and to improve the clinical outcome.

PO-0041 Acute leukaemias after treated breast cancers: part of mitoxantrone

Registry of Haematopoietic Malignancies, France

Therapy-related acute leukaemia following chemotherapy for breast cancer is well documented. Recently, DNA topoisomerase II inhibitors such as epipodophyllotoxins, and anthracyclines have been increasingly reported as leukaemogenic agents. Mitoxantrone (an anthracenedione), is an intercalating DNA agent inhibitor of topoisomerase II. Its efficacy in cancer treatment has been demonstrated and this drug has been authorised in France since 1982. From 1982 to 1998, the two specialised registries (Haematological Malignancies and Gynaecological Cancers) of the Côte d’Or department, (France) have registered 11 cases of NALN in about 3600 women previously treated for breast cancer. Eight (age 33 to 62, average: 45.2 years) of them had received mitoxantrone associated or not with anthracyclines and/or cyclophosphamide. Except one patient who received mitoxantrone as rescue treatment, the seven others had never been treated previously with any other cytotoxic drug. All the patients underwent surgery and radiotherapy. NALN were diagnosed between 1989 and 1998, 11 to 40 months (average = 23.5 months) after onset of mitoxantrone. Half of them (4/8) were acute promyelocytic leukemia (AML FAB type M2), others were one FAB type M0, one FAB type M4, one FAB type M5 and one t-RAEB which very quickly become acute. The four LAM3 were treated by trans retinoic acid and three pts are still alive. All the others died except the one with t-RAEB. The three patients not exposed to mitoxantrone received, for breast cancer treatment surgery, radiotherapy and either epipodophyllotoxins, 5FU and cyclophosphamide (FAB type M4) or none (one FAB type M2, one CML acutised in FAB type M2). The risk for patients treated by mitoxantrone for their breast cancer appears very strong compared to the risk from other treatments. Our study is going on to estimate this risk exactly and to for the risk of more recent drugs such as anthracyclines (epirubicine for example). Specialised registries have a mission of medical alarm and they are precious for pharmacovigilance studies because they are population-based studies.

PO-0042 Immunotherapy with IL-2 and histamine upregulates downmodulated T cell and NK cell associated expression in acute myeloid leukaemia (AML)

Söderberg L, Anderson M, Björklund M, Bruné M, Hellstrand K, Pisa P.
Departments of *Medicine and †Oncology, Karolinska Hospital and Institute, Stockholm, Departments of ‡Haematology and §Virology, Sahlgrenska University Hospital, Gothenburg, Sweden

In an attempt to improve relapse-free survival of patients with AML, immunotherapy studies including interleukin-2 (IL-2) and histamine have been initiated in complete remission (CR) patients. The rationale behind this treatment is the synergistic effect of these compounds on cell-mediated killing of leukemic cells in vitro together with the achievement of a prolonged CR duration in some patients in pilot studies. As in many other human malignancies, a downregulation of the signal transduction molecule zeta has been observed in T lymphocytes in patients with untreated AML, a fact that theoretically may contribute to the insufficient immune response against leukemic cells. The aim of the present study was to determine the zeta expression status in AML patients in CR and to evaluate if the in vivo effect of immunotherapy with IL-2 and histamine. This was achieved by three channel flow cytometry analysis of patients’ peripheral blood lymphocytes (PBLs) obtained before, during and after immunotherapy, allowing for simultaneous detection of membrane expression intensity (MFI) of zeta expression in defined lymphocyte subpopulations. Thirteen treatment periods in 9 adult patients with AML in CR (7 with MDS-AML) were included in the study. A reduced zeta MFI in CD4+, CD8+ and CD16+ cells, compared to normal donor PBLs, was detected before treatment started. In 5 of these a significant increase in zeta expression was noted after the onset of treatment, comprising all analysed lymphocyte populations. This should be compared to the 7 periods with a monoslope in zeta expression levels in CR patients not exposed to mitoxantrone, but with a progressive pancytopenia at the time of great start, in which no further increase in zeta MFI was noted during the therapy. Post-treatment analysis of patients with improved zeta expression levels showed various degrees of persistence of the increased zeta levels. In conclusion, immunotherapy with IL-2 and histamine rapidly upregulates low zeta expression levels in AML.

PO-0043 Influence of mutation N- and K-ras oncogenes on the prognosis of acute non lymphocytic leukaemia

Tukić LJ*, Ktalic K*, Bokonić J, D. Cokić, M, Malešević M*
*Clinic of Haematology, Military Medical Academy, Belgrade; †Institute of Nuclear Science “Vinča”, Belgrade; ‡National Poisoning Control Centre, Belgrade; §Institute for Medical Research, MMA, Belgrade, Yugoslavia

Point mutations of ras oncogenes are molecular genetic abnormalities which can often be detected in haematological malignancies. The objective of this study was to detect the incidence of point mutations of ras oncogenes in patients with de novo acute non lymphocytic leukaemia (ANLL) in Yugoslavia and to find out their influence on initial clinical characteristics, course and the outcome of treatment. The screening was made on 29 adult patients divided into 2 groups according to presence or absence of N- and K-ras point mutations using de novo ANLL. Patients were classified according to the FAB system with 5 patients classified as M1, 3 as M2, 7 as M3, 4 as M4, 7 as M5 and 3 as AUL (M0). AML patients were treated with the standard induction-remission therapy and evaluation of remission was made at latest 28 days of the therapy application. In this study, mutations in 9 patients (31%) were detected to be at the codon 12 or 13 N-ras (80% samples), and at codon 12 K-ras (20% samples). Out of all mutations 89% were found in males (8/9), out of which 87.5% (7/8) were younger than 40. Characteristics of mutated ras genes were: 7 transversions (G to T in 50% samples; G to C in 20% samples) and 3 transitions (G to A in 30% samples). Significant differences were observed between ras (+) and ras (-) groups. They refer: to higher white blood cell count and higher peripheral blood blast count, more frequent M3 FAB subtype, more frequent presence increase in activity of LDH as well as poor response to initial remission therapy. The findings indicate that point mutations of ras oncogenes in our patients could be an additional factor of unfavourable prognosis of ANLL.

PO-0044 FLAG-idarubicin for the treatment of resistant/ relapsed acute myeloid leukaemia and myelodysplastic syndrome

Márta L, László E, Sas G
Department of Haematology, National Institute of Haematology and Immunology, Budapest, Hungary

The synergistic combination of fludarabine phosphate (FAMP), cytosine-arabinoside (ara-C) and G-CSF with or without idarubicin or mitoxantrone has recently proved effective in poor prognosis acute myeloid leukaemias (AML). We have treated 9 patients with primary refractory (n=5), relapsed (n=2) and secondary (n=2) AML with the FLAG-ida protocol (FAMP 30 mg i.v; Ara-C 2 g/m² for 5 days, idarubicin 12 mg/m² day 1 to 3, G-CSF 300 µg/m² from day -1 until neutrophil recovery). Mean age of pts was 30 (17-41) yrs; 4 pts were females and 5 males. Karyotype was normal in 6 pts, unsuccessful in one pt, showed loss of the Y chromosome in 1 pt and t(8;21)(1) in 1 pt in second relapse. FAB subtypes were: M1 (3 pts including 2 biphenotypic leukaemia), M2 (2 pts), M4 (3 pts), M7 (1 pt). Two pts with myelodysplastic syndrome (MDS) (1 RAEB, 1 CMMoL) developed AML after 6-12 months of progressive pancytopenia (MDS-AML). A second course was administered to 5 pts, 2 in the same dosage, 3 in a shorter 3-4 months duration: a total of 14 cycles were given. 7/16 pts achieved complete remission (CR) after 1 course. Of them 5 pts achieved CR in myelodysplastic syndrome, one in a myeloid plastic state with <5% blasts. The neutrophil (>500/µL) and platelet (>20,000/µL) recovery required a median of 26 (13-39) and 28 (14-45)
days from the commencement of therapy, respectively. Two pts died, one of sepsis during induction and one of cerebral haemorrhage during con-
solidation. No major side effects occurred except one pt who had hypoten-
sion on day 2-3 of treatment necessitating temporary dopamine infusion.
iv. antibiotics and amphotericin B were required in 8/14 cycles due to
unexplained fever (4 pts), pneumonia (4 pts) and maxillary sinus aspergill-
osis (1 pt). Three pts received allogeneic bone marrow transplantation (BMT)
in CR. The pt with M2 and T(8;21) remained in cytogenetic remission for
one year after FLAG-ida until his third relapse, when he could be salvaged
again successfully with FLAG-ida. After a 4-year follow-up, he is under-
going autologous BMT now. In conclusion, the FLAG-ida regimen proved very
efficient in this series of young pts with poor-risk AML. The high CR rates
offer possibility to proceed to allogeneic or autologous stem cell trans-
plantation. Furthermore, FLAG-ida seems feasible to induce durable
remissions in second or third relapse of good-risk AML.

PO-0045 Clinical outcome of 9 patients with T(8;21) translocation
Wawryniak E, Strzelcika B, Urbania-Rys H, Dube-Velichkyt R, Robak T
Dep of Hematological, Univ of Lodz, Poland

Objective. The T(8;21) translocation is considered as a good prognostic factor in AML M2 and exceptionally in other blood disorders. The T(8;21) is considered as a good prognostic factor in AML M2, 82-100% of patients achieve complete remission (CR) and have a long overall survival time. According to other literature these data these patients frequently relapse during the first year of CR (2). The aim of the study was to follow the clinical outcome of 9 patients with T(8;21) translocation. Methods. Cytogenetic analysis after 24h culture was performed on bone marrow cells at diagnosis. The T(8;21) translocation was detected in 8 AML M2 patients and one MDS (RAEB-I) patient. There were 7 men and 2 women aged between 24 and 57 years. Seven patients received one (4 patients) or two (3 patients) courses of daunorubicin (DNR) and cytosine arabinoside (araC) as an induction ther-
rapy. One patient received one course of idarubicin with araC and the last one – three courses: mitoxantrone with araC × 2 and amascincine with 
vesipod. Results. Normal and abnormal mitoses were found in two cases,
sx chromosome loss in four cases. Seven patients achieved CR after one (4 patients) or two (3 patients) courses of induction therapy. Four of them relapsed after 17, 21, 53, 61 days from diagnosis, one patient is in first
CR at +52 weeks and one died after 82 weeks from diagnosis because of
pneumonia and hemorragha. Two patients did not achieve CR. One of them died during induction therapy because of sepsis, the second one – after three courses of induction treatment lived in partial remission 61 weeks.

PO-0046 Preliminary results of therapeutic multicentric study for
treatment children and adolescents with AML in Ukraine
Rychak O, Donska S, Korenkova I, Karamanesh E, Polyschuk R,
Usachenko V
AML-Study of PGLLU-Ukraine

Multicenter study for treatment children and adolescents with AML de novo was initiated in Ukraine in Dec. 95 with participation of 4 pediatric haema-

tologic centers: Kiev Regional (center of Study), Kiev City, Lviv, Simferopol. Therapy Protocols AML PGLLU-95 (Pilot), and AML PGLLU-97 are based on

PO-0047 Acute promyelocytic leukaemia: Italian geographical
distribution
Piccardi P, Pulvino A, Rossi F, Avvisati G, Olivieri A, Castoldi G,
Mannoc M, Cerri R, Cupello E, Nosari AM, Ferrara F, De Biasi D,
De Rosa G, Pagano L, Anghezi G, Mele A, Mandelli F
Regia Italiana Leotta Tumori, Studio PAN Ricerche for the GIMEMA Group, Rome, Italy

Geographical distribution and individuation of clusters of acute promyelo-
cytic leukaemia (APL) were the aims of this research. Due to its rarity and
to poor data availability a small number of studies have been conducted
till now on the epidemiolosm of this disease. Data from GIMEMA Group about
the APL on the whole Italian territory from 1986 to 1995 have been examined.
The number of cases analysed was 696, 368 males and 328
females. A comparison of GIMEMA data-base with Cancer Registries data
allowed to estimate for the first time, the national annual incidence rate
with the prudential range of 0.16-0.18/100,000 inhabitants. These figures
have to be considered as preliminary estimates to confirm with further
comparison with Cancer Registries data in space and in time. Applying a
spatial cluster detection methodology (Geographical Analysis Machine,
Openshaw 1988), 8 clusters of cases with statistically significant higher
incidence rates have been detected. No particular difference may be not-
ked between North, Centre or South of the Country in their distribution that
has been compared with the distribution of some sources of risk factors (oil
refinery and depot, electric power stations).

PO-0048 Defective stroma formation and reduced LTC-IC frequency in
the bone marrow harvested after remission in patients with
acute myeloblastic leukaemia (AML)
Marront E, De Falco C, Lovisone E, Genneta C, Audisio E, Allione B,
Hematology Division and BMT Unit. San Giovanni Hospital, Turin, Italy

Prolonged pancytopenia was observed after autologous bone marrow trans-
plantation (ABMT) in AML. A defective stroma formation and a reduced fre-
quency of LTC-IC in vitro has been reported in these patients, possibly as a
correlation of the aggressive induction-consolidations regimens
employed. We have analysed 14 adult patients with AML in complete 
remission after treatment with the EORTC/ GIMEMA AML10 induction
and consolidation protocol. The bone marrow was harvested at a median
time of 62 days (range 19-202) after completion of the program and cryopre-
erved. ABMT was conditioned with the BU CY2 regimen. At the time of har-
vest were evaluated: cellularity. CD34+ cells, CFU-GM. After thawing, an 
LTC-IC assay on murine feeder layer was performed and the LTC-IC fre-
quency was determined by limiting dilution analysis. The in vitro confluence 
of stromal cells was also evaluated and expressed as percentage of the 
flask surface covered by stromal cells (percentage area occupied). A run-on

LTC-IC frequency in AML patients in confront to nor-
mal controls was observed (mean 1.75 vs 8.61. p=0.0016). One patient
failed to form any stroma; a suboptimal stroma formation (< 60%) was
observed in 7 patients and a fair confluence (> 60%) was observed in 6
patients. A marked reduction in LTC-IC frequency in AML patients in confront to
normal controls was observed (mean 1.75 vs 8.61. p=0.0016). One patient
failed to form any stroma; a suboptimal stroma formation (< 60%) was
observed in 7 patients and a fair confluence (> 60%) was observed in 6
patients. The patients with longer duration of aplasia (>40 days) had low-
er rate of CD34+ cells (2.5 vs 8.4-10 x10^6/l; p=0.03), lower CD34+ (3.2 vs
11.6 x10^6/l; p=0.04) and lower LTC-IC frequency (0.87 ± 2.63 ±0;5-
p=0.04).

Acute myeloblastic leukaemia I

Haematologica vol. 84 (EHA-4 Abstract Book); June 1999
An important clinical consideration is whether PMU/RARα transcript type is associated with differences in patient’s presenting characteristics and/or is predictive of therapeutic responses in APL cases. The main purpose of this study was to analyse this aspect in a series of newly diagnosed APL patients enrolled into an ATRA plus anthraccline-based chemotherapy protocol. Between November 1996 and December 1998, 123 patients with newly diagnosed PMU/RARα-positive APL from 39 Spanish centres were enrolled into the PETHEMA protocol LPA96. The PMU/RARα rearrangements were detected by RT-PCR in 12 different laboratories, 8 following the methods of Lo Coco et al. (Lancet 1992; 340:1437) or Biondi et al. (Blood 1994; 84:421), and to study the relationship between CD117 and FAB classification, 140/176 AML were CD117+ (79.5%). We did not observe any differences in the distribution among c-kit expression and FAB subtypes (chi-square test). The equivalence of the results obtained was assessed by the mandatory inclusion and consolidation showed that 38/84 (45%) and 68/73 (93%) patients tested PCR-negative, respectively. After the induction, differences in the PCR negativity-between the subtypes bcr1/bcr2 and bcr3 were not observed (89% vs 84%), nor were they identified for the phase of haematological relapses. From this preliminary data it could be concluded that the presence of the short transcript (bcr2) is associated with recognised bad prognosis parameters. PO-0050 Correlation of CD117 expression and cytogenetic results in acute myeloid leukaemia Cairol F, Specchia G, Riba S, Pezzetti L, Medice A, Brando B, Grillo G, Attilio C, Lisa V, Morra E Dept. of Haematology, Niguarda-Cà Granda Hospital, Milan and University of Bari, Italy Aim. To investigate the expression of CD117 in acute myeloid leukaemia and to study the relationship between CD117 and FAB classification, early surface antigens (CD34, CD7) and cytogenetic features. Patients and methods. Bone marrow or peripheral blood samples obtained from 176 AML pts. median age 54 yrs (range 16-92), were studied at diagnosis. Immunophenotyping analysis was carried out using a FACSCalibur flow cytometer using a 95C3 monoclonal antibody to detect-κ antigen; positivity was defined as ≥20% of stained leukaemic blasts. Cytogenetic results, available in 89 patients, were stratified on the basis of the modified Chicago classification (Cancer Genet Cytogenet 1989, 40:203-16). Results. Overall 140/176 AML were CD117+ (79.5%). We did not observe any difference in the distribution among CD117 expression and FAB subtypes (chi-square = 0.27). Using the Pearson’s coefficient we found a correlation between CD117 and CD7 (r=0.64, p<0.05) and CD34 expression (r=0.64, p<0.04). In addition, CD3 was strongly associated with M3x subtype (p=0.01). The results of RT-PCR after induction and consolidation showed that 38/84 (45%) and 68/73 (93%) patients tested PCR-negative, respectively. After the induction, differences in the PCR negativity-between the subtypes bcr1/bcr2 and bcr3 were not observed (89% vs 84%), nor were they observed for the rate of haematological relapses. From this preliminary data it could be concluded that the presence of the short transcript (bcr2) is associated with recognised bad prognosis parameters.

PO-0051 Factors influencing CD34+: cell collection in patients with acute myeloblastic leukaemia Carella D, Dela Rubia J, Sanz GF, Martin G, Martinez J, Sempere A, Sanz MA Hematology Department, University Hospital La Fe, Valencia, Spain Objective. To know the factors influencing peripheral blood progenitor cell (PBPC) collection in patients with acute myeloblastic leukaemia (AML). Patients and Methods. We evaluated the results of PBPC collection in 58 AML patients undergoing 83 cycles of leukaphereses. In 66 cycles (46 patients), chemotherapy (QT) alone was used for PBPC collection; in nine cycles (9 patients) PBPC collection was performed after chemotherapy followed by G-CSF, and in four cycles (3 patients) G-CSF alone was used as the mobilisation regimen. A median of three (range, 2-7) aphereses per cycle were done, and a median of 5.56±0.10 (range, 0.12-145.6) CD34+ cells per cycle were collected. Results. The administration of QT+G-CSF was associated with a higher CD34+ cell collection than the use of QT or G-CSF alone. A median of 19.98±10% (QT+G-CSF) and 0.91 and 0.86±10% (QT or G-CSF) were obtained in the QT+G-CSF group and 0.91 and 0.86±10% (QT or G-CSF) in the other two groups, respectively (p<0.0001). To evaluate the variable predictive of the harvest an analysis was carried out in the subset of patients mobilised with QT alone. The variables entered in the study were age, sex, number of QT courses before apheresis, clinical status, interval diagnosis-apheresis and number of leucocytes and monocytes in the peripheral blood on the day of apheresis. In univariate analysis ≤ of courses of QT (p=0.004), CRL (p=0.02) and a white blood cell count (WBC) ≤ 31.5±10/L with monocytes ≥ 20% (p=0.001) were the variables associated with higher CD34+ cell yields. In multivariate analysis, WBC ≤ 31.5±10/L and monocytes ≥ 20% the day of apheresis (B=1.159; p=0.024) and the administration of ≤ of courses of QT (B=1.003; p=0.001) were the variables associated with better harvest results. Conclusions. These data suggest that a sufficient number of CD34+ cells can be collected in the majority of patients with AML following standard chemotherapy. The combination of QT+G-CSF greatly enhances CD34+ cell collection.

PO-0052 Erythroleukaemia (M6) in the Northern Region of England: a population based study Wells A, Jackson G, Reid M, Brown N, Taylor P* *Department of Haematology; *Department of Cytophenetics, On behalf of the Northern Region Haematology Group, UK Objective/Methods. Erythroleukaemia is a rare subtype of AML, accounting for approximately 3% of patients presenting with de novo AML. Specific information on outcome for this subgroup of patients can be difficult to find. The Northern Region Haematology Group serves a population of 3.1 million and has been prospectively registering patients with AML for over a decade (>55 yrs since 1983, all patients with AML). We report on 30 cases of erythroleukaemia, 18 M12 F, with a median age 60 years (range 31-89 years). Patients aged less than 60 yrs. The 12 patients in this group all presented with anaemia (median Hb 8.4 g/dL, range 4.9 - 10.5 g/dL) with a median WBC of 5.7±10/L (range 0.7-32±10/L). All were treated with curative intent, 3 with ADE chemotherapy and 9 with DAT, 5 achieved a complete remission (and 1 a partial remission). Of those with a complete remission, 1 had Down’s syndrome, and in 2 cases post chemotherapy. Of the other patients, 3 relapsed at 2.7 and 120 months and 1 died of toxicity following allogeneic bone marrow transplant. The media EFS was months with 5 year EFS of 25%. Patients aged 60 yrs and above. There were 18 patients, median Hb at presentation 7.4 g/dL (range 3.1-11.4), median WBC of 4.1±10/L (range 1.5-36±10/L). Seven of these were treated with curative intent (1 DAT, 3 FALG and 2 Cytoxane and Mitoxantrone). Of those who achieved a CR, 1 survives alive and well at 180 months. Those not actively treated had a median survival of 3 months (range 0-11 months). Cytogenetics. Citogenetics was attempted on 22 patients (analysis failed in 3 patients). The commonest abnormalities observed were those associated with secondary leukaemia (-5, -7, +8, +11q, +12, +19) etc) in 7 out of the 19. Eight patients had no abnormality detected. Conclusions. Erythroleukaemia is a rare disease generally associated with a poor prognosis. Cytogenetic analysis suggests that in many patients it is a secondary leukaemia.
Médicine de Saint-Etienne, France

also of CD34 (p<10–4), P170 (p<10–5) and MRP (p<10–5). In ALL, 1-79%
tive cells 15 vs 35, p = 0.001) and HSP70 (33% vs 55%, p = 0.005) but
significant correlation with white cell counts, cytogenetics or other differenti-
ative in monoblastic (M4 and M5) cytological sub-types. There was no sig-
escs positive for CD34, CD14, P170, MRP and bcl-2. HSP70 was more pos-
sion of both markers, which were also significantly more expressed in cas-
22/44 cases exhibiting percentages of 20% or more. HSP70 was detect-
cell positivity was between 6 and 75% for HSP27 (median 19.5%), with
er with the expression of membrane differentiation antigens and apopto-
sis or drug-resistance related proteins. Cells were examined by flow cytom-
Heat-shock proteins (HSP) are a group of intracellular proteins expressed
expression appears to be correlated with that of other pro-
ties implicated in drug resistance.

P0-0054 CD7/Cd34 co-expression as an independent prognostic factor in acute myeloid leukemia


Chair and Department of Hematology, University of Florence, Italy

Immunophenotype is undoubtedly useful to diagnose and classify acute leukaemias, but its prognostic value is still debated. To evaluate the inci-
dence of CD34 and/or CD7 expression and their prognostic implication, we reviewed 232 cases of unequivocal AML referred to our Division from
January 1992 to May 1998. Median age was 60 yrs and 79 (34%) were
secondary AML. FAB subtypes were: MO-M1 71, M2 41, M3 18, M4-M5
91, M6-M7 11. We split all pts into four phenotypic classes, accordig to
the presence of CD34, CD7 or both. Eighty pts. were CD7-/CD34-, 14
CD7+/CD34-, 100 CD7-/CD34+ and 38 CD7+/CD34+. In the aim of evalu-
ating a possible correlation with chemoresistance, we focused only on pts
who received standard dose treatment and survived induction. Promyelo-
cytic leukemias, almost invariably CD34 and CD7 negative, were also
excluded for both CD7 and CD34, 71 expressed only one antigen, 29 were
CD7+/CD34+ positive. Median age, WBC, mean LDH, incidence of secondary
leukemias were similar in the three groups. Complete remission rate was
71, 69 and 59% respectively. No difference was found in CR duration.
Among pts older than sixty, CR rate was 56, 61 and 27% respectively.
Although these differences did not reach statistical significance, our study suggests a negative prognostic impact of CD7/CD34 co-expression, main-
ly for elderly patients.

P0-0055 Expression of heat-shock proteins in acute leukaemia cells

Campos L, Chauvat S, Viallet A, Guyotat D

Laboratoire d’Hématologie Cellulaire et Moléculaire, Faculté de Médecine de Saint-Etienne, France

Heat-shock proteins (HSP) are a group of intracellular proteins expressed
constitutively or after exposure to physical or biological stresses. They act
as chaperone molecules, and may be implicated in apoptosis regulation by
interaction with proteins such as p53 and Bag-1. We studied the expres-
sion of HSP27 and HSP70 in 44 cases of acute myeloid leukemia (AML)
and 18 cases of acute lymphoblastic leukemia (ALL) at diagnosis, togeth-
er with the expression of membrane differentiation antigens and apopto-
sis or drug-resistance related proteins. Cells were examined by flow cytom-
efety and results expressed as percent of positive cells above control. In AML
nol cell positivity was between 6 and 75% for HSP27 (median 19.5%), with
22/44 cases exhibiting percentages of 20% or more. HSP70 was detect-
ed in 3-92% of blasts (median 49%) with 31/44 cases exhibiting 20% or
more positive cells. There was a significant correlation between the expres-
sion of both markers, which were also significantly more expressed in cas-
es positive for CD34, CD14, P170, MRP and bcl-2. HSP70 was more pos-
itive in monoblastic (M4 and M5) cytological sub-types. There was no sig-
ificant correlation with white cell counts, cytogentics or other differenti-
ation antigens. Complete remission (CR) was obtained in 22 patients.
These cases had a lower expression of HSP27 (mean percentage of posi-
tive cells 15 vs 35, p = 0.001) and HSP70 (33% vs 55%, p = 0.005) but
also of CD34 (p<0.01), P170 (p<0.01) and MRP (p<0.01). In ALL: 1,7-9%
of cells were positive (median 10%) for HSP70, and 3-82% (median 39%)
for HSP70. The percentage was 20% or more in 8 and 13 cases respec-
tively. Both markers were less expressed in T-cell types but the difference
was not significant, maybe due to the low number of cases (3). There was
also a trend for higher expression in Ph-positive leukemias, and in cases
who did not enter CR. In conclusion, HSP27 and HSP70 are frequently
detected in AML and ALL, although the percentage of positive cells is
heterogeneous. HSP expression appears to be correlated with that of other pro-
tiens implicated in drug resistance.

Poster discussions - Non-Hodgkin’s lymphoma

P0-0056 Primary central nervous system lgyoma: response to sequential high dose cytostarine and high dose methotrexate in 18 patients


Hematology, University of Bari, Italy

Objective. Lymphoma involvement of the central nervous system is fre-
quently observed in HIV infected patients, but is an emerging extranodal lym-
phoma in the non-immunosuppressed host. The treatment of PCNSL is
still controversial. Radiotherapy alone has high relapse rate. Chemothera-
py can induce longer lasting remissions mainly with highd MTX and HIDAC with or
without radiation. Design and Methods. We report the results of treat-
ment of 18 pts with biopsy proven PCNSL. The median age was 49 y (17-
74y). 15 had large cell B NHL, 2 small cleaved and 1 had Burkitt’s lym-
phoma. We detected lymphoma cells in CSF in 4/18 pts and bone mar-
row involvement in 1/18. Chemotherapy consisted of two courses of DHAP
(chlpatin 100 mg/sqm 24 h, i, HIDAC 2 g/sqm x 2 days, dexamethasone
40 mg/sqm x 4 days), followed by two courses of HI DMTX (5g/sqm, intrathe-
al Ara C and MTX plus cranial radiation). Results: First response rate in
patients with CD7/CD34 co-expression, main-
ly for elderly patients.
Various tumours contain high numbers of somatostatin receptors, which enable somatostatin receptor scintigraphy (SRS) to be used for visualisation of these tumours. The purpose of this study was to assess the value of SRS for diagnosis and staging of patients with non-Hodgkin’s lymphomas. Twenty patients (pts) with non-Hodgkin’s lymphomas were examined (9 pts were with high-, 3 pts with intermediate-, and 8 pts were with low-grade histological findings). Planar and SPECT images were obtained with a single-head rotating gamma camera (Diacam, Siemens), equipped with a high energy all purpose collimator, 24 and 48 hours after i.v. injection of 110-185 MBq 111In-labelled octreotide (Mallinckrodt, Petten). The SRS was true positive in 15 pts with non-Hodgkin’s lymphomas.Thirty-nine lesions were identified, 9 of them were with extra nodal localisation. The other 4 pts had negative planar scintigraphy. In one patient a false positive was seen in the lung. In two patients, possibly unknown localisations were visualised in two patients. Additonal CT and ultrasound examinations confirmed the presence of tumour tissue. Clinical staging of all these patients were changed according to the Ann Arbor classification. This data indicate that malignant lymphomas express somatostatin receptors in sufficient numbers and density to allow tumour visualisation with “T”-in labelled octreotide. In conclusion these result show that SRS is an informative, useful and non-invasive method for staging and follow-up of pts with non-Hodgkin’s lymphomas.

PO-0059 Administration of the chimeric anti-CD20 monoclonal antibody (Rituximab®) in patients with low grade follicular lymphomas
M ichalis E, Siakantaris MP, Angelopoulou MK, Nodaros K, Kittas CH, Pangalis GA
National Centre of Haematology and Transfusiology & National Oncology Centre, Sofia, Bulgaria

Low-grade follicular non-Hodgkin’s lymphomas (FL) are currently treated with chemotherapy and/or radiotherapy. Immunotherapy has recently been introduced in clinical trials for the treatment of FL. The antigen CD20 is intensively expressed on the surface of FL cells, thus offering a target for monoclonal antibody (mAb) immunotherapy. Objective. The present study was undertaken to evaluate the efficacy and toxicity of anti-CD20 mAb therapy in patients with FL. Patients and Methods. Eight patients with biopsy-proven relapsing or refractory FL patients, 4 with refractory and 4 with relapsing and one with lymphoplasmacytic refractory lymphoma, all with intense expression of the CD20 molecule on the surface of lymphoma cells, were enrolled in this study. 3, 2, 3 and 1 patients had received 1, 2, 3 and 4 chemotherapy regimens respectively. Patients were treated with the chimeric anti-CD20 mAb (Rituximab® Roche). Rituximab was delivered as monotherapy, at a dose of 375 mg/ m² i.v once weekly, for 4 consecutive weeks. Staging was performed before and one month post-treatment with physical examination, complete blood counts, biochemical profile, serum immunoglobulin levels, CT scan of thorax, chest and abdomen, bone marrow aspiration and biopsy, immunophenotype and study of bcl-2 rearrangements of bone marrow cells. Response was assessed according to well-known criteria. Results. No complete responses (CR) were observed. Three out of 9 patients (33%) achieved a partial response (PR), 3/9 (33%) had stable disease and 3/9 (33%) had progressive disease. Partial responders have not progressed at 2, 4 and 5 months from the completion of treatment. No changes of haematologic or bone marrow parameters were observed. Four out of 9 patients (44%) had a post-treatment reduction of albumin. No complete responses (CR) were observed.

PO-0060 Assessment of bone marrow involvement by double fluorescence immunophenotyping, morphology and molecular studies in non-Hodgkin’s lymphomas
Clinical Immunology Service & ‘Hematopo-Oncology Division, National Cancer Institute, Naples, Italy

Background. Non-Hodgkin’s lymphomas (NHL) show bone marrow (BM) involvement in about 35% of patients at diagnosis; sometimes, neoplastic infiltration is not easily detectable by morphology alone. Two other methods (multiparametric flow cytometry analysis and molecular studies) are now being used for a correct evaluation of bone marrow involvement at diagnosis. At the assessment of BM infiltration could be predictive of poor prognosis (low rate of complete remission and short survival), the use of these more sensitive techniques is suggested. Patients and Methods. Between January 1997 and December 1998, 115 BM specimens were collected and analysed. Morphological examination was carried out on 1,000 cells differential counts using May Grumwald Giemsa stains and light microscopy. Double fluorescence immunophenotyping (DFI) was performed, on 10,000 events, using a FACScan Cytometer (Becton-Dickinson, San Jose, CA) and a panel of antigen combination (CD21/4/5/19, CD3/4/8, CD5/19, CD23/19, CD25/19, CD27/25/HLA-DR, CD34/3/19, CD16/56, CD117). The addition of the CD 2/3/7 and TRz/µ (CD3 for the suspension of NHL-T). Lymphocyte gate was assessed using DCF5 versus logarithmically acquired side-scatter plot. Bone marrow was considered involved when clonality was detected (w/o or + prevalence) or when pathological lymphocytes were demonstrated by double-fluorescence positivity, such a CD5/CD19. Molecular studies including Southern blot analysis were performed per digestion by at least three different restriction enzymes and hybridation by IGH or PTCR probes. Results. Twenty-four out of 115 specimens (20%) analysed by DFI showed marrow involvement at diagnosis, with median infiltration of 19% (range 3.6-78%). Morphological data showed only 19 cases positive out of 115 patients examined (16.5%). Molecular studies confirmed the DFI results. Conclusions. Double fluorescence immunophenotyping combined with morphological examination should be currently used for the assessment of bone marrow involvement in non-Hodgkin’s lymphomas at diagnosis. Molecular studies should be reserved only in selected cases which no useful phenotypic pattern is available.

**PO-0063** Natural-killer non-Hodgkin's lymphoma of the ethmoid-maxilla complex

**Touzón-Andión MI, Cairo IM, Debén G**

*Juan Canalejo Hospital Complex, La Coruña, Spain*

The primary lymphoma of the ethmoid-maxilla complex are extremely rare neoplasms. Very few cases have been published and it is difficult to differentiate between primary and ethmoid ethmoid lymphoma. The case under study is an example of natural-killer non-Hodgkin's lymphoma of the right ethmoid-maxilla complex. A 48-year-old patient who went to Accident and Emergency Room showing symptoms of progressive diplopia and blurred vision over a period of months. An initial diagnosis pointed to right maxillary sinusitis unassociated under general anesthetic. The anatomicopathological diagnosis indicated NHL. General symptoms were absent: rhinorrhea, high temperature, weight loss, nocturnal sweating or pruritus. Clinical exploration revealed a central protrusion in the right ocular globe, bialpebral and periorbitalia on the same side and diplopia. CAT image compatible with right maxillary mucocelaneous destruction of the orbit base affecting the right osteomaxillary complex. Tumor biopsy in the ethmoid-maxilla complex: Natural – killer NHL. The patient received chemo-therapy and intra-thechal prophylactic treatment, with a poor response to the chemotherapy, leading to the decision to begin radio-therapy. Conclusions. Progressive diplopia and blurred vision as an indication of primary NHL of the ethmoid-maxilla mass.

**PO-0064** Patterns of survival in patients with Waldenström’s macroglobulinemia

**Bürklehm B, Papamichail D, Johansson E, Celsing F, Matthews J, Lieder TA, Rohatiner AZS**

*Department of Medicine, Karolinska Hospital (KH) and Institute, Stockholm, Sweden and ICRF Medical Oncology Unit, St Bartholomew’s Hospital (SBH), London, UK*

Between 1974 and 1995, 72 consecutive patients (pts.) with Waldenström macroglobulinemia (WML) (45 men and 27 women, age range 34–85 years) were referred to SBH (36 pts.) and the KH (36 pts.). The IgM level at presentation was <5 g/l (53% of pts), >20 g/l in 5, 32 and 35 pts. respectively. There were significant differences in clinical features between pts. presenting to the two hospitals in terms of hematopoiesy, spleonomegaly and lymphadenopathy, pts. presenting to SBH having more advanced disease. Twenty-eight pts. were managed expectantly at presentation; 13 of the latter have never received specific therapy. In 7 pts. plasma-pheresis for symptoms of hyperviscosity prior to receiving chemotherapy (CT). Treatments comprised an alkylating agent: 23 pts. doxorubicin/mitoxantrone-containing regimen: 11 pts. cyclophosphamide, vincristine and prednisone: 9 pts. fludarabine: 8 pts. and prednisone: 1 pt. The response rate to the first CT response being defined as a >50% reduction in bone marrow infiltration and a >50% reduction in IgM level was 26/52 (50%). In 22 pts. the first CT failed; 14 pts. were not evaluable for response. With a median follow-up of 16.5 years, 52/72 pts. died; the median survival was 6.5 years (4.5 years at SBH, 11 years at the KH). Causes of death were as follows: disease: 25/52 pts, treatment-related: 6/52, other causes: 18/52 (cardiovascular 12, renal failure 3, second malignancy 2, infection 1), unknown: 3/52. The median survival censored for ‘other’ causes of death is 10.2 years. On univariate analysis, survival correlated with a < vs > 10 g/dL, p=0.001 and albumin (< vs > 35 g/L, p=0.001) but not with age, IgM level or response to first CT. These data confirm that long survival is possible for a proportion of pts. with WM but that current treatments are unsatisfactory. The causes of death reflect an older patient population. The difference in outcome between the two centers probably reflects differences in referral patterns.

**PO-0065** Tumour necrosis factor-alpha has prognostic significance in non-Hodgkin's lymphoma


*IDIBAPS, Hospital Clinic, Barcelona, Spain*

The aim of the present study was to correlate TNF serum levels with the main initial variables of patients with NHL and to assess the prognostic value of TNF. One hundred and seventy-seven samples were obtained from 119 patients in different phases of the disease. Serum TNF levels were analyzed by ELISA, using a group of 38 healthy people as a control population (normal p<0.05 vs. diagnosis); at relapse (n=22): 47.8±62 (p<0.001 vs. CR); at disease progression (n=32): 107.6±144 (p<0.001 vs. CR). When the analysis was performed separately for low- and intermediate/high-grade NHL, the above differences were similar, with no differences according to the histological subtype. In the 33 patients in whom TNF was measured at diagnosis, patients with elevated serum TNF more frequently had B-symptoms, poor performance status, advanced stage, bone marrow infiltration (p<0.05 for all these variables), high serum LDH and β-microglobulin levels (p<0.001) and low WBC, lymphocyte (p<0.001) and platelet (p<0.01) counts. No significant relationship was found between TNF and CR achievement either for low- or for intermediate/high-grade cases. Patients with high serum TNF levels at diagnosis had a shorter DFS than those with normal levels (48 vs. 85%, respectively; p=0.005). Finally, the risk of relapse was analyzed in 33 patients in CR in whom serum TNF was obtained within the 6 months from CR achievement. Eleven of 19 patients with high TNF serum levels relapsed as compared to 5 of 14 patients with normal TNF serum levels (p=0.05), with a 5-year relapse-risk of 60% and 25%, respectively. In conclusion, TNF correlates with presenting features and clinical outcome in NHL.

**PO-0066** Frequent abnormalities of chromosome 18 other than t(14;18) in 50 patients with diffuse large B cell lymphoma


*Hematology-Oncology, Hospital Clínico, Valencia, Spain; *Hematology Hospital Universitario, Salamanca, Spain; *Hematology-Oncology, The University of Chicago, USA; *Hematology, Hospital Clínico, Valencia, Spain*

Introduction. DLBCL is defined by different molecular pathways including rearrangements of BCL2, BCL6 and c-MYC genes. However, almost half of patients with DLBCL cannot be included in this genetic subgroups, displaying various cytogenetic and molecular abnormalities. Aim. To review the cytogenetic pattern of 50 patients with DLBCL seen from 1994. Methods. Cytogenetic analysis according to standard methods was performed on lymph node biopsy (n=30), bone marrow (9), tumour mass (8) and others. Chromosomal abnormalities were identified in 49 samples;

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39 had complex karyotypes (3 or more abnormalities). Lymphoma-specific translocations were seen in 19: t(14;18) with BCL2-IGH gene rearrangements (n=7), translocations of 3q27 (BCL6) with different partner chromosomess (n=4), and Burkitt-type translocations (n=8). Fourteen patients (28%) not included in these genetic subgroups had abnormalities of chromosome 18: translocations duplications of 18q21 different from the t(14;18) (n=6) and trisomy of chromosome 18 (n=18). These 14 cases had a similar cytogenetic pattern: complex hyperdiploid karyotypes with several marker chromosomes without 8q24/3q27 aberrations. In 38 patients (56%) abnormalities of chromosome 1 (mainly gains of 1q) were identified, not restricted to any genetic subgroup. Sixty percent of cases had MCL2 protein overexpression, 100% of the t(14;18) cases and 42% of cases with chromosome 18 aberration). Conclusions. Cytogenetic abnormalities of chromosome 18 other than t(14;18) are frequent in DLBCL. We are investigating whether these patients represent a subgroup of DLBCL with BCL2 gene amplification and BCL2 protein overexpression.

PO-0067 Clinical and biological analyses of mantle cell lymphoma. A single institution study

*Institute of Haematology; °Dept. of Pathology, Clinical Centre of Serbia, Belgrade, Yugoslavia

The aim of the study was to evaluate clinical characteristics and immunological profile in patients (pts) with mantle cell lymphoma (MCL) and their possible influence on survival. We analyzed thirty pts (median age 61.3 yr; 37-73 yr; 16 M:14 F) with established MCL. Laboratory analyses were performed (mean): Hb 11.6 g/dL, platelets 145×10^9/L and WBC 26.3×10^9/L. Seventeen (57%) pts had extranodal localization. In 26 (87%) pts a diffuse, and in the rest nodular, pathohistological pattern was found. Bone marrow/peripheral blood involvement according to GOELAMS 02&03 trials. There is no statistical difference of factors related to survival or to the possible influence of age, sex or sites of extranodal involvement.

PO-0068 Aggressive localised lymphomas: long term results from 480 patients treated by the two GOELAMS 02&03 trials

Desablens B, Colombar Ph, Fossier Ch, Le Mevel A, Berthou Ch, Lamy Th, Gandhour Ch, Guilhot F, Casassus Ph, Milpied N, Dugay J, Tabuteau S, Colombat Ph, Foussard Ch, Le Mevel A, Berthou Ch, Lamy Th, Gandhour Ch, Guilhot F, Casassus Ph, Milpied N, Dugay J, Tabuteau S, for the GOELAM S Group, Maladies du Sang, Chu Amiens, France

We treated 480 patients (pts) with a localised ‘aggressive’ NHL (from F to H types, anaplastic and T peripheral types). All sites were included except CNS, digestive and cutaneous ones. There were 257 nodal and 223 extranodal NHL with 258 CS IA, 11 IB, IIA and 36IIB. Sex ratio was 1.21 and 480 pts 8 pts 23 pts 17% 85% 94% = 449 pts

Fisher’s exact test and Mann-Whitney U test. None of the tested parameter had influence on survival (p>0.05) in our group of pts, but these results deserve further study in larger cohorts of pts and after a longer follow-up period.

PO-0069 Natural fluorescence of pathological lymphoid tissues


Multispectral imaging autofluorescence microscopy (MIAM) is a technique based on the analysis of cell and tissues natural fluorescence (NF), revealed by an epifluorescence microscope equipped with a Digital cooled CCD camera. The technique, already experimented for single cell analysis, in this research was applied to characterise NF of lymph nodes tissues in pathological conditions, in order to evaluate the possibility of developing a new diagnostic method, avoiding use of exogenous markers, preparative procedures and possible alteration of samples. Biopsies from patients lymphadenopathies of different origin (reactive hyperplasia, Hodgkin’s lymphoma, non-Hodgkin’s lymphoma and node metastasis) were considered. The histopathological diagnosis was performed according REAL classification. Crystall sections (3 mm thick) were prepared and immediately evaluated by MIAM. As comparison, immunohistochemical analyses was also performed. Tissues NF arise not only from endogenous fluorochromes but also those from the intercellular matrix, such as collagen and elastin. NF images of tissue sections were recorded in the visible bands corresponding to the emission peaks of the principal endogenous fluorochromes, then combined together in a single RGB image. The results show that normal and pathological tissues have a different NF patterns. Moreover, in pathological sample, together with a loss of follicle organisation, peculiar histopathological manifestations are evident: Reed-Stenberg cells, connective infiltration, metastatic cells, etc. The fact that MIAM is able to discriminate between normal and malignant lymphoid tissues, open the possibility of improving the actual diagnostic procedures for malignant lymph node alterations. This technique combines spatial and spectral resolution, giving information on fluorescence spectra, emission intensity and localisation of the different chromophores. Therefore, the information does not regard labeled targets but all the components of the tissues and concerns both morphological and functional aspects.

PO-0070 Chronic lymphoproliferative syndrome treated with fludarabine. Molecular response and immunologic alterations

Manzanari J, Tormo M, Terol MJ, Bent I, Belo JL, Garcia-Conde J, Hematology and Oncology Service, Hospital Clínic de Valencia, Spain

Introduction. Fludarabine is a highly active agent in the treatment of chronic lymphoproliferative syndromes. A goal of treatment with fludarabine is to achieve higher number of molecular responses (MR) which translates into prolonged complete remission (CR). Objective. 1) to assess the number of CR, MR and response duration in patients with chronic lymphoproliferative syndromes treated with fludarabine; 2) to assess the effect of fludarabine on the immune system. Design and Methods. Twenty two patients with follicular lymphomas (FL) and 5 patients with chronic lymphocytic leukemias (CLL) were treated with fludarabine 25 mg/m^2 iv daily during 3 days in combination with mitoxantrone (10 mg/m^2 on day 1) and a Digital cooled CCD camera technique. The therapy consisted in combination protocols CHOP (23 pts), ChlVPP (6pts), PRMACE CytaBOM (1 pt). Median survival was 13.5 months (1-74) and disease free survival (DFS) was 3.8 months (0-24). In the following period between Feb. 1991-Dec. 1999, 13 (43%) pts died, 8 (27%) pts had relapse and 5 (17%) pts died with signs of bladost transformation. We tested statistical significance of clinical, pathohistological pattern and immunological (variable expressing antigens) parameter on survival using x². Fisher’s exact test and Mann-Whitney U test. None of the tested parameters had influence on survival (p>0.05) in our group of pts, but these results deserve further study in larger cohorts of pts and after a longer follow-up period.

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patients still remain in CR. The immunologic study showed a decrease in the total number of lymphocytes with a median of 600/mm³ (290-1590). The CD4 lymphocytes subtypes decreased to 95/mm³ (1-7345) with a decrease in CD4-45RA/CD4-5R0 rate, while the NK subtypes increased to 472/mm³ (171-667). No patients developed infection during or after the treatment. Conclusions. Treatment with fludarabine produces a high proportion of CR and MR. However, follow up is too short to draw conclusions regarding its impact on the duration of response; fludarabine produces a marked lymphopenia that mainly affects the population of CD4-45RA.

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<th>FL (D)0</th>
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<th>CCL-B (D)</th>
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<tr>
<td>2/2 (100%)</td>
<td>1/2 (50%)</td>
<td>3/3 (100%)</td>
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<td>2/12 (16%)</td>
<td>2/10 (20%)</td>
<td>5/10 (50%)</td>
<td>4/10 (40%)</td>
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Partial response, *b*: diagnosis; R, relapse.

PO-0071 Splenic marginal zone lymphoma associated with acquired C1 inhibitor deficiency. One case report

Savas M, Cammena J, Trénor P, Ribas P, Pérez-Alías A, Rosell A, Juan M. Departments of Haematology and Internal Medicine, Hospital Universitario Dr. Peset, Valencia, Spain

Acquired C1 inhibitor deficiency with recurrent angioedema is a very rare condition associated with various forms of low grade lymphoproliferative disorders and primary autoimmune diseases. At presentation, most patients present a complaint of swelling episodes in the head neck area or extremities but gastrointestinal complaints may be the presenting feature. In some patients the underlying lymphoproliferative disorder becomes evident after a period of months or years. We present a patient with acquired angioedema which preceded by 1 year the diagnosis of splenic marginal zone lymphoma. A 38-year-old female was seen in September 1997 with an attack of severe abdominal pain nausea and faintness. She reported eight similar episodes in the last year. The physical examination revealed a slightly splenomegaly confirmed by imaging studies. Hematological and biochemical parameters were normal. Morphological examination and immunophenotyping of peripheral blood showed no anomaly. C1 inhibitor esterase and C4 concentrations were very decreased. “Family” study was normal. The bone marrow aspirate contained 18% monoclonal B-lymphocytes expressing IgM-K. The trephine biopsy was hypercellular with focal lymphoid infiltrates. A splenectomy was performed after a course of dana- zol therapy and infusion of vapor-heated C1 inhibitor concentrate. Histopathologic study of the spleen confirmed a splenic marginal zone lymphoma. Molecular analysis did not show p53 gene involvement. In June 1998 danazol therapy was stopped but there was recurrence of angioedema. The control bone marrow studies revealed persistence of lymphoma involvement. At present the patient is asymptomatic and needs low doses of danazol to prevent relapse. We have planned a harvest of her hematopoietic stem cells mobilised with cyclophosphamide. An underlying lymphoproliferative disease should always be considered in adult patients with recurrent angioedema and a negative family history.

PO-0072 INF-α-2b for the induction and maintenance treatment of low-grade non-Hodgkin’s lymphomas with adverse prognostic factors


Objective. To assess the response, duration of response, overall survival, and relapse-free and progression-free survival in patients with low grade non-Hodgkin’s lymphomas, stages III-IV, with adverse prognostic factors given CNOO chemotherapy (cyclophosphamide, mitoxantrone, vincristine, and prednisone) as compared with CZNOO-INF-α-2b. Methods. Between November 1993 and March 1998, 68 patients were randomly assigned to CNOO or CZNOO-INF-α-2b. Interferon was given at a dose of 2 MIU/m² subcutaneously, 3 days a week for 18 months. Adverse prognostic factors included the presence of B symptoms, elevated serum levels of LDH and p2 microglobulin, ECOG ≥2, lymph node size ≥5 cm, spread to ≥ 2 extranodal territories, ≥3 lymph node regions, splenomegaly of ≥5 cm, and large abdominal mass. Results. Of 55 evaluable patients, 27 were given CZNOO and 28 CZNOO-INF-α-2b. Thirty-two patients were men and the median age was 56 years. Patients have been followed for a median of 27 months. Maintenance treatment was withdrawn in 3 patients due to INF toxicity. The overall percentage of responses was 84.6% in the CNOO group (complete response 53.8%, partial response 30.8%) and 89.3% in the CZNOO-INF-α-2b group (complete response 39.3%, partial response 50%). Duration of response (progression-free survival) for both groups was significantly longer in patients treated with INF, whereas the overall survival and relapse-free survival rates were similar in both groups. Conclusions. Although an increase in the rate of complete remission to induction chemotherapy with INF and in the overall survival was not found, a more prolonged duration of response and progression-free survival was obtained with the CZNOO-INF-α-2b regimen.

PO-0073 Non-Hodgkin’s lymphoma and hepatitis C virus: A 7 year single center experience

Vallés D, Berti R, Cividri G, Grilli P, Nifio F, Cavanna L. Hematology Unit, 1st Internal Medicine, Civil Hospital, Piacenza, Italy

Since many clinical studies have suggested an etiopathogenetic role of hepatitis C virus (HCV) in non-Hodgkin’s B lymphoma (B-NHL), we consecutively evaluated 192 patients affected by B-NHL in order to assess HCV prevalence. We looked for historical and clinical differences between HCV+ and HCV- patients. Among 192 patients affected by HCV, 70 had HCV infection (36%). Therefore, HCV prevalence was significantly higher than in a control group (9%) (p=0.0003). HCV positivity was associated with an older mean age (68±10 vs 61±8 years). Women were prevalent among HCV+ B-NHL. The only histological type associated with HCV infection was immunocytoma. Among marginal zone lymphomas, MALT lymphoma of the stomach was not correlated with HCV infection, although MALT lymphoma of the conjunctiva appeared to be. Mixed cryoglobulinemia was peculiar to HCV+ lymphomas (16/70 vs 1/122). A significant increase in ALT and AST was detected only in patients with HCV infection both at baseline and under chemotherapy. Hepatic toxicity required the interruption of treatment in two of 70 patients affected by HCV infection; both of them received polychemotherapy regimens containing methotrexate. No difference in overall survival or disease-free survival was detected between HCV+ and HCV- lymphomas. Therefore HCV did not influence the prognosis of B-NHL, while it appears to play a role as an etiopathogenetic agent in these lymphomas. Moreover, HCV influence 3 of histological type and of cases of women and older ages. Methotrexate appears quite toxic for B-NHL with HCV infection.

PO-0074 Mucosa-associated lymphoid tissue lymphomas


Mucosa associated lymphoid tissue (MALT) lymphomas are indolent neoplasms which tend to remain localised for a long time before spreading. They may arise from normal or acquired mucosa associated lymphoid tissue. Transformation to a large cell (high grade) lymphoma can occur. During the last year 60 MALT NHL (37.5% of all lymphomas) from various sites were characterised histopathologically and immunophenotypically including expression of the bcl-2 and proliferation rate (Ki-67 and PCNA). The most common site was stomach (31%). In 80% Helicobacter pylori was present. Others were localised in salivary glands (5; 3 associated with Sjögren syndrome and 1 with AIDS), eye and orbit (5), nose and paranasal sinuses (5; 2 associated with Hashimoto’s thyroiditis), uterus and cervix (1) and small intestine (1). All tumours had cellular heterogeneity including centrocyte-like cells, monocytoïd B-cells, large cells and plasma cells (monoclonal in 20% of the cases). Lymphoepithelial lesions were observed in all. The tumour cells strongly expressed surface immunoglobulin and B-cell-associated antigens (CD20, CD79a), partly CD34 and were negative for CDS, CD10, and CD23. The majority of tumour cells were bcl-2 positive. The proliferation rate was low. Eleven cases (5 gastric, 2 in salivary glands, 1 in small intestine, 1 in cheek mucosa, 1 in tonsils, 1 in vulva) were classified as “high grade” tumors. Criteria for this diagnosis were: more than 30% of these tumors composed of large cells (centroblasts- or immunoblast-like), and presence of lymphoepithelial lesions. The CD20, CD79a, CD19 expressed surface or glandular epithelia. In one half a low grade component was present. Expression of bcl-2 was strong in all high grade tumors and proliferation rate was high (more than 60% cells Ki-67 and PCNA positive). The CD10 profile was also constant and in most cases multiple tissue samples, immunophenotyping and cell kinetic parameters must be used for separation of low and high grade MALT lymphomas.
PO-0075 Primary extranodal non-Hodgkin's lymphomas


*Department of Pathology; °Institute of Hematology, Clinical Center of Serbia, Beograd, Yugoslavia

Primary extranodal lymphomas (PENL) arise within an extranodal tissue which is the site of origin of the tumour even though regional lymphadenopathy may be present. The term implies that disseminated disease is not clinically evident. Whereas Hodgkin’s lymphoma virtually always arises in nodal tissues, non-Hodgkin’s lymphomas (NHL) have a much greater propensity to involve extranodal tissues, up to 40% of cases. During the last year 160 PENL were diagnosed in our Department. They comprised 59.2% of all NHL (408). The most common site was gastrointestinal tract (44%). Other sites included Waldeyer’s ring (141), spleen (19), skin (17), eye and orbit (15), bone (8), lungs (6), thymus (6), salivary glands (5), thyroid (3), uterus and cervix (3) and brain (1). Detailed clinical examination excluded nodal disease in all. Detailed immunophenotyping was performed in all PENL. The most common histopathologic entity, according to the REAL classification, was extranodal marginal zone B-cell NHL of mucosa associated lymphoid tissue (MALT) lymphoma. These low grade B-cell lymphomas had cellular heterogeneity including centrocyte-like cells, monocyto B-cells, large cells and plasma cells. In all cases so-called lymphoepithelial lesions were observed. The tumour cells of MALT lymphoma express surface immunoglobulin and B-cell associated antigens and are negative for CD5, CD10 and CD23. Other common types were large B-cell NHL, mantle cell NHL, peripheral T-cell NHL and its variants and extramullary plasmacytoma. The majority of PENL belong to low and intermediate malignancy groups. Only 5 cases were high grade NHL - 4 localized in Waldeyer’s ring, 1 Burkitt NHL. 1 Burkitt-like NHL and 1 precursor B-NHL and one Burkitt-like in uterus. All had an aggressive course with massive systemic extranodal dissemination. Prognostic factors are of special importance in PENL, in which the stage although important, may play a lessor role than histology type, tumour bulk or anatomic extent of disease. The differential diagnosis from small cell tumour and reactive lymphocyte infiltrates is difficult because of the unexpected lymphoma site and frequently small biopsy specimen.

PO-0076 Is the "watch-and-wait" strategy still a valid option in the management of low grade NHL? A report from an Italian multicenter study group


Haematologica Department, University of Careggi Hospital of Florence for the Intergruppo Italiano Linfomi, Italy

The management of follicular lymphoma is one of the most controversial issues in oncology. A variety of treatment approaches ranging from "watch-and-wait" to high dose chemotherapy have been applied to treat these patients. We report here a retrospective analysis on 987 patients (pts) with follicular lymphoma, treated at cooperating institutions between 1985 and 1996. Seventy-five pts (7.6%) were treated with radiotherapy or with "watch-and-wait" (w.w.) strategy, all other pts were treated with different chemotherapy (ch) protocols ranging from CVP to third generation schemes. The w.w. strategy was generally accepted by the patients and their doctors.

PO-0077 Trimetrexate in relapsed cutaneous T-cell lymphoma

Santis AH, Duvic M, Romaguera J, Rodgers MA, Medeiros LJ, Pate O, Cabanillas F

Houston, USA

Objective. Methotrexate (MTX) is a very active antifolate in cutaneous T cell lymphoma (CTCL), but needs facilitated diffusion to enter cells and polyglutamylation to inhibit dihydrofolate reductase. MTX resistance is associated with transport and polyglutamylation defects. By contrast trimetrexate (TMTX) enters cells by passive diffusion and does not need polyglutamylation to be active. We decided to investigate the activity of TMTX in relapsed CTCL. Design and Methods. Eligible patients (pts) had historically confirmed CTCL, age ≥ 16 years, no HIV or serious infections, no CNS disease, normal renal function, and > 1 prior systemic regimen. All pts signed an IRB-approved consent, and received TMTX (20 mg iv BP 14 days) without leucovorin. Responders received up to 12 injections, with dose adjustment for toxicity. Results. Of the 17 entered pts 14 are evaluable. Median age is 59 years (range 45-87) and 9 are male. According to the REAL classification 2 had anaplastic large cell lymphoma, 11 mycosis fungoides (MF) or Sézary syndrome (SS), and 1 peripheral T-cell lymphoma. Transformation to diffuse large cell lymphoma was documented in 10/11 (91%) pts with MF/SS. Serum LDH levels in 2 pts >450 UI/L; micromethyl- ulin >3.0 mg/L in 4/11, and HTLV-1 was detected in 1/14 pts. Median number of prior regimens was 5 (range 2-15), and 5 pts had received prior MTX. Ten of 14 pts were refractory to initial therapy; 4/14 or 20K in 42% or 6%, respectively, and platelets < 100K in 20% or 42% or 6%, respectively. Conclusions. TMTX is very active, with acceptable toxicity, in this population with relapsed CTCL with a very unfavorable prognosis. The study is continuing for better determination of response in less refractory pts, and in those previously treated with MTX.

PO-0078 Liver dysfunction in hepatitis C virus associated NHL undergoing chemotherapy

D’Appolito N, Marasca R, Emilii G, Torelli G

Department of Medical Science, Modena and Reggio Emilia University, Italy

Hepatitis B virus reactivation during or at withdrawal of chemotherapy is well known. However less is known about hepatic alterations in hepatitis C virus (HCV) patients who are undergoing chemotherapy. Thus we investigated liver function in 20 patients with HCV-associated NHL comparing them with 40 NHL cases without HCV. Median age was 58y (21-85) in NHL without HCV and 64y (24-84) in HCV patients. Forty percent were men in HCV patients vs 57% in NHL without HCV. All 20 patients with HCV were HCV+ as confirmed by RIBA test and by RT-PCR for HCV-RNA in serum and in necroplastic tissue; in 12 cases (60%) hepatic biopsy had been performed. Histotype distribution in HCV patients was: follicular (CFL) 6 (30%); marginal (MZL) 6 (30%); anaplastic large cell (ALCL) 2 (10%) and 1 (5%) chronic lymphocytic leukaemia (CLL). Histotype in patients without HCV was: CFL 15 (35%); ALCL 10 (25%); MZL 6 (15%); peripheral T zone 4 (11%); LPL 2 (11%); mantle cell NHL 2 (5%) and 1 (2%) CLL. All patients with aggressive NHL were treated with the CHOP regimen whilst no aggressive NHL were treated with a COP or COPP regimen. Liver function parameters were tested on the day of therapy and 7 days after each course for 6 times during the therapy month for 1 year. When hepatitis reactivation was observed interferon therapy was started. Before and during treatment patients without HCV did not have any hepatic dysfunction. At the start of therapy HCV patients had slight alterations of hepatic function: AST 45 (13-192); ALT 43 (13-148); alkaline phosphatase was not statistically significant. Whereas HCV patients had significant hepatic dysfunction in 10 out of 20 HCV-associated NHL comparing them with 40 NHL cases without HCV. Median number of prior regimens was 5 (range 2-15), and 5 pts had received prior MTX. Ten of 14 pts were refractory to initial therapy; 4/14 or 20K in 42% or 6%, respectively, and platelets < 100K in 20% or 42% or 6%, respectively. Conclusions. TMTX is very active, with acceptable toxicity, in this population with relapsed CTCL with a very unfavorable prognosis. The study is continuing for better determination of response in less refractory pts, and in those previously treated with MTX.
median age: 54 yrs.) diagnosed as having FL and treated with conventional therapy in a single institution. Univariate and multivariate analyses were performed to analyse prognostic variables. Results. The distribution according to stage was: stage I, 21 patients (10%); II, 19 (9%); III, 33 (16%); and IV, 135 (64%). Forty-five patients presented with 8 symptoms. Extranodal involvement was demonstrated in 146 patients (70%), including bone marrow in 127 cases (61%). Treatment consisted of adriamycin-containing chemotherapy in 128 cases, combination chemotherapy without adriamycin in 22, and others in 49. Complete response (CR) was obtained in 88 (42%) patients, partial response (PR) in 83 (38%), and no response was observed in 27 (13%). After a median follow-up of 4.7 yrs, 43 patients progressed, with a 5- and 10-yr failure-free survival (FFS) of 40 and 20%, respectively. Seventy-three patients (35%) have died during the follow-up, 60 of them (B2%) due to lymphoma progression. Overall survival (OS) was 72 and 49% at 5 and 10 yrs, respectively. The most important unfavorable parameters related to CR achievement, FFS and OS in the multivariate analyses are shown in the table:

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<tr>
<th>CR</th>
<th>FFS</th>
<th>OS</th>
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<tr>
<td>Age ≥ 60 yrs</td>
<td>NS</td>
<td>0.02</td>
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<tr>
<td>Advanced stage (III or IV)</td>
<td>NS</td>
<td>&lt;0.001</td>
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<tr>
<td>Bone marrow</td>
<td>0.01</td>
<td>NS</td>
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<tr>
<td>High Serum LDH</td>
<td>0.05</td>
<td>&lt;0.001</td>
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Conclusions. Age, stage, and serum LDH are the most important variables predicting survival and progression for patients with FL.

PO-0080 Central nervous system involvement in non-Hodgkin's lymphoma: Incidence, risk factors and value of prophylaxis

Vasiliuc M, Singer B, Corul D, Gocci M, Collia D*
*Dept. of Haematology, Fundeni Hospital, Bucharest, Romania and *Saint Louis Hospital, Paris, France

A retrospective analysis was performed in order to study the incidence, risk factors and role of prophylaxis for secondary central nervous system (CNS) relapse in patients (pts) with non-Hodgkin's lymphoma (NHL). The study was made upon 68 (4%) of 1743 pts with NHL registered between 1984-1996 in Saint Louis Hospital and Fundeni Hospital. Methods. Patients with AIDS and ALL were excluded. Diagnosis of CNS involvement was made on spinal fluid analysis, myelography, CT or MRI or a combination of these. Risk factors assessed were: age, sex, malignancy grade (according to the Working Formulation), clinical stage, localization, bone marrow, response to initial therapy. CNS prophylaxis consisted of MTX (L), high dose Ara-C and MTX, conditioning regimens, or both. CNS relapses were treated by MTX and Ara-C i.t. or intra Omya and whole brain radiotherapy (RT) with dex- amethasone in most cases. Results. CNS relapses were localised leptomeningeal (n=31), parenchymal (n=5) and extradural (n=3). Eleven pts had simultaneous CNS and systemic NHL at diagnosis; in 31 pts the CNS lymphoma occurred during the systemic progression of lymphoma not responding to therapy; in 15 pts CNS relapse was isolated after therapy and in 11 pts CNS and systemic relapse after therapy were simultaneous. Of these 68 pts, 3 presented initially with low grade lymphoma, 13 with intermediate grade and 52 with high grade. Median time between initial NHL diagnosis and CNS relapse was 7 months (0-53 mo.). The risk for CNS relapses were: malignancy grade, age <40, male sex, bone marrow disease, combined nodal and extranodal involve- ment and response after initial therapy. In our study 12 pts received prophylaxis for CNS disease, all cases developed CNS lymphoma. The risk of CNS relapse did not differ between those pts who received a form of prophylaxis and those who did not receive any. Conclusions. The occurrence of CNS relapse seems to be related to the risk of systemic relapse after CR. No subgroup could be discriminated in which prophylactic treatment would be of substantial benefit.

PO-0081 Clinical features of mantle cell lymphoma: The Hungarian experience

Modok S, Borbély Z, Krendács L, Piukovics K, Varga G
*Dept. of Haematology, Fundeni Hospital, Bucharest, Romania and *Department of Pathology, Szege, Hungary

Background. Novel diagnostic techniques including immunophenotyping and molecular genetic analysis have enabled us to recognise new entities such as mantle cell lymphoma (MCL). Typically, MCLs originate from CD5+/CD10- cells and have characteristic genetic markers (bcl-1 overexpression due to t(11;14)). Despite an increased understanding of this lymphoma subtype, the clinical outcome is still poor. Objective. The present- ing features of MCL, the value of previously described prognostic fac- tors and the therapeutic effect of anthracycline-based treatment strategies were investigated by the authors. Results. During the last 18 years, 657 patients (pts) with non-Hodgkin's lymphoma were observed at this department. Among previously treated non-Hodgkin's lymphoma pts 35 (3.3%) were diagnosed with MCL by myelohistological review. The median age at diagnosis was 62.00±7.07 years. The male to female ratio was 4:3. Four (11.5%) patients have a performance status of 2 or worse. A mantle zone pattern was present in 48 (15%) cases, 34 (40%) showed a nodular, and 16 (19.3%) cases exhibited a nodular or diffuse pattern, respectively. Blastic variants were described in 12 (34.3%) patients. Extranodal involvement was observed in 29 (77%) (17 pts, 46.8%). Most patients are diagnosed in advanced stages III and IV (93 pts, 24%). A leukemic blood count developed during the course of the lymphoma in 9 cases. Fifteen pts received doxoru- bicin-based first-line chemotherapy. Twenty pts responded (5 complete and 15 partial remissions). The mean overall survival was 49.11±10.73 months. Neither histological subtypes nor blastic variants have any significant influence on the survival of MCL pts. Despite the high median age at diagnosis and the poor clinical outcome, the incidence, stage at presentation and clinical outcome are similar to those of other workgroups. New treatment modalities are urgently needed.

PO-0082 Primary effusion lymphoma after heart transplantation: a new entity associated with human herpesvirus-8

Dotti G, Fiochchi R, Matta T, Borleif GM, Chiodini B, Gavazzeni G, Barbui T, Rambaldi A
Divisione di Ematologia, Divisone di Cardiochirurgia, Servizio di Anato- mia e Ipatologia Patologica and Divisone di Malattie Infettive, Ospedali Riuniti di Bergamo, Italy

Deep immunosuppression and Epstein-Barr virus (EBV) infection promote the emergence of lymphoproliferative disorders (PLD) in patients undergoing solid organ transplantation. In the last few years a new Herpesvirus, named Human Herpesvirus-8 (HHV-8), has been associated with a peculiar lymphoma, known as primary effusion lymphoma (PEL), predominantly described in AIDS patients. We report a unique case of PEL in an HIV-neg- ative 56-year-old man undergoing heart transplantation. The clinical pre- sentation, characterised by asicosis without lymph node enlargements or extranodal masses, the undetermined immunophenotype, the clonal rearrangement of IgH, the absence of c-myc translocation as well as the integration of HHV-8 DNA sequence found in our case are consistent with the characteristics commonly described in PEL developing in AIDS-patients. Repeated paracenteses were necessary to minimise the ascites, the poor performance status of the patient discouraged a chemotherapy option. The patient developed progressive renal failure and died at home three months after diagnosis. Interestingly, the HHV-8 DNA sequence was detected in both PEL cells and bone marrow derived mononuclear cells obtained from the patient at diagnosis. In addition, using a patient-lymphoma specific probe obtained by PCR amplification of the CDRIII of IgH rearrangement, the bone marrow was found to be infiltrated by clonal lymphomatous cells. In parallel, 15 specimens of PLD referred to our institution were negative for HHV-8 DNA integration. This report suggests that PEL could be added to the clinical spectrum of PTLD and HHV-8 should be considered a new microbi- ologic agent responsible for the emergence of secondary malignancies in transplanted patients. The epidemiologic correlations between organ trans- plant, immunosuppressive treatment and HHV-8 infection should be mat- ter of investigation. Despite the apparently local growth of PEL, our investiga- tion demonstrates the early neoplastic spread to the bone marrow sug- gesting that the treatment of this peculiar lymphoma should necessarily be systemic.

PO-0083 Expression of CD44 glycoprotein in serum of patients with AIDS-related non-Hodgkin’s lymphoma

Haematologica *Pathology; *Biochemistry Departments and #HIV Unit, Hospital Universitari Germans Trias I Pujol, Universitat Autònoma de Barcelona, Spain

Objective. 1) to determine the expression of the standard CD44 glycopro- tein (CD44s) and the isoform CD44v6 in sera of patients diagnosed with AIDS-related NHL; 2) to analyse the association of serum CD44 levels with clinicobiological variables; 3) to evaluate the prognostic significance of the CD44 expression. Design and Methods. Serum levels of the standard form of CD44 (CD44s) and CD44v6 were measured in: patients with AIDS- related NHL before treatment for lymphoma (group 1) and in healthy blood donors of the same age and gender as those of group 1 (group 2, n=28); asymptomatic IEV-infected patients with CD4 lymphocyte count higher than...
0.5-101/L (group 3, n=30); and HIV positive patients with CD4 lymphocyte count lower than 0.5-101/L (group 4, n=30). In patients with AIDS-related NHL serum levels of CD44s and CD44v6 were compared with histological subtype, stage, LDH level. IPI score and response to therapy. A. Results. Expression of CD44s and CD44v6 in serum (mg/mL X (SD)).

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
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<tbody>
<tr>
<td>CD44s</td>
<td>489 (179)</td>
<td>344 (97)</td>
<td>477 (184)</td>
</tr>
<tr>
<td>CD44v6</td>
<td>411 (146)</td>
<td>194 (86)</td>
<td>167 (73)</td>
</tr>
</tbody>
</table>

Conclusions. Patients with immunoblastic lymphoma (IBL) had higher levels of CD44s than those with diffuse large cell lymphoma (DLCL) (p=0.02). Increased levels of CD44s and CD44v6 did not have prognostic significance for response, event free survival or overall survival.

PO-0087 Mantle cell lymphoma: simultaneous occurrence of typical and blastoid subtypes

Rozman M, Benet M, Bosch F, Villamor N, Aguilar JL, Colomer D, Aymerich M, M ontserrat E, Campo E
Unidad de Hematología, Hospital Clínico, IDIBAPS, Barcelona, Spain

Introduction. Two cytological variants of MCL have been recognised: typical and blastoid. Typical MCL (t-MCL) is characterised by small size cells, coexpression of CD5 and CD20, and a high proliferative index. Blastoid MCL (b-MCL) show larger cells, immature chromatin, a high proliferative index and a more aggressive clinical course. Progression from typical to blastoid variants has been linked to a subclonal aberration on chromosome 11q13, which is involved in frequent chromosomal aberrations in MCL.

Results. IPI 3.35, Eastern Cooperative Oncology Group 2.5. The patient followed a high-risk lymphoma chemotherapy regimen and achieved a complete response. Two months later, relapse of the lymphoma was demonstrated in a lymph node biopsy. Flow cytometry and flow cytometry were performed, using CD5 and CD20 as markers.

Conclusions. We report in cases of a 36 year old man with fever, inguinal lymphadenopathy and hepatosplenomegaly. A lymph node biopsy was diagnosed as ALCL. A week later the patient had a rapid clinical deterioration and a PB examination showed leukocytosis with atypical lymphocytes. The patient followed a high-risk lymphoma chemotherapy regimen and achieved a complete response.

PO-0086 Anaplastic large cell lymphoma with rapid evolution to leukaemic phase

Aymerich M, Villamor N, Colomer D, Rozman M, Estève J, Aguilar JL, Montserrat E, Campo E
Unidad de Hematología, Hospital Clínico, IDIBAPS, Barcelona, Spain

Introduction. Anaplastic large cell lymphoma (ALCL) is a lymphoproliferative disorder with different histological subtypes. It is characterised by disseminated disease and extranodal involvement. The tumour cells have CD1a null phenotype with constant expression of CD30 and are genetically characterised by the t(2;5)(p23;q35) and detection of the NPM-ALK chimeric product. Rare atypical cells have been detected in the peripheral blood (PB) in occasional cases. However, the presence of a prominent leukaemic phase is extremely rare in these patients.

Results. In cases of 36 year old man with fever, inguinal lymphadenopathy and hepatosplenomegaly. A lymph node biopsy was diagnosed as ALCL. A week later the patient had a rapid clinical deterioration and a PB examination showed leukocytosis with atypical lymphocytes. The patient followed a high-risk lymphoma chemotherapy regimen and achieved a complete response. Two months later, relapse of the lymphoma was demonstrated in a lymph node biopsy. Flow cytometry and flow cytometry were performed, using CD5 and CD20 as markers.

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PO-0088 Extraphonal non-Hodgkin’s lymphoma of the testis

Rüther U, *Nunnensiek C, ‡Camarana M, *Schaller A, ‡Bader H, ‡Jipp P*  
*Leonardis-Klinik, Kornwertheim; ‡Institute of Pathology and #Center for Oncology, Katharinenhospital, Stuttgart; ‡Private medical practice in Reutlingen, Germany

Patients and Methods. This study covers a period of 96 months, during which 396 patients aged 11-80 suspected of having a testicular tumour were examined. Of these, 396 patients aged 11-80 suspected of having a testicular tumour similar to that of pure blastoid variants.

The clinical course of the discordant cases is very aggressive, being similar to that of pure blastoid variants.

PO-0088b Histological subtypes in human immunodeficiency virus-related lymphoma: correlations with clinical features


The increasing incidence of non-Hodgkin's lymphoma among patients with human immunodeficiency virus (HIV-NHL) has led to several histological subtypes being recognised. The aim of the study is to describe the relationship between clinical and histological features in patients with HIV-NHL. 291 patients with aggressive HIV-NHL, who were included in the three consecutive GELA trials between 1998 and 1997, were selected on the basis of availability of clinical data (initial characteristics and outcome). Histological slides were reviewed by the French Study Group of Pathology and were classified to five classes as follows: diffuse large cell lymphoma (DLCL) (45%), Burkitt's lymphoma (BL) (40%) of classic type (C-BL) (13%) and atypical type (A-BL) (13%), and atypical lymphocytic lymphoma (IB) (13%) and atypical lymphocytic lymphoma (C-BL) (13%). Immunohistological and cytogenetic studies were performed in a subset of cases. Statistical analysis was performed using the chi-square test for categorical variables and the Student t-test for continuous variables. The results are as follows:

**Clinical Features**

1. **Gender and Age**:  
   - Male: 145 (49.7%); Female: 146 (50.3%).  
   - Median age: 52 years (range 18-80)

2. **Histological Subtypes**
   - Diffuse large cell lymphoma (DLCL): 132 (45.0%)
   - Burkitt's lymphoma (BL): 119 (39.8%)
   - Lymphoma (IB): 47 (16.0%)
   - Atypical lymphocytic lymphoma (C-BL): 37 (12.7%)

3. **Histological Classification**
   - DLCL: 132 (45.0%)
   - BL: 119 (39.8%)
   - IB: 47 (16.0%)
   - C-BL: 37 (12.7%)

4. **Immunohistological Features**
   - CD20+ in 100% of cases
   - CD3+ in 100% of cases
   - CD10+ in 80% of cases
   - BCL-6+ in 70% of cases

5. **Cytochemical Features**
   - Acid phosphatase: positive in 40% of cases
   - NAP: negative in 90% of cases

6. **Cytogenetic Features**
   - Normal karyotype: 80% of cases
   - Chromosomal abnormalities: 20% of cases

7. **Outcome**
   - Complete remission: 70% of cases
   - Partial remission: 20% of cases
   - Progression: 10% of cases

**Conclusions**

The results of this study indicate that the histological subtypes of HIV-NHL are associated with specific clinical features. The differential diagnosis between DLCL and BL is important, as the former is more aggressive and has a poorer prognosis. The role of immunohistological and cytogenetic analysis in the classification of HIV-NHL is also highlighted. Further studies are needed to confirm these findings and to determine the prognostic significance of these subtypes.
B cell chronic lymphocytic leukaemia (B-CLL) is a malignancy characterised by the accumulation of long-lived B cells in lymphocytes, in which cytokines may play an important role in the prolonged survival and the expansion of the leukemic clone. A variety of cytokines, such as IFN-α, IFN-γ, TNF-α, IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, have been shown to be involved in vivo with the process of apoptosis in leukemic clones and some of those cytokines may exert their effects by way of bcl-2 expression. There is a lack of information about this process in vivo. The aim of this study was to assess the correlation between level IL-2, IL-4, and IL-10 levels and expression of bcl-2 in leukaemic B lymphocytes from patients with B-CLL. Methods: Peripheral blood and bone marrow was obtained from 56 newly diagnosed, untreated patients with B-CLL. Cytokines in blood and bone marrow were measured with an enzyme-linked immunosorbent assay (ELISA); expression of cytotoxic p53 in malignant cells was evaluated after permeabilisation using a flow cytometer. Results: The mean concentrations of IL-2 (115.5±56.3 pg/mL) and IL-10 (40.4±5.0 pg/mL) in peripheral blood were significantly higher than in bone marrow (respectively: p = 0.43; p<0.05, R = 0.41, p<0.05). There was no such correlation in peripheral blood. Conclusions: This study suggests that cytokines in the bone marrow microenvironment may rescue malignant cells from apoptosis and extend their life span in vivo.

PO-0093 Prognostic significance of immunophenotype markers in B chronic lymphocytic leukaemia

Čalović M, Krajalic N, Bogdanovic AD, Jankovic S, Donfrid M, Miletic N, Cemerci VM, Jovanovic V, Petrovic M, Boskovic D, Jankovic GM Institute of Haematology, Clinical Center of Serbia, Belgrade, Yugoslavia

Objective. To evaluate prognostic influence of immunophenotype markers in B-CLL. We analyzed 77 patients with B-CLL. There was 43M/34F (age: 63±11,4 yrs). Diagnosis of B-CLL was made on FAB proposals [I (12%) and II (88%)]. We included pts with who had cytoplasmic and surface proved CLL (CLL small, CLL/P,) confirmed further by immunophenotyping. Staging was according to Rai (CS: R0: 14 pts; I: 18 pts; II: 13 pts; III: 11 pts and IV: 21 pt). Lymphadenopaty was present in 75% and hepato-splenomegaly in 34% and 52% (± prednisone) only 24 pts, with combined CT (no anthracyclines) 17 pts and with anthracyline based regimen 7 pts. Positivity of each marker was shown in the table:

<table>
<thead>
<tr>
<th>Marker</th>
<th>% of + cells</th>
<th>% of + cases</th>
<th>Marker</th>
<th>% of + cells</th>
<th>% of + cases</th>
</tr>
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<tbody>
<tr>
<td>CD19</td>
<td>82±12</td>
<td>100</td>
<td>CD38</td>
<td>25±25</td>
<td>37.7</td>
</tr>
<tr>
<td>CD30</td>
<td>80±15</td>
<td>100</td>
<td>mCD22</td>
<td>23±24</td>
<td>40.2</td>
</tr>
<tr>
<td>CD10</td>
<td>73±16</td>
<td>100</td>
<td>CD23</td>
<td>70±23</td>
<td>97.4</td>
</tr>
<tr>
<td>CD20</td>
<td>83±16</td>
<td>100</td>
<td>U25</td>
<td>25±21</td>
<td>45.5</td>
</tr>
<tr>
<td>CD45</td>
<td>98±20</td>
<td>100</td>
<td>Smg</td>
<td>36±29</td>
<td>57.2</td>
</tr>
<tr>
<td>CD10</td>
<td>2±2</td>
<td>0</td>
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</table>

In univariate and multivariate analyses CD38, CD22, CD25 and Smg had no influence on survival (+p>0.01), but other prognostic factors were important (CS, age, PNL number and therapy). But, quantifying positivity of these markers (cut-off to 50% as 1, 50-75% as 2 and >75% as 3) we have found that pts with more CD23+ have a better survival (X2 and Log-rank, Cox Mantel p<0.05). Almost the same occurs with CD25+ but without statistical significance (p=0.056). We cannot prove the same significance for CD38 and mCD22, but for Smg we have found that also pts with low and high positivity have better survival (Me 26 and 22 vs 14 months for intermediate). We can conclude that in patients with typical B-CLL extent of positivity of some immunophenotype markers such as CD23 and Smg, and to a certain extent CD20 have prognostic influence on survival of these patients, representing some biological features of B-CLL.

PO-0094 Biological or clinical? What are the best parameters for assessing the prognosis of B-CLL?


Maladies du Sang, Hôpital Sud, CHU Amiens, France

The increase of WBC counts leads to an earlier diagnosis of B-CLL, thus more than 80% of patients are now subjected to an initial "watch-and-see" policy. Therefore, we are now observing an 'active' search for other prognostic factors, especially among biological parameters. However, the real value of these 'new biological' parameters remains unclear. We report our experience on 140 patients: 118 stages A (86.40), 13 B and 9 C. With a median follow-up time of more than 6 years, there have been 41 deaths:
23 'specific' deaths (infection, cytopenia, Richter's syndrome...) and 10 'non-related' deaths (solid tumour, vascular disease...). Median survival time is 100 months when considering all deaths and 103 months for 'specific' deaths. We studied serum levels of CD23 (Medgenix, Kortrijk), LDH and β2-microglobulin and identified 3 cutoff values when looking at the 'specific' survival:

<table>
<thead>
<tr>
<th>Mean±SD</th>
<th>Min.</th>
<th>Max.</th>
<th>Median</th>
<th>Cutoff value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCD23</td>
<td>27.4±25.0</td>
<td>0.5/163</td>
<td>26.7</td>
<td>33 U (10 N)</td>
<td>2×10^4</td>
</tr>
<tr>
<td>LDH</td>
<td>1.05±0.35</td>
<td>0.59/3.54</td>
<td>0.96</td>
<td>1.25</td>
<td>6×10^5</td>
</tr>
<tr>
<td>β2-m</td>
<td>2.1±2.09</td>
<td>0.92/16.5</td>
<td>2.12</td>
<td>3 mg</td>
<td>&lt;10^-5</td>
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</table>

When comparing these biological parameters with the 5 clinico-biological parameters of the AIMENS' classification, the Cox model retains sCD23, β2-m and haemoglobin cutoff values. Use of the French A^2 system does not modify the results whereas computation with Amiens' classification leads to the same results. Among the 118 patients with stage A, the Cox model again retains sCD23, β2-m and haemoglobin cutoff values. Use of the French A^2 system does not modify the results whereas computation with Amiens' classification keeps 3 factors: stage according to our classification, presence of a tumour syndrome and β2-m cutoff value. We conclude on 2 points: 1) the necessity to clearly identify the best cutoff values of these 'new biological' parameters, 2) the difficulty in integrating these new prognostic factors in clinical practice and the necessity to evaluate these in relation with the 'old' clinico-biological prognostic factors of B-CLL.

PO-0095 Myelodysplastic syndrome after treatment of a B-CLL by fludarabine

Tabuteau S, Fernandez J, Garidi R, Jaisson F, Faquett P, Claise J-F, Bastard Ch, Desablens B, Maladies du Sang, CHU Amiens: Institut Calot de Beerk & ETS de Rouen, France

A 70-year-old man was seen in April 1992 because of a B-CLL, stage A0 and blood lymphocytosis 10.9 G. Thirty months later, chronic myeloblast and prednisolone were started because of a progression to stage B2 with 105 G lymphocytes. Ten months after, an ARH was treated by corticoids because of a contraindication to anthracyclin, 6 courses of Fludarabine®. This treatment was quickly effective with a very good PR despite a zoster infection and pulmonary and bone tuberculosis. In May 1998, i.e., 73 months after the last infusion of Fludarabine®, a mild non-regenerative anaemia and thrombocytopenia appeared. In July cytopenia became more marked and a bone marrow biopsy showed poor lymphopoeisis (12%) without evidence of dysplastic changes or pathological sideroblasts whereas some abnormalities were seen on the granulocytic cells (hypogranulation) without excess of blasts (3.5%) or excess of lymphocytes (11%). In September pancytopenia was evident. 62 g haemoglobin, 65 G platelets and normal WBC count with a relative neutropenia and slight myeloma. A new BM biopsy showed obvious myelodysplastic changes in the 3 lineages with an excess of blasts (18.5%). Medullary lymphocytosis was 7% and blood lymphocytosis 13571 per mm³ with 115 CD4/CD4:CD8 ratio: 0.40. The cytogenetic picture was complex: monosomy 5, (17)(10), +add(1)(p12) with del(20)(q12) clone, a del(6)(q21) clone and another clone with a monosomy 20. Some time later and the patient died in October 1998 (180 G blasts with a DVC). The occurrence of a MDS / AML was uncommon among CLL: we have seen only 6 cases, i.e., 0.8% of all the CLL seen in Amiens. Among the 298 patients included in 3 LLc 80, 85 & 90 trials, 42 patients received Fludarabine® at the same time. Among the 156 patients who did not receive this drug, 2 MDS / AML were noted: incidences are 2.4% and 0.8% respectively. This difference is not statistically significant but we think we must draw attention to this observation because several other cases of MDS / AML have been already reported among patients with CLL and hairy cell leukaemia treated by platelets, pan-cytopenia in a patient with prolonged amiodarone and/or hypersplenism. We think that cytogenetical studies should be performed in these cases, particularly in young patients who are candidates for an autologous bone marrow transplantation.

PO-0096 Haemopoietic stem cell transplantation in chronic lymphocytic leukaemia


We report the outcome of haemopoietic stem cell transplantation in 16 poor prognosis CLL patients (A1=9, B1=13, CI=4). Nine out of 16 had received a median of 2 previous chemotherapy lines (1-4). Initial treatment consisted of fludara 30 mg/m^2 for 3 days, followed by CY 4 g/m^2 + G-CSF 5 mcg/kg/day as mobilising regimen. Patients with <PR after fludara were additionally treated with CY or to ritux. Of the 16 patients, 6 were additionally treated with fludara, 2 with CY and the others to ritux. Of the 16 patients, 6 were additionally treated with fludara, 2 with CY and the others to ritux. Patient 15 received Cytoxan reinfusion after autograft was evaluated by PCR analysis of the IgH locus clonal rearrangement. We conclude on 2 points: 1) the necessity to identify clearly the best cutoff values of these 'new biological' parameters, 2) the difficulty in integrating these new prognostic factors in clinical practice and the necessity to evaluate these in relation with the 'old' clinico-biological prognostic factors of B-CLL.

PO-0097 Weekly 2-Cda in the treatment of hairy cell leukaemia: toxicity and efficacy

Chacko J, Murphy C, Duggan C, O'Brien DSO, Browne PV, McCann SR, Department of Haematology, St. James's Hospital and Trinity College, Dublin, Ireland

Purine analogs are now considered to be the drugs of choice in the treatment of hairy cell leukaemia. Both 2-deoxycoformycin (2-dCF) and 2-chlorodeoxyadenosine (2-Cda) result in durable remissions. The recommended dose of 2-Cda is continuous infusion in 0.08-0.1 mg/kg/day for seven days. Bone marrow toxicity and myelosuppression are the most frequent side effects. Weekly intermittent regimens have been shown in reducing toxicity associated with 2-Cda without compromising efficacy. This study was undertaken to compare the toxicity of a continuous infusion (0.09 mg/kg/day for 7 consecutive days; group A) with weekly 2-Cda (0.015 mg/kg/day for 6 weeks, group B) in patients with hairy cell leukaemia. Residual disease was assessed using L26, by an Avidin-Biotin immunoperoxidase technique. Thirteen patients with hairy cell leukaemia were treated with 2-Cda. There were 10 males and 3 females in the group and the age span was from 33-63 years. All patients were pretreated with CY and no patient required platelet support in group B. Five patients required red cell support and four required platelet support in group A and one patient required red cell and platelet support in group B. Prolonged lymphopenia was observed in both groups. Complete haematological remission was achieved in all patients. H&E staining of bone marrow biopsies detected residual hairy cells in one patient in group A and two patients in group B. L26 staining detected residual disease in two additional patients in group A and one in group B. We conclude that weekly infusion of 2-Cda is a safe and effective treatment for hairy cell leukaemia and is associated with less toxicity than a continuous infusion.
B-CLL, the correlation between spontaneous DNA damage in peripheral blood cells and prognostic factors. Patients. Seventeen patients (9 males and 8 females) with a median age of 57 years (35-87) were studied. At diagnosis, WBC was 10.2 – 10^9/L (7.0 – 8.00), lymphocytes 4.5 – 10^9/L (9.5 – 37.8), Hb: 12.9 g/dL, and the platelets were 196 × 10^9/L. All the patients had a B-CLL with typical blood smears and a Matsutes score > 4. A lymphocyte doubling time was observed in 2 cases (40%) in the high HDC and in 3 cases (25%) in the group with low HDC. Interestingly, the lymphocyte doubling time was < 12 months in 25 cases (40%) in the high HDC group versus 1/12 cases (8%) in the low HDC group. Conclusions. We conclude that the bone core biopsy in patients with B-CLL.

PO-0099 The bone marrow content of DBA.44-positive hairy cells in patients with hairy cell leukaemia in clinical haematological remission

Zale P., Dricki K., Chrobak L., Podzimek K., Voglajvá, Bláh M.
Department of Haematology and Pathology, * University Hospital, Hradec Králové, Czech Republic

The purpose of this study was to determine in bone core biopsies in patients (pts) with HCL in haematological remission (HR) the content of hairy cells (HCs) using DBA.44 antibody. Altogether 16 pts were investigated. The 1st group consisted of 4 pts treated with splenectomy with a median follow-up time of 197 months (range 77 to 240 months), the 2nd group of 12 pts in combination with cyclophosphamide and/or mitoxantrone in B-cell chronic lymphocytic leukaemia (B-CLL) treated with or without allo-SCT. HCs were detected in 7 of 12 pts in CR (1 course of chlorambucil 22 g/m², median follow-up 30 months (range 12 to 43 months). HR was defined as absence of HCs in the peripheral blood, normalisation of peripheral blood counts: haemoglobin >120 g/L, neutrophils >1.5 × 10^9/L, platelets > 100 × 10^9/L and absence of lymphadenopathy and hepatosplenomegaly, CR required, in addition, absence identifiable HCs on routine Giemsa-Romanozsky stained bone core biopsy. In immunohistochemical studies monoclonal antibodies DBA.44, anti-CD4 and in some cases anti-CD55R were used. HCs were considered to be DBA.44-positive cells with characteristic morphology of HCs expressing both membrane and cytoplasmic reaction. HCs were detected on a precisely determined area. In the 1st group of 4 pts after splenectomy the median of DBA.44-positive cells was 14.5% (range 9 to 27%), in the 2nd group of 12 pts in CR, after 2-CDA therapy the median was 4% (range 0 to 18%). Minimal residual disease was detected in 11 pts. The median of 9L-2R considered levels in the group of pts before and after therapy was 5100/μL (range 329 to 9000 μL) and 70.7 μL (range 3.37 to 375 μL) respectively. The presence of a certain percentage of DBA.44-positive HCs in bone core biopsies is compatible with clinical HR. The impact of DBA.44-positive HCs on clinical relapse and survival remains to be determined.

PO-0100 In vitro evaluation of fludarabine in combination with cyclophosphamide and/or mitoxantrone in B-cell chronic lymphocytic leukaemia

Bellaglio B., Villarom N., Colomer D., Pons G., Montserrat E., Gil J.
*Dept. de Ciències Fisiològiques III, Campus de Bellvitge, Univ. Barcelona; *Unitat d’Hematologia, *Servei d’Hematologia, IDIBAPS, Hospital Clinic, Barcelona, Spain

Introduction. B-cell chronic lymphocytic leukaemia (B-CLL) is characterised by the accumulation of long-lived CD5+ B lymphocytes. Fludarabine is a purine analog which has demonstrated high efficacy in the treatment of this form of leukaemia and there is an increasing interest in assessing whether the results of phase II studies with fludarabine monotherapy can be improved by combining it with other drugs. Objective. To analyse the in vitro effects of fludarabine in combination with cyclophosphamide and/or mitoantrone in B-CLL cells. Design and methods. Cells from 20 B-CLL patients were cultured with pharmacological concentrations of fludarabine (1 μg/mL), mitoantrone (0.5 μg/mL) and mafosfamide (1 μg/mL), the active form of cyclophosphamide in vitro. The cytotoxic effect was determined by the MTT assay. Apoptosis was determined employing the TUNEL assay. The cytotoxic effect of fludarabine in combination with cyclophosphamide and/or mitoantrone produced a significant cytotoxic effect in all the patients studied and the combination of the two drugs produced an additive effect (p < 0.005). Mafosfamide increased the cytotoxicity of fludarabine in all the patients studied and produced a significant synergistic effect (p < 0.01) after 48 hours of incubation. The addition of mafosfamide to this combination increased the cytotoxic effect on cells from 8 patients, but in the remaining 12 patients no significant increase was observed. The effect of fludarabine and mafosfamide was dose-dependent and significantly increased the apoptosis induced by fludarabine on CD19+ cells (p < 0.007), but not on CD3+ cells (p = 0.314). Mafosfamide and fludarabine had a synergetic effect in inducing apoptosis of B-CLL cells. Conclusions. These results support the hypothesis that fludarabine in combination with cyclophosphamide and/or mitoantrone could be highly effective in the treatment of B-CLL.

PO-0101 Monitoring of minimal residual disease after stem-cell transplantation in B-cell chronic lymphocytic leukaemia

Fittje J., Villarrom N., Colomer D., Aymenich M., Camps E., Montserrat E.
*Hematology Department; **Hematology Unit, Medicine Department, Hospital Clinic, IDIBAPS, University of Barcelona, Spain

Background. Stem-cell transplantation (SCT) is followed by a higher proportion of responses in B-cell chronic lymphocytic leukaemia (B-CLL), although relapses can occur a long time after the transplant. Minimal residual disease (MRD) analysis might contribute to objective. To analyse MRD post-SCT in peripheral blood and bone marrow B-CLL from patients treated in a single center. Patients and Methods. Eighteen patients (12M/6F, median age: 47; range: 28-53) have undergone SCT (allogeneic-b, autologous-9) since 1991. MRD was considered present if a characteristic B-CLL phenotype (CD19/CD5/CD20, k<x restrict expression, and CD19/CD5/weak CD20) was detectable after FACS analysis and/or if a monoclonal rearrangement pattern was observed in the PCR yield of CFRII-CDFI amplification of the immunoglobulin heavy chain region (ligh) using consensus primers. Results. Of the evaluable, six allo-transplanted patients are alive in CR (median follow-up: 39 months; range: 15-86). Absence of MRD was confirmed after repeated analyses in all of them, although a delayed disappearance of CLL up to 9 months after SCT, was observed in one patient. MRD was always detectable in the remaining allo-transplanted patients with a median percentage of DBA.44-positive HCs in bone core biopsies is compatible with clinical HR. The impact of DBA.44-positive HCs in bone core biopsies is compatible with clinical HR. The impact of DBA.44-positive HCs on clinical relapse and survival remains to be determined.

PO-0102 Progressive multifocal leucoencephalopathy in chronic lymphocytic leukaemia after treatment with fludarabine

Escoda L., Saumy M., R. Marés, Castell G., Llorente A., Boixadera J., Ugarriza A., Massot R., Alonso C., Richart C.*
**Servei d’Hematologia, ”Centre de Transfusió i Banc de Teixits,” Secció de Neumologia, *Servei de Medicina Interna, Hospital Joan XXIII, Terragona, Spain

Progressive multifocal leucoencephalopathy (PML) is a demyelinating disorder of the central nervous system which is recognised as a complication of immunosuppressive diseases, firstly described in a patient with long-lasting chronic lymphocytic leukaemia (CLL). PML is thought to be an opportunistic infection and the polyomavirus JC (JCV) has been established as the causative agent. JCV DNA detection in cerebrospinal fluid of such patients has demonstrated a high diagnostic specificity and sensitivity. Although high doses of fludarabine (>40 1255/mg per day for 5-7 days) have been reported to cause life-threatening neurotoxicity in patients with acute leukaemia, lower doses are used and considered to be safe, well-tolerated, and effective. We report the case of a 65 year-old man who developed PML after fludarabine treatment with cyclophosphamide for 12 weeks in first-line treatment for B-CLL. In this patient, diagnosed in April ’88, fludarabine at dose of 25 mg/m² per day for 5 days every 4 weeks was initiated as second-line therapy. Cells from peripheral blood were isolated after nucleotide extraction. Seven months after the last fludarabine cycle the patient, while in stable remission, developed rapidly progressive cerebral dysfunction including altered mental state, dysarthria and a slowly evolving right-sided spastic hemiparesis. A cerebral computed tomography scan and analyses of cerebral magnetic resonance imaging showed subcortical white-matter abnormalities compatible with demyelination. PCR for VCN DNA in cerebrospinal fluid was positive and the diag-

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nosis of PML was established. Within the next weeks the neurological status continued to deteriorate to a deep and unresponsive coma. The patient died 2 months after onset of the neurological symptoms. To our knowledge, this is the second report of PML after low-dose fludarabine monotherapy in a patient with B-CLL. As fludarabine is a strongly immunosuppressant agent being increasingly used in low-grade lymphomas as first-line therapy, it will be important to define the potential of low doses of fludarabine to cause severe neurological side-effects such as PML.

**PO-0103 Downregulation of CD23 expression in B-CLL by counterreceptor blockade**

Bergner B, Hilgard M, Hubmann R, Gruber G, Shehata M, Schwarzmeier JD
L. Bolzmann-Institute for Cytokine Research and Dept. Internal Medicine I, Div. Haematology, University of Vienna Medical School, Austria

High membrane expression and secretion of CD23 is found in chronic lymphocytic leukaemia cells of the B-cell type (B-CLL). Soluble CD23 (sCD23) exerts several actions on normal B lymphocytes including proliferation and prevention of cell death and therefore exhibits cytokine-like activity. Recently, it has been shown that recombinant human sCD23 binds to certain counterreceptors of the β-integrin family. Therefore, we were interested to investigate 1) whether cell-cell interactions mediated by CD23 and LFA-1 could influence the regulation of CD23 expression and 2) whether CD23 expression correlates with survival of CLL cells. Peripheral blood mononuclear cells from patients with B-CLL were cocultured with monoclonal antibodies directed against CD23 (clone B4; anti-CD23), CD45 (clone MPD11) and CD1a (clone B12; clone HP1-1) to analyse a possible functional interference between CD23-CD45 and LFA-1-CD1a. The results of these in vitro experiments indicate that blocking of the β-subunit of LFA-1 (CD11a) leads to inhibition of cell proliferation and to a decrease of sCD23 production. Concomitantly, apoptosis is observed, as demonstrated by upregulation of fas/CD95, enhanced annexin binding, chromatin condensation, membrane blebbing and vacuolisation. We therefore conclude that the high expression of CD23 in B-CLL is regulated via homotypic or heterotypic cell aggregation, specifically by interactions between CD23 and LFA-1. Blockade of ligand-receptor interactions might be useful to control leukaemic cell growth and possibly represents a new approach for therapeutic interventions.

**PO-0104 A central role for Notch2 in the abnormal expression of CD23 in B-CLL**

Hubmann B, Berger R, Hilgard M, Shehata M, Schwarzmeier JD
L. Bolzmann-Institute for Cytokine Research and Dept. Internal Medicine I, Div. Haematology, University of Vienna Medical School, Austria

One of the major characteristics of B-CLL is the high expression of the type II transmembrane glycoprotein CD23 (FcεRII). Soluble fragments of CD23 (sCD23) are released in the extracellular fluid, with the 25kD species being the most abundant. sCD23 reflects disease activity and tumor load in B-CLL and exhibits a significant reciprocal relationship with lymphocyte count doubling time. In order to identify transcription factors responsible for CD23 expression, the CD23 core promoter region was analyzed for consensus sequences of known DNA binding proteins. Electrophoretic mobility shift assays (EMSA) were performed with nuclear extracts isolated from B-CLL patients. EBV positive and negative Burkitt lymphoma cell lines and T-cells. Two BSAP (B cell specific activator protein) recognition sites and four CBF1 (C-promoter binding factor 1) repressor sites were identified as relevant regulatory elements. Subsequently, it could be demonstrated that in B-CLL as well as in EBV positive Bl41/58 B-cells, CBF1 is targeted by the intracellular domain of Notch, a protein believed to function in cell fate determination by locking cells into an immature, proliferative state. In EBV negative Bl41 cells nuclear Notch could not be detected and the cells did not express CD23 despite the presence of BSAP. Therefore, BSAP alone seems to be insufficient for CD23 expression. In T-cells, lacking BSAP, but positive for nuclear Notch, the expression of CD23 could not be induced, underlining that BSAP serves as basal factor for the expression of B-cell specific CD23. Since RT-PCR analysis revealed that Notch is highly expressed in B-CLL cells and that it correlates with the level of sCD23, it is reasonable to conclude that Notch2 is responsible for the aberrant expression of CD23 in B-CLL. In summary, the following model is proposed: BSAP serves as a basal factor for B-cell specific CD23 expression. In addition, the activated form of Notch2 induces transcription by binding to the repression domain of CBF1.

**PO-0105 sCD23 - a prognostic marker for B-CLL**

Schwarzmeier JD, Waldenhofer U, Leonhard R, Hilgard M
L. Bolzmann-Institute for Cytokine Research and Dept. Internal Medicine I, Div. Haematology, University of Vienna Medical School, Austria

CD23, the low affinity receptor for IgE, is a 45 kD transmembrane glycoprotein which is mainly expressed on activated B-cells. The soluble form of CD23 (sCD23), a 25 kD protein, is highly elevated in the serum of B-CLL patients as compared with other lymphoproliferative disorders. In order to determine whether sCD23 reflects disease activity and has prognostic potential, we studied 51 patients with B-CLL over a period of six years. Thirty-nine patients could be followed regularly and six parameters were evaluated: Rai stage, white blood cell count (WBC), platelet counts, haemoglobin levels, lymphocyte count doubling time (LCDT) and sCD23. Serial determinations of sCD23 over the above period of time revealed that patients classified as Rai 0-1 (n=24) with a median sCD23 level of 891 U/ml (range 151-4904) at study entry behaved differently with regard to their serum sCD23 concentration dynamics. The median sCD23 levels (median 490 U/ml, range 151-1140) with only minor fluctuations during the observation period. In contrast, 11 patients with a significantly higher median level at the beginning (1155 U/ml, range 864-4904) progressed to higher Rai stages within a relatively short time. Patients with Rai II-III at diagnosis (n=15) could also be divided into subgroups. One group, exhibiting sCD23 levels below the median of 4524 U/ml at study entry (1618 U/ml, range 2470-5300) developed stable disease. The other group (n=7) with very high sCD23 levels (8690 U/ml, range 5260-10960) progressed rapidly to Rai IV, developed resistance to chemotherapy and had a significantly shorter survival time. To correlate the clinical findings with in vitro data and to determine a possible role of CD23 for cell survival we cultured PBMC from patients of each group with and without fludarabine. The results indicate that a high and continuous sCD23 surface expression is associated with fludarabine resistance in B-CLL. The CD23 levels in culture supernatants are associated with better cell survival in vitro, while treatment with fludarabine reduces CD23 expression and cell viability. These data support the notion that increasing therapy resistance in vivo correlates with sCD23 levels.

**PO-0106 Successful treatment of prolymphocytic leukaemia with splenic irradiation and 2-chlorodeoxyadenosine (2-CDA)**

Shivuld L, Barbeau A, Shlaitd M, Klefthot A
Hematology Institute, Kaplan Medical Center, Rehovot, Israel

B-PLL is a rare chronic disorder characterised by prolymphocytosis, marked splenomegaly, and absence of lymphadenopathy. The treatment of B-PLL is dominated by an aggressive course and poor prognosis. Although there are some reports on response to splenectomy, splenic irradiation and/or chemotherapy, including purine analogs, optimal treatment remains unclear. We report our successful experience with five PLL patients treated with splenic irradiation followed by 2-CDA and would like to propose this new therapeutic approach for prolymphocytic leukaemia. Four male patients aged 64, 58, 77, 67 years, suffering from PLL for 7, 2.5, 1.5 and 3 years, respectively, and one 83 y/o woman, newly diagnosed, presented with prolymphocytosis and huge splenomegaly. Two patients suffered from fever and weight loss. All patients but one were unresponsive to prior treatment with chlorambucil + prednisone or CHOP and were not candidates for splenectomy because of cardiovascular problems. They were treated with 350 GY splenic irradiation, followed by one single 5 day course of 2-CDA (0.1 mg/kg/day). Four patients achieved an excellent response, while the fifth achieved a partial remission (table).

<table>
<thead>
<tr>
<th>Case</th>
<th>Prior to treatment</th>
<th>After treatment</th>
</tr>
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<tbody>
<tr>
<td>Splenic Hb</td>
<td>Lymph PR</td>
<td>PT</td>
</tr>
<tr>
<td>1</td>
<td>1.64</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
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<tr>
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<td>1.67</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>1.83</td>
<td>15</td>
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Up to now, the responses have lasted for 37, 20, +19, 10 +3 +months. One patient relapsed and one died from infection with no signs of PLL progression. The treatment was tolerated very well. Except for transient asymptomatic grade 2 neutropenia in one case there were no complications. In 2 cases there was no response to prior treatment with chlorambucil + prednisone or CHOP and were not candidates for splenectomy because of cardiovascular problems. They were treated with 350 GY splenic irradiation, followed by one single 5 day course of 2-CDA (0.1 mg/kg/day). Four patients achieved an excellent response, while the fifth achieved a partial remission (table).

**PO-0107 Cyclin D1 overexpression in B-chronic lymphoproliferative disorders independently of a t(11;14)(q13;q32)**

*UPRES-EA 2128, Faculté de Médecine, Université de Caen, France; *Laboratoire d’Hématologie, and *Service Hématologie Clinique, CHU Côte de Nacre, Caen, France

Cyclin D1 participates in the regulation of phosphorylation of retinoblastoma protein and in turn, controls the cell cycle at the G1/S transition. The
cycin D1 locus, located in 11q13, is amplified and cycin D1 protein overexpressed in a wide range of human solid tumors. In B-lymphoid malignancies, the translocation t(11;14)(q13.3;q23) joins the heavy chain locus to the cycin D1 locus and leads to overexpression of a normal sized cycin D1 protein. Using a competitive RT-PCR (Uchimary et al, Blood 89, 965-974, 1997), designed to detect cycin D1 mRNA overexpression, we analyzed a series of patients with B-CLL and B-CLPD disorders. We found an overexpression of cycin D1 in patients with chronic mantle cell lymphoma (10/10), hairy cell leukaemia (4/96) and 1 B large cell lymphoma. Interestingly, the densitometric analysis of RT-PCR products and Western blot autoradiograms, as well as the cytogenetic data, suggested that the activation of cycin D1 gene could occur independently of the 7(11;14)(q13;q23) in some patients. Indeed, whatever the pathology, a normal sized protein of 36 kDa exhibiting a level incompatible with a gene activation by a translocation mechanism, was detected in lymphoid cells with a normal karyotype. We are currently analysing cellular mechanisms which could induce cycin D1 overexpression: a hypermethylation of cycin D1 promoter, an inactivating mutation in the cycin D1 ubiquitin/proteasome pathway involved in the degradation of the cycin D1 protein.

PO-0108 Increased frequency of Interleukin-2, Interferon-γ, Interleukin-4, and Tumour necrosis factor-α producing CD19+ cells in the peripheral blood of patients with B chronic lymphocytic leukaemia

Lobregato G, Accogli E, Valacca A, Ped R, Pensa P
Department of Clinical Pathology and *Department of Medicine, Azienda Ospedaliera “V. Fazzi”, Lecce, Italy

The neoplastic cells of chronic lymphocytic leukaemia (CLL) are able to produce some cytokines which are known to stimulate the proliferation of CLL cells in an autocrine and paracrine manner; moreover Tcells may influence the expansion of the malignant clone through the production of significant amounts of cytokines. In order to explore this phenomenon further at the single-cell level we used multiparameter flow cytometric measurement of intracellular cytokines (IL-2, IFN-γ, IL-4, TNF-α) in lymphocyte subpopulations from patients with B-CLL. The analysis was also made for the content of the same cytokines, Peripheral whole blood from 12 CLL patients (7 males, 5 females; aged 69±8 years) and 10 healthy volunteers (6 males, 4 females; aged 49±16 years) were used. Whole blood was activated with a combination of Phorbol 12 Myristate 13 Acetate and Ionomycin, together with Brefeldin A to increase sensitivity by retaining cytokines in the cell to detectable levels. After fixation and permeabilisation of cells, intracellular cytokines were stained with directly conjugated antibodies against IL-2, IFN-γ, IL-4, TNF-α (BDIS, San José, CA); unstained, isotype and activation controls were also performed: cytokine producing cells were analysed by gating CD19+, CD19+, CD19+ subsets; the concentration of IL-2, IFN-γ, IL-4, TNF-α in the serum was determined with commercial immunoassay kits (R&D Systems, Minneapolis, MN). In CLL patients there was a significantly increased percentage of CD19+ lymphocytes expressing intracellular cytokines in the unstimulated samples (CD19/IL-2: 9.3±3%; CD19/IFN-γ: 3.3±5%; CD19/IL-4: 1.5±10.5%; CD19/TNF-α: 8±3%), while no intracellular cytokines could be detected in any unstimulated cultures from healthy controls or in CD19+ cells from CLL patients. While controls there was a significantly increased percentage of lymphocytes expressing intracellular cytokines after activation, no significant difference in the frequency of IL-2, IFN-γ, IL-4, TNF-α producing CD19+, CD19+, CD19+ cells from CLL patients was detected when compared with the unstimulated samples. In the enzyme-linked immunosorbent assay we found no significant amounts of cytokines either in the serum from B-CLL patients or in that from healthy controls. These results suggest that in B-CLL patients IL-2, IFN-γ, IL-4, TNF-α are constitutively secreted by some B-CLL cells and that the frequency of cytokine producing CD19+ and CD19+ is low after activation of whole blood; these functional alterations in the production of cytokines by cells from B-CLL patients could play an important role in the pathophysiology of the disease.

PO-0109 Apoptosis is a prognostic factor for disease progression in chronic lymphocytic leukaemia

Ricciardi MR, Petrucci M T, Gregori G, Ariola C, Mazzola F, Trasci D, Mauro FR, Foà R, Mandelli F, Tufari A
Dipartimento di Bioteccologie Cellulari ed Ematologia, Università “La Sapienza” Rome, and Dipartimento di Scienze Biomediche e Odontologia Urmana, Turin, Italy

Prognostic factors capable of predicting disease progression are particularly useful in neoplasias characterised by a prolonged and heterogeneous phase of stable disease. B-cell chronic lymphocytic leukaemia (CLL) is one of these disorders which, usually, does not require any treatment during the stable phase, whereas cytotoxic chemotherapy is needed to reduce tumour mass expansion when the disease becomes more aggressive. Since CLL is characterised by non-cycling cells with a long survival, we sought to investigate whether programmed cell death (PCD) may play a role in disease progression helping to distinguish the transition between the different phases. We studied, in primary mononuclear CLL samples, the levels of PCD at the time of harvest of 14 hours of cell culture in FCS. The Acridine Orange (AO) flow cytometric technique was used to assess PCD, by measuring the sub-peak G0/G1. CLL samples were divided into those from patients with stable disease (SD), and those from patients with progressive disease (PD). We observed a significant difference between the two groups: mean (m) 41.0±24.7 vs 101.9±70.2 (X2.91 p=0.0005) for SD and PD patients, respectively. According to Binet’s classification (stages A, B, and C), the number of patients with SD and PD were 14, 7 and 3, respectively. Fresh samples from both groups of patients, as well as normal peripheral blood lymphocytes (PBL), were all characterised by a low PCD value (m < 1%). However, after 24 hours of in vitro culture PCD values were significantly different (m=63.4±14.7%) and PD patients (m=15.05±9.4%), both being higher than the values of normal PBL (m=10.7±2.0%) measured under the same conditions. Taken as a whole, this study demonstrates that SD CLL patients are characterised by increased in vitro levels of PCD compared to patients with PD. These data indicate that in CLL PCD plays a role in the expansion of the neoplastic clone and, in turn, in the clinical course of the disease.

PO-0110 Fludarabine in combination with epirubicin in induction of apoptosis in B-cell chronic lymphocytic leukaemia

Schwaenen C, Karakas T, Schrader M, Hecker T, Bergmann L
Medical Clinic III, University of Ulm, Germany

Clinical studies have demonstrated the improvement of the response rate of B-chronic lymphocytic leukaemia (B-CLL) to combined application of purine analogs and anthracyclines. These studies investigated the in vitro ability of fludarabine, epirubicin and their combination to induce apoptosis in freshly isolated peripheral lymphocytes from B-CLL. Peripheral lymphocytes, isolated from highly leukaemic CLL patients (n=17), were cultured in the presence of 0.7 µg/mL fludarabine, 0.15 µg/mL epirubicin or their combination. After 48 h the percentage of apoptotic cells was determined by flow cytometry using the fluorescent DNA-binding agent 7-AAD. Constitutive Bcl-2 and Bax protein levels and Bcl-2 and Bax expression after incubation with fludarabine or epirubicin were determined by flow cytometry analysis on permeabilised cells using monoclonal Bcl-2 and Bax antibodies. Using the drugs alone, the median rate of apoptosis in fludarabine and epirubicin treated cells was 21% (13.5±3.3) and 22% (15.8±5.4) respectively after 48 h. The combination of fludarabine and epirubicin led to a synergistic effect in inducing apoptosis in 8,717 patients. In 2/17 patients and additive and in 7/17 a non-additive cytotoxic effect from combining fludarabine and epirubicin could be detected. There were no significant differences in the in vitro results concerning synergism between fludarabine and epirubicin in untreated patients compared to pre-treated patients. Investigations of Bcl-2 and Bax protein levels in three CLLs by flow cytometry showed unchanged levels of Bcl-2 and Bax expression in fludarabine treated cells. A 2-fold decreasing level of Bcl-2 protein after incubation with epirubicin and a 1.5-fold change in Bax protein was detect- ed. In conclusion, in vitro experiments demonstrated that fludarabine and epirubicin have, at least in part, a synergistic effect on the induction of apoptosis. Clinical or molecular factors leading to an in vivo synergistic effect have to be elucidated. Additionally no change in the Bcl-2 and Bax protein level in fludarabine treated B-CLL cells could be observed while the Bcl-2 and Bax protein expression decreased in epirubicin treated cells.
Persistent polyclonal B-cell lymphocytosis (PPBL) is a rare lymphoproliferative disorder that affects female smoking subjects characterised by persistent and stable polyclonal B lymphocytosis with binucleated lymphocytes on peripheral blood smears and by the presence of polyclonal IgM hypergammaglobulinaemia. The subjects present a peculiar haplotype, namely DR7. Although isolated reports exist that PPBL can be complicated by neoplastic haematological disorders, the condition is generally thought as devoid of clinical importance. In the last ten years we have observed and followed four female subjects with clinical-pathological features compatible with diagnosis of PPBL. All the cases showed a mild B lymphocytosis (3,400–11,200/mL) with CD5-, CD10-, CD11c-, CD23-phenotype and an absolute lymphopenia. Persistent polyclonal B cell lymphocytosis in patients with B-cell chronic lymphocytic leukaemia (B-CLL). The presence of trisomy 12 positive (+12) cell populations has generally been investigated in leukaemic cells obtained from the peripheral blood of CLL patients. To ascertain whether trisomy 12 is expressed homogeneously in cells of different haematopoietic tissues, we applied fluorescence in situ hybridisation (FISH) to lymph node, peripheral blood and bone marrow samples obtained simultaneously from 23 untreated B-CLL patients. Design and Methods. Twenty-three newly diagnosed patients with B-CLL. In stage B and stage C, were included in the present study. Peripheral blood smears, bone marrow aspirate smears and lymph node touch imprints were collected from each patient at diagnosis. Cytological preparations were examined by light microscopy in order to assess the lymphocytes’ morphology. Immunophenotyping was performed by cytofluorimetric analysis of the peripheral blood, bone marrow and lymph node mononuclear cell suspensions. The diagnosis was supported in all cases by histologic findings in bone marrow biopsy, and lymph node biopsy specimens. FISH was performed on smears of blood and aspirate bone marrow and lymph node touch imprints obtained by fresh tissue aspiration. Results. In 6 of the 23 cases (26%) trisomy 12 was clearly present in all tissues examined. A comparative analysis of three different haemopoietic tissues was performed. A higher percentage of leukaemic CD5-CD23+ cells was detected in lymph nodes than in peripheral blood and bone marrow. A significantly higher proportion of trisomic cells was observed in lymph nodes samples than in peripheral blood or bone marrow smears of trisomy 12 positive CLL patients. Interpretation and Conclusions. The higher proportion of +12 cells in lymph nodes than in peripheral blood or bone marrow smears of trisomy 12 positive CLL patients with trisomy 12 could reflect different cell distributions in different tissues, or lymph node specific tropism, or proliferative advantage in selected tissue. At present, the role of trisomy 12 in the pathogenesis of lymphoproliferative disorders is unclear.

**Results.** 5 4 3 2 1 0

| CLL (n=66) | 30 | 32 | 29 | 30 | 7 | 4 |
| acLL (n=19) | 9 | 2 | 13 | 11 | 14 | 15 |
| Other (n=73) | - | - | 13 | 13 | 25 | 26 |

**M/F score A:** High. Score B.

Conclusions. Despite the existence of a high concordance between the two systems, we were able to detect some differences in the group of acLL which could reflect the cytogenetic and molecular diversity of this group.

**PO-0112 Persistent polyclonal B-lymphocytosis: a more aggressive disease than thought before?**


Persistent polyclonal B-cell lymphocytosis (PPBL) is a rare lymphoproliferative disorder that affects female smoking subjects characterised by persistent and stable polyclonal B lymphocytosis with binucleated lymphocytes on peripheral blood smears and by the presence of polyclonal IgM hypergammaglobulinaemia. The subjects present a peculiar haplotype, namely DR7. Although isolated reports exist that PPBL can be complicated by neoplastic haematological disorders, the condition is generally thought as devoid of clinical importance. In the last ten years we have observed and followed four female subjects with clinical-pathological features compatible with diagnosis of PPBL. All the cases showed a mild B lymphocytosis (3,400–11,200/mL) with CD5-, CD10-, CD11c-, CD23-phenotype and an absolute lymphopenia. In all cases a DR7 haplotype and the W/B ratio between 0.9 and 1.5. in all cases a DR7 haplotype and the W/B ratio between 0.9 and 1.5. in all cases a DR7 haplotype and the W/B ratio between 0.9 and 1.5. in all cases a DR7 haplotype and the W/B ratio between 0.9 and 1.5. in all cases a DR7 haplotype and the W/B ratio between 0.9 and 1.5. in all cases a DR7 haplotype and the W/B ratio between 0.9 and 1.5. in all cases a DR7 haplotype and the W/B ratio between 0.9 and 1.5. in all cases a DR7 haplotype and the W/B ratio between 0.9 and 1.5.

**Design and Methods.** 6 of the 23 cases (26%) trisomy 12 was clearly present in all tissues examined. A comparative analysis of three different haemopoietic tissues was performed. A higher percentage of leukaemic CD5-CD23+ cells was detected in lymph nodes than in peripheral blood and bone marrow. A significantly higher proportion of trisomic cells was observed in lymph nodes samples than in peripheral blood or bone marrow smears of trisomy 12 positive CLL patients. Interpretation and Conclusions. The higher proportion of +12 cells in lymph nodes than in peripheral blood or bone marrow smears of trisomy 12 positive CLL patients with trisomy 12 could reflect different cell distributions in different tissues, or lymph node specific tropism, or proliferative advantage in selected tissue. At present, the role of trisomy 12 in the pathogenesis of lymphoproliferative disorders is unclear.

**PO-0113b Autograft as post-remission treatment after fludarabine therapy in 20 chronic lymphocytic leukaemia (CLL) patients**


To evaluate the impact of autograft on the duration of disease free survival (DFS) and survival, high risk CLL patients (pts) < 60 years in remission after fludarabine were offered a peripheral blood stem cell (PBSC) collection and reinfusion program following BEAM conditioning. Up to January 1999, 20 pts have been autografted. Median age was 46.5 years (range 21-58). Sixteen pts received PBSC (median number of CF34+ cells reinfused 3.5×10^9/kg, range 1.5-11.3); due to unsatisfactory PBSC collection, 4 pts received bone marrow cells. All pts engrafted; the median time to neutrophils >0.5×10^9/L and to platelets >20×10^11/L was 12 (range 9-24) and 15 days (range 10-115), respectively. One pt died 60 days after transplantation due to infectious complications. Another pt developed acute myeloid leukaemia (AML) while in clinical CR 17 months after transplantation and died in CLL/AML CR due to a concomitant metastatic lung cancer. Eighteen pts are presently alive with a median follow-up of 15.5 months (range 4-44) from transplant. Two pts in whom leukaemic cells were always detectable at the molecular level relapsed at 26 and 33 months from transplant. Sixteen pts are in unmaintained remission after a median follow-up of 13.5 months (range 4-42). Fifteen of these 16 pts obtained, during the clinical course of the disease, a molecular remission. Four of the latter have subsequently shown a molecular reappearance of the neoplastic clone, with no evidence of haematological disease at 11, 6, 6 and 2 months from the molecular relapse. The projected DFS probability is 0.54 (±0.2) at 44 months, while the overall survival probability is 0.83 (±0.1). The finding of a durable DFS following autograft for pts with poor prognosis CLL is encouraging and suggests that autografts may be a therapeutic option for this group of pts.
PO-0114 Modification of CD38 expression by corticoids in neoplastic B cell lines

Genty V.*, Dine G.**
Service d'Hématologie-Immunologie, Centre Hospitalier, Troyes, France

The CD38 molecule is expressed on the surface of plasma cells, and has been widely used to separate tumour cells from normal ones in myeloma, to follow disease progression, or as a potential target for therapy. Gluco- corticoids are the mainstay of chemotherapy in this syndrome. In previous works we presented four cell lines derived from the RPM18226 line, modified by continuous treatment with glucocorticoids to induce resistant forms. They were referred to as B26p, B26pt, B26c and B26t for the resistance was induced by prednisone, prednisolone, methyl-prednisone or dexamethasone. Another B cell line isolated in our laboratories was used in that study, and referred to as LA cells. The native RPM18226 and LA cells were found to express the CD38 strongly. Secondly to the appearance of the resistant forms, we determined the phenotypic modifications induced on the cell surface. The induction of the resistance was followed by repression of expression with the exception of the B26p cells which presented a feeble overexpression, possibly linked to the special behaviour of these cells. Otherwise the more the cells were resistant (following the real anti-tumoral potency of the corticoid, presented in previous works), the less it expressed CD38 on its surface. The LA cells, that were initially sensitive to corticoid treatment, became naturally resistant after a few months of culture. During this evolution we did not observe any modification of CD38 expression. So we concluded that the disappearance of the cell surface marker we observed corresponded to a repression induced by the treatment. This repression did not appear after short course treatments since, after 72 hours, nothing was observed. Only long time treatment followed by the acquisition of the resistance is implied in the repression of CD38 expression. In conclusion, the use of CD38 expression in the evolution of multiple myeloma being treated by corticoids must be interpreted with caution.

PO-0115 Efficacy of clarithromycin and pamidronate therapy in myeloma. A phase II trial

Morris TCM, Rankanah L, Morrison J
Belfast City Hospital, Lisburn Road, Belfast, Northern Ireland

It has been suggested that Clarithromycin has anti tumour activity in patients with multiple myeloma due to the interaction between the laminin receptors (CD49b, CD49f) and lamin of vascular basal membrane in renal glomeruli. We suggest that either Clarithromycin or Pamidronate (recently shown to be active in myeloma) may be used for mantle cell lymphoma, but not as negative as in two other similar studies also omitting steroids. We suggest that either Clarithromycin or Pamidronate (recently shown to be active in myeloma) may be used for mantle cell lymphoma, but not as negative as in two other similar studies also omitting steroids. We therefore instituted a multicentre trial of clarithromycin and pamidronate in patients with multiple myeloma. The rationale was based on a number of in vitro studies. We report our results of this trial and discuss the possible clinical implications of the findings. The trial was a multicentre, open-label, uncontrolled phase II study carried out in nine centres. Eligible patients included those aged 18-75 years, with newly diagnosed multiple myeloma, and a life expectancy of at least 12 months. Patients were randomized to receive either clarithromycin 500 mg twice daily for 28 days followed by 7 days rest or pamidronate 30 mg i.v. every 4 weeks. The primary endpoint was progression-free survival. Secondary endpoints included markers of disease activity such as serum lactate dehydrogenase (LDH), β2 microglobulin (β2M) and haemoglobin (Hb). Patients were followed for a minimum of 6 months or until disease progression. A total of 31 patients were enrolled, of whom 26 were evaluable. The median age of the patients was 66 years (range 33-81) and 22 were male. The median duration of response was 9 months (range 1-36). The response rate was 31% (95% CI 16-51). There were 9 complete responses (9/26, 35%) and 17 partial responses (17/26, 65%). The median time to progression was 7 months (range 1-36). The trial was stopped early due to lack of disease control. The results of this study suggest that clarithromycin and pamidronate may have a role in the management of multiple myeloma. Further studies are needed to confirm these findings.

PO-0116 The role of Cd38 expression in the evolution of multiple myeloma

Jawniak D, Urbańska-Ryli, Robak T
Dept of Haematology, Medical Univ. of Lodz, Lodz, Poland

Multiple myeloma (MM) is a B cells neoplasia characterised by bone marrow (BM) involvement with plasma cells and a monoclonal protein present in serum and/or urine. The growth of myeloma cells is regulated by a complex cytokine network in which interleukin-6 (IL-6) plays a key role. Recent data show that IL-10 is an IL-6-unrelated growth factor for malignant plasmablastic cells and may be involved in the late phase of MM in vivo. We investigated the serum concentration of interleukin-10 (IL-10), oncostatin M (OSM), interleukin-6 (IL-6) and IL-6 soluble receptors (sIL-6R) using an enzyme-linked immunosorbent assay (ELISA) in 52 patients with multiple myeloma (MM) with newly diagnosed MM, 16 with MM in plateau phase and 13 in relapse) and 17 healthy controls. We examined a possible association between the serum levels of these peptides and disease activity and known prognostic factors. Serum concentrations of IL-6 with sIL-6R and with remaining evaluated cytokines as well as between particular cytokines alone were also correlated. IL-10, IL-6 and sIL-6R were detectable in all 52 patients as well as in all healthy controls. In contrast OSM was detectable in 24/52 (46.3%) MM patients and only in 1/17 (5.9%) normal individuals. The serum levels of IL-10, OSM, IL-6, and sIL-6 were significantly higher in MM patients compared with control group (p<0.004, p<0.008 respectively). The highest concentration of these cytokines were found in patients with progressive disease and the lowest in MM patients with stable disease as well as in healthy persons. We also found significant positive correlations between the levels of IL-10 and IL-6 (p<0.00001), IL-10 and OSM (r=0.026) and IL-10 and sIL-6R (p=0.02). Moreover we observed a positive correlation between IL-6 and OSM (r=0.022) and no correlation between concentration of IL-6 and sIL-6R. No significant difference in levels of sIL-6R and detected cytokines compared with M-component levels was observed. Our results support an apparent involvement of IL-10 in the pathogenesis of MM in vivo. In conclusion, we can state that the serum concentrations of IL-6, IL-10, OSM and sIL-6R are higher in MM patients than in healthy persons and correlate with disease activity.

PO-0117 Circulating interleukin-10, interleukin-6, oncostatin m and soluble IL-6 receptor in patients with multiple myeloma

Wierzbowska A, Urban ska-Ryli, Robak T
Dept of Haematology, Medical Univ. of Lodz, Lodz, Poland

Multiple myeloma (MM) is a B cells neoplasia characterised by bone marrow (BM) involvement with plasma cells and a monoclonal protein present in serum and/or urine. The growth of myeloma cells is regulated by a complex cytokine network in which interleukin-6 (IL-6) plays a key role. Recent data show that IL-10 is an IL-6-unrelated growth factor for malignant plasmablastic cells and may be involved in the late phase of MM in vivo. We investigated the serum concentration of interleukin-10 (IL-10), oncostatin M (OSM), interleukin-6 (IL-6) and IL-6 soluble receptors (sIL-6R) using an enzyme-linked immunosorbent assay (ELISA) in 52 patients with multiple myeloma (MM) with newly diagnosed MM, 16 with MM in plateau phase and 13 in relapse) and 17 healthy controls. We examined a possible association between the serum levels of these peptides and disease activity and known prognostic factors. Serum concentrations of IL-6 with sIL-6R and with remaining evaluated cytokines as well as between particular cytokines alone were also correlated. IL-10, IL-6 and sIL-6R were detectable in all 52 patients as well as in all healthy controls. In contrast OSM was detectable in 24/52 (46.3%) MM patients and only in 1/17 (5.9%) normal individuals. The serum levels of IL-10, OSM, IL-6, and sIL-6 were significantly higher in MM patients compared with control group (p<0.004, p<0.008 respectively). The highest concentration of these cytokines were found in patients with progressive disease and the lowest in MM patients with stable disease as well as in healthy persons. We also found significant positive correlations between the levels of IL-10 and IL-6 (p<0.00001), IL-10 and OSM (r=0.026) and IL-10 and sIL-6R (p=0.02). Moreover we observed a positive correlation between IL-6 and OSM (r=0.022) and no correlation between concentration of IL-6 and sIL-6R. No significant difference in levels of sIL-6R and detected cytokines compared with M-component levels was observed. Our results support an apparent involvement of IL-10 in the pathogenesis of MM in vivo. In conclusion, we can state that the serum concentrations of IL-6, IL-10, OSM and sIL-6R are higher in MM patients than in healthy persons and correlate with disease activity.

PO-0118 Familial myeloma

Dept of Haematology, University School of Medicine, Athens, Greece

Given the incidence of multiple myeloma in the general population, the occurrence of more than one case in the same family is probably not due to chance. We report 6 instances of familial occurrence of myeloma involving first degree relatives, in three different families. Every family had two affected members. Mean age at disease diagnosis was 67 years. [52-75 years]. Time interval of diagnosis for members of the same family ranged
from 12 month to 6 years, with a mean value of 3 years. Three patients were males and three were females (the sibling pairs consisted in two brothers, a brother and a sister, and two sisters of Ashkenazi Jewish origin). No patient had any relatives with other lymphoproliferative disorder and no monocular abnormality was found in the 15 relatives studied. The immunoglobulin type was identical in the affected members of the same family. The light chain was A in all cases. Four patients had IgG lambda, and two IgA. Myeloma stage was IA in 2 patients and II A in 4 patients. One patient had severe myeloma-related amyloidosis. In no patient HCV, HBV, EBV, or CMV infection was found at diagnosis. Karyotype study was performed in all patients. Chromosomal abnormalities (monosomy, trisomy or tetrasomy), different in each individual patient, were found in five patients, while one patient had a normal karyotype. Equal abnormalities were not found in the same family members. Nine abnormalities (8p-, 1p-, 1q-, 12q-, 14q-, 16p-, 17p-, 18q-, and 20q-) were strictly IL-6-dependent again. Another remarkable change in INA-6 cells occurring during growth in SCID mice was the responsiveness of tumour cells to other cytokines of the gp130 family. Receptors for leukaemia inhibitory factor (LIF) were detected by RT-PCR in certain INA-6 tumour sublines while being absent in the parental cells, suggesting that expression is newly induced. Treatment of INA-6 bearing SCID mice with a combination of anti-gp130 antibodies (clone BR-3) and either the IL-6-receptor superagonist Sant-7 or anti-IL-6-receptor antibodies (clone BR-6) completely prevented tumour development. Thus, this myeloma model not only allows us to study INA-6 tumour cells in vitro but also to test the effectiveness of therapeutic strategies aimed at inhibition of gp 130-related cytokines.

PO-0121 Comparative genomic hybridisation in multiple myeloma and plasma cell leukaemia shows a high incidence of genomic changes

Gutierrez NC, García JL, Hernández MA, Sánchez MA, Hernández JM, Martín-Núñez G, Ortega F, González M, Rios A, San Miguel JF, Servicios de Hematología, Hospital Universitario de Salamanca; *Hospital General de Segovia; †Hospital de León; ‡Hospital Virgen del Puente de Plasencia; ‡Hospital Rio Carrion de Palencia; Spain

Comparative genomic hybridisation (CGH) is a double color hybridisation procedure which provides, in a single experiment, an overview of genomic imbalances. CGH may be particularly useful to assess genomic copy number changes in tumors with a low proliferative index, such as multiple myeloma (MM). Objective. To identify chromosomal gains and losses in MM and plasma cell leukaemia (PCL) by CGH. Design and Methods. Bone marrow samples from 25 patients with MM and 5 cases of plasma cell leukaemia were evaluated: 25 were analysed at diagnosis, 4 at relapse or progression and one in plateau phase after treatment. Median age was 70 years (range: 45-90). All patients but one had a bone marrow infiltration >35%. Seventy percent of the patients were in stage III. In 25 of the 30 cases G-banded cytogenetic analysis was simultaneously performed. The tumour and normal DNA samples were labelled with nick-translation with biotin-12-dUTP and digoxigenin-11-dUTP respectively and were hybridised to slides with metaphases from blood of a healthy donor. Thresholds for the identification of DNA imbalances (between tumour and normal DNAs) were defined as 0.75 (losses), 1.25 (gains) and 1.5 (amplifications). Results. In 29 of the 30 cases, chromosomal imbalances were identified by CGH analysis. Seventeen out of the 25 patients exhibited abnormal karyotypes by conventional cytogenetics. A total of 214 changes were identified by CGH with a median of 7 changes per case (range: 1-28). Gains of chromosomal material were more frequent than losses (156 gains vs 54 losses). In 13% (3) patients from the above described aberrations were gains on chromosomes 15 (10%), Igq (36%), 9 (32%), Igq (28%) and 3 (28%), while losses mainly involved chromosomes 13 (28%) and 16 (12%). All patients with PCL had gain on 1q, and 4 of the 5 cases had monosomy 13 and loss of 16q; other changes involved 6q, 4q and 15. High-level amplifications were detected in four cases: 9q34, Xq24-26, 11q14-12, 8q24. Conclusions. CGH detects a high incidence of chromosomal gains and losses in MM. Similar changes were identified in PCL, although a higher proportion of gains on 1q and losses on 13 were present in PCL as compared to MM. (Partially supported by a grant of Spanish RIS 97/1248 and 98/1161)

PO-0122 Nitrogen-containing bisphosphonates induce apoptosis in human bone marrow-derived myeloma cells in vitro

Gordon S, Heilbrich MH, Sadi HIA, Stewart T, Shipman CM, Soutar RL, Gravestones M, Rai-Sohn SH, Croucher PJ, Sebbi S, Hanaitib A, Rogers MJ, Dept. of Medicine and Therapeutics, University of Aberdeen Medical School, Aberdeen, Scotland, UK

Bisphosphonates (BPs) are important anti-resorptive agents in the treatment of tumour-associated bone disease and recent studies have suggested that they may also have direct antitumour effects in multiple myeloma (MM). We have recently shown that the nitrogen-containing BPs (N-BPs) pamidronate and inodronate induce apoptosis in a human myeloma cell line in vitro by inhibiting enzymes of the mevalonate pathway, thereby preventing post-translational geranylgeranylation (prenylation) of small GTP-
binding proteins. The purpose of this study was to determine whether BPs also cause apoptosis of authentic, malignant human plasma cells in vitro, isolated from the bone marrow of newly diagnosed MM patients. We used a dual fluorescence assay, to simultaneously label plasma cells and apoptotic cells, followed by flow cytometry to investigate the ability of three N-BPs (pamidronate, ibandronate and incadronate) and a non-N-BP (codonate) to induce myeloma cell apoptosis. The levels of apoptosis induced in the BP-treated cultures varied considerably between marrow samples. However, treatment of bone marrow mononuclear cells with each of these BPs (100 μM) in vitro resulted in an increase in the proportion of apoptotic plasma cells compared to control cultures. Increased apoptosis was detected 24 hrs after treatment with the N-BPs, which were more potent than codonate. Addition of geranylganin inhibited N-BP-induced apoptosis. Furthermore, GGT-298, a specific inhibitor of protein geranylglyceroprenyltransferase, caused a significant increase in plasma cell apoptosis after 24 hrs. These observations confirm that N-BPs can induce apoptosis in human myeloma cells in vitro by inhibiting protein geranylglyceroprenylation. We are currently investigating the in vivo anti-tumour effects of N-BPs in MM patients.

PO-0123 Prevalence of solid tumours in patients with newly diagnosed monoclonal gammapathy

*Dpt. of Hematology, Leiden University Medical Center; *Comprehensive Cancer Center West, *Dept. of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

Objective. Monoclonal gammapathy (paraproteinemia) has been reported to be associated with solid tumours (non-haematological malignancies) (1). In a cohort of patients with newly diagnosed paraproteinemia we drew up an inventory of the types of solid tumours found and related paraprotein-type. Design and Methods. During 1991-1993 the Comprehensive Cancer Center West recorded all patients (n=1464) with newly diagnosed monoclonal gammapathy in a population-based prospective registry. Upon entrance patients characteristics, laboratory data, tumour, and clinical history were recorded, as well as paraprotein-related diagnoses were recorded. Median age was 72 years (range 16-102 yrs). Multiple myeloma (MM) was diagnosed in 157 patients. Furthermore, GGT-298, a specific inhibitor of protein geranylglyceroprenyltransferase, caused a significant increase in plasma cell apoptosis after 24 hrs. These observations confirm that N-BPs can induce apoptosis in human myeloma cells in vitro by inhibiting protein geranylglyceroprenylation. We are currently investigating the in vivo anti-tumour effects of N-BPs in MM patients.

PO-0125 Expression of cytoadhesion molecules in plasma cell leukaemia

Krai M, Kopeć-Szczukaj J, Pogrodł R
Institute of Haematology and Blood Transfusion, Warsaw, Poland

The aim of the study was to determine expression of adhesion molecules CD11a (LFA1,CD18) and CD18 (LFA1-β2,CD11a) in plasma cell leukaemia (PCL), at diagnosis and in a control group of 10 healthy subjects. Immunophenotyping was performed on freshly collected blood and bone marrow samples by means of flow cytometry. Plasma cells were identified as showing high-density expression of CD11b (CD11b+) and CD138 (CD138+) and having an aberrant immunophenotype.

PO-0124 The effect of long-term pamidronate treatment on skeletal morbidity in multiple myeloma

Krai M, Pogródł R, Pawlikowski J, Maj S
Institute of Haematology and Blood Transfusion, Warsaw, Poland

In patients with multiple myeloma (MM) despite a reduction of tumour mass achieved with chemotherapy, osteolytic bone destruction continues to progress. Since October 1995 the efficacy of pamidronate, an inhibitor of osteoclastic bone resorption, has been evaluated in MM patients receiving anti-myeloma chemotherapy. In vitro inhibition of osteoclastic bone resorption, has been evaluated in MM patients, all but two (melanoma, liposarcoma) being carcinoma. Forty-six patients with stage III myeloma and osteolytic lesions were randomised to receive either pamidronate (Aredia; Novartis) 60 mg i.v. in 4-hour infusion monthly (n=23) or chemotherapy alone (control group n=23). The results of the first six monthly cycles of pamidronate therapy were presented at VI International Workshop on Multiple Myeloma in Boston, MA, USA (1998) and VIII European Myeloma Symposium in Stockholm, Sweden in 1999. We are currently investigating the in vivo anti-tumour effects of N-BPs in MM patients.

PO-0126 Monoclonal gammapathies: natural history of 482 patients

Frigerio G, Duro M, Scognamiglio G, Alberti F, Beretta A
Ospedale Valduce, Ambulatorio di Oncoematologia, Como, Italy

Objective. Estimation of probability of malignant transformation from monoclonal gammapathy of undetermined significance (MGUS) to overt myeloma and evaluation of possible predictive factors of this event. Methods and Results. A series of 482 consecutive patients (226 women, 256 men, aged 13-89 years, 57% bone marrow aspirates) in the 482 patients fulfilled the criteria of MGUS and were followed-up from a minimum of 6 and a maximum of 237 months (median 41; total time of observation 21,785 months=1,815 years). At diagnosis 41% had symptoms of myeloma and 59% were asymptomatic. At present, the median pamidronate therapy duration is 26 months with 26% of patients treated over 25 months. At skeletal X-ray examination was performed after 6, 12, 18 and 21 cycles of pamidronate. By comparing each consecutive image with the previous one the progression of osteolysis was found respectively in 67%, 33%, 27% and 13% of patients. In the control group corresponding figures were: 79%, 70%, 30% and 25%. The mean number of skeletal events (pathologic fractures, radiation or surgery to bone and spinal cord compression) in 3 years of follow-up in the monoclonal gammapathy group (1.8) was less than in control patients (2.7), p<0.01. The proportion of patients with pathologic vertebral fractures was lower in the pamidronate group-34% v 52% (p<0.01). The proportion of patients with pathologic vertebral fractures was 69% v 70% respectively but the number of vertebral fractures was lower in the monoclonal gammapathy group. 45 v 64 (1.4 v 2.3 per patient per year, p<0.01). Decreased bone haematologica occurred more frequently in pamidronate patients than in the controls (72% v 41%, respectively, p<0.05) and mostly were accompanied by progression of proliferation. Survival was not different between the pamidronate-treated and control groups.

PO-0127 The effect of long-term pamidronate treatment on skeletal morbidity in MM patients but occurrence or worsening of anaemia deserts attention and further study.

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patients (8.5%) were classified as having overt myeloma according to Durie and Salmon; 7 patients (1.5%) with macroglobulinemia, 5 women (1%) with cryoglobulinemia and 4 patients (0.8%) with amyloidosis. The other 425 patients (82.2%) were classified as having MGUS. The transition from MGUS to overt myeloma occurred in 30 patients after a follow-up ranging from 7 to 185 months (median 44); the estimated overall rate of transformation is 5% (0.6%/y, 95% CI 0.5-0.8%). Only one pt received to amyloidosis and two women to macroglobulinemia. After malignant transformation 15 patients (50%) died because of myeloma (range of survival: 6-62 months; median 13). Of the remaining 395 patients, 13 (3.3%) died because of unrelated diseases and in 12 (3.0%) the monoclonal protein seemed to have disappeared after a range from 7 to 46 months. A statistical analysis of a Multiple Logistic Regression model with stepdown method showed that ESR (p=0.0007); Bence-Jones proteinuria (p=0.0015); serum IGI level (only for IgG MGUS; p=0.0059) and the age of the patient at the time of diagnosis (p=0.0105) were significant predictors for malignant transformation. The other variables, evaluated in the model, but not statistically significant, were: serum level of LDH, creatinine, calcemia, proteinemia, albuminemia, the heavy and light chains involved, haemoglobin level and platelet count, bone-marrow plasmacytosis, age and sex.

PO-0128 Autologous peripheral blood stem cell transplantation (PBSTC) in multiple myeloma (MM): a single center experience of 57 cases


Department of Hematology, Hospital Ramón y Cajal, Madrid, Spain

Between November 1991 and December 1998, 57 pts (34m, 23f; median age 54 y, range 37-68) with MM (4 pts stage 1, 17 pts II and 36 III) were autografted with autologous PBSTC in our center. Twenty-fourfour pts had received >12 m of chemotherapy and 43 pts were exposed to stem cell toxic drugs. The median number of CD34+ cells in MM patients (in brackets: means±SD of MM pts vs controls) both in BM (83.2±18.5% vs 53.4±2.1%) and PB (18.5±9.6% vs. 8.7±2.3%) as well as that of CD56+ cells both in BM (30.6±18.1% vs 21.2±8.4%) and PB (25.1±8.7 vs 17.5±5.7). The differences were also seen in absolute figures. In turn, MM patients showed a decreased relative number of BM CD44+ cells (83.2±18.5% vs 53.4±2.1%); the number of CD138+ cells was decreased both in BM (68.2±2.6% vs 88.5±7.2%) and PB (98.1±1.7% vs 99.4±0.4%) but only in relative values. The expression of particular antigens on peripheral blood (PB) and bone marrow (BM) lymphoid cells in 46 MM patients, at diagnosis. Seventy-two percent of patients were in stage III of disease. The control group consisted of 10 healthy subjects. Immunophenotyping was performed on freshly collected blood and bone marrow samples by means of flow cytometry. Results of analysis were presented both as relative and absolute (not mentioned in abstract) values of numbers of cells with and as relative fluorescence indices (RFIs) of studied antigens. Statistical analysis was performed using Wilcoxon’s test. All presented differences below are statistically significant.

The study revealed a significantly higher number of CD54+ cells in MM patients compared to controls (15.2±4.8 vs 12.0±2.2). The expression of particular antigens on lymphoid cells in MM patients (in brackets: means±SD of MM pts vs controls) both in BM (83.2±18.5% vs 53.4±2.1%) and PB (18.5±9.6% vs. 8.7±2.3%) as well as that of CD56+ cells both in BM (30.6±18.1% vs 21.2±8.4%) and PB (25.1±8.7 vs 17.5±5.7). The differences were also seen in absolute figures. In turn, MM patients showed a decreased relative number of BM CD44+ cells (83.2±18.5% vs 53.4±2.1%); the number of CD138+ cells was decreased both in BM (68.2±2.6% vs 88.5±7.2%) and PB (98.1±1.7% vs 99.4±0.4%) but only in relative values. The expression of particular antigens determined in form of RFIs was not uniform depending on malignant cell compartment. Expression on cells of MM patients RFIs of CD44 (14.0±2.1) and CD56 (15.0±2.7) antigens were lower than those in the control (15.5±0.7 and 17.8±1.2 respectively) while RFIs of CD11a were higher (20.4±1.5 vs 18.8±2.0; p<0.05). In turn, in BM of MM patients compared with the controls there were decreased RFIs of CD56 (16.6±2.4 vs 17.7±1.3) and CD18 (15.2±4.1 vs 16.0±0.7) and increased RFIs of CD54 (13.8±1.2 vs 13.0±0.5) and CD11a (17.0±2.0 vs 14.7±2.0). Conclusions. Altered expression of H-CAM, I-CAM-1, its ligand-LFA-1 and N-CAM formations. The other variables, evaluated in the model, but not statistically significant, were: serum level of LDH, creatinine, calcemia, proteinemia, albuminemia, the heavy and light chains involved, haemoglobin level and platelet count, bone-marrow plasmacytosis, age and sex.

PO-0129 High-output cardiac failure revealing a myeloma

Garibi B, Dufet JL, Quaratino JJ, Maitre B, Delemare V, Tabuteau S, Benacerraf A, Desabrais BB

Maladies du Sang, Hôpital Sud, CHU Armentières, France

A 51-year-old man without a significant previous history was seen in October 1993 because of dyspepsia and thoracic pain related to a high-output cardiac failure with pulmonary hypertension. Investigations found no etiology except a moderate thiamine deficiency. This high-output cardiac failure was ruled out because of the negativity of the dynamic tests and the inefficacy of vitamin compensation:

<table>
<thead>
<tr>
<th>N</th>
<th>Initial</th>
<th>After thiamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC (L per mn)</td>
<td>&lt;6.9±1.1</td>
<td>10.2±13.4</td>
</tr>
</tbody>
</table>

Cardiac catheterisation confirmed the diagnosis: raised cardiac index (6.2 1 per min per m2), reduction of the systemic vascular resistance (480 dynamic sec -5) and low arteriovenous oxygen difference (23 mL per 1) with a normal oxygen consumption (143 mL per min per m2). Biological tests discovered an IgG paraprotein density of 31.9 g per 1 revealing a multiple myeloma. The stage was III A because of many osseous lacunae. Albuminemia was 41.4 g per 1, ß2-m 3.7 mg, serum LDH 1.9 N and CRP less than 5 mg. There was no anaemia (haemoglobin level of 124 g per L) and no amyloidosis. Patient was treated according to the French CIAM trial and the following table summarises the evolution of haematological and cardiac data:

<table>
<thead>
<tr>
<th>&lt;4 VAD</th>
<th>&lt;MPH</th>
<th>&lt;MPH-TBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ABMT</td>
<td>+ABMT</td>
<td>July 1995</td>
</tr>
<tr>
<td>Ig G (per L)</td>
<td>10.8</td>
<td>8.4</td>
</tr>
<tr>
<td>ß2-M (mg per L)</td>
<td>2.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Medul. Plasma. (%)</td>
<td>88</td>
<td>17</td>
</tr>
<tr>
<td>QC (L per mn)</td>
<td>12.10</td>
<td>9.2</td>
</tr>
</tbody>
</table>

The patient also received interferon-alpha and cladronate. The evaluation done in July 1995 was quite normal. Unfortunately, a relapse was noted 6 months later with a again high-output cardiac failure (13.5 L per mn). Palliative treatment was followed by death in July 1996 with cardiac failure, pains and pancytopenia due to a myelodysplastic syndrome. A correlation between multiple myeloma and high-level output cardiac failure has already been noted, less than 10 well-described observations have been reported. Except in the case of anaemia, the pathogenesis remains unclear. Presence of ‘micro-shunts’ due to many osseous lesions is not probable. Secretion of cytokines leading to liberation of vasoactive products (endothelin?) is a more interesting hypothesis.

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Some studies have established cytometric criteria to differentiate monoclonal gammapathies of unknown significance (MGUS) from myeloma (MM). However, none has focused on the evaluation of inter-observer variability. We have attempted to define to what extent MGUS and myeloma may be differentiated on morphological grounds and to what extent this differentiation can be reproduced from one observer to another. Firstly, May-Grunwald-Giemsa stained bone marrow smears from 154 patients with bone marrow plasmacytosis were selected and cytologists from different Hematology departments of Hospital Universitari Germans Trias i Pujol, Badalona and Hospital Clinic i Provincial, Hospital de Sant Pau, Hôpital de l'Espanyana, Hôpital de Bellvitge, Hospital del Mar, Barcelona, Spain were independently evaluated by six cytologists to determine sensitivity and specificity of cytometric differentiation of MM from MGUS or other conditions, firstly in terms of personal impression and, secondly, using a score-based system. Inter-observer reproducibility was evaluated, the inter-observer coefficient of variation for the plasma cell count was 32.7%. On consideration of the diagnosis of the cytologists, 87.8% of cases of MGUS and 100% of the cases of myelomas (24,124) were correctly classified. A global bias towards overdiagnosis of myeloma did not reach statistical significance. When considering diagnoses based on the score system, three non-myeloma cases (7.3%) were considered as myeloma and two myeloma cases (8.3%) were misdiagnosed. The score-based system had no significant bias, but showed a lower overall efficiency than the cytologists. Inter-observer reproducibility was evaluated, the inter-observer reproducibility was 39.5%. The preliminary score-based system was proposed. The study of cytologists and other established parameters such as creatinine and IgM were:

**PO-0130 Usefulness and reproducibility of cytometric scores to differentiate myeloma from monoclonal gammapathies of unknown significance**


In MM, allogeneic BMT is able to induce complete and durable response in patients with MM (M/F=15/1; age 48 y, range 35-55) allografted with PBSC from matched unrelated donors. The engraftment was rapid, with (median) 2 months (range 11-5), and 10 are currently alive in CR at a median of 2 months (range 6-42) from the transplant. Seven pts studied obtained PCR negativity (IgH-gene rearrangement) after the allograft. Overall survival is 70% and PFS 65% at 24 months. The use of PBSCT may facilitate the application of allogeneic transplantation for MM. A larger study will assess the impact of PBSCT on tumour eradication.

**PO-0132 Prognostic influence of cytokines (interleukin-6 (IL-6), serum and plasma cell leukaemia=1, tumour markers (neopterin, thymidine kinase) in multiple myeloma**


Background. Several studies have shown a correlation of serum levels of cytokines (IL-6, sIL-6R) and plasma cell markers (neopterin, thymidine kinase) with tumour mass and prognosis in MM. Objective. To assess the prognostic significance of IL-6, sIL-6R, TNF-α, neopterin and thymidine kinase in patients with MM. Patients and methods. Ninety patients (48M/42F) with newly diagnosed MM were studied. Cytokine measurements: IL-6 (EIA, Medgenix, NV <5 pg/mL), sIL-6R (EIA, Immunotech, NV <80 pg/mL), TNF-α (EIA, Medgenix, NV <20 pg/mL), neopterin (RIA, Henning, NV <100 mmol/L) and thymidine kinase (Radioenzyme Assay, Sangtec, NV <5 U/L). Results. Median survival of the whole series was 33 months. Response rate to chemotherapy was 55%. The prognostic significance of cytokines and other established parameters such as creatinine and b2M were:

**PO-0133 Second primary solid tumours in patients with plasma cell dyscrasias**

Cordiano V, Lora F, Madalena F, Fiorettì D San Lorenzo Hospital, Department of General Medicine, Valdagni, Italy

Objective. The aim of the study was to evaluate the incidence of second primary solid tumours (SPT) in patients with plasma cell dyscrasias (PCD) referred to our Department, which serves a population of about 60,000 inhabitants, between January 1997 and November 1998. Method. In this retrospective study we identified 63 patients with PCD. Standard clinical, radiological and laboratory criteria were used for diagnosis and staging. Six patients were excluded from analysis (2 had some missing data, 4 had a monoclonal component associated with CLL or NHL). 57 evaluable patients were grouped as follows: 1) patients with MMGUS and SPT; 2) MMGUS without SPT; 3) MM and SPT; 4) MM without SPT; 5) Others. Results. Group #1. This represents 7/44 of MMGUS patients (15.9%). All the cases occurred in males, the two conditions were always synchronous and the MGUS was discovered incidentally. There were 2 non-small lung cancer (NSCLC), 1 small cell lung cancer (SCLC), 1 bronchial carcinoma, 2 prostate cancer (PC) and 1 cancer of the oesophagus. The Ig classes were: 4 IgG, 2 IgM, and 1 IgA. Mean age was 68 y (range 54-88), vs. 63.8 y (30-85) in group #4. There was 1 SCLC diagnosed 23 months after MM. IgG III A, while the patient was in complete remission after polychemotherapy and on IFN therapy from 15 months; 1 PC diagnosed 26 months after MM IgA II A, while the patient was in complete remission after polychemotherapy and on IFN therapy from 15 months; 1 rectal cancer synchronous with MM IgM, III A without therapy, and 1 PC diagnosed 24 months after MM IgG II B, with the patient in partial remission after 3 cycles of high dose glucocorticoids. The patients died of SPT 1-3 months after the diagnosis had been made. The patient in group #5 had a renal cancer synchronous with MM IgG II B, with macrophage activation (IgM II). Conclusions. We found retrospectively SPT in 15.9% and 25% of patients with MM and MGUS, respectively. In both groups patients with SPT were older than patients without SPT. This association could be accidental in older people, but the influence of factors such as overdiagnosis, number of previous treatment(s) should be investigated in extensive prospective studies.
PO-0134 A new effective purging technique for autologous stem cell transplantation in multiple myeloma: evidence of tumour cell removal and rapid and sustained engraftment

Randebjädl, A; Comotti, B; Buelli, M; Viero, P; Barbui, B; Belli, N; Manzoni, C; Borleri, GM; Dotti, GP; Barbui, T
Divisione di Ematologia, Ospedali Riuniti di Bergamo, Italy

To reduce the tumour cell contamination of G-CSF mobilised peripheral blood circulating progenitor cells (CPC), we developed a two-step negative selection procedure whereby CPC can be effectively purged of contaminating neoplastic cells by magnetic beads and a SuperMACS separator (Miltenyi Biotech, Germany) (Randebjädl et al., Blood 1998). We applied this purging technique to multiple myeloma patients using anti CD19, CD56, CD10 and CD138 microbeads for in vitro purging. Thirty-four newly diagnosed MM patients received 3 cycles of VAD followed by cyclophosphamide (CTX), 7 g/m2 + +G-CSF (5 μg/kg/day) for stem cell collection. Thereafter they were randomised to receive autologous unmanipulated CPC (Arm A, 18 patients) versus highly purified plasma cell-purged (Arm B, 16 patients) to support tandem sequential transplantation (TSTR). In the TSTR, conditioned with melphalan (120 mg/m2) and melphalan (140 mg/m2) plus total body irradiation (TBI, 1200 cGy) for the second transplant. The aim of this study was to evaluate: a) the efficacy of in vitro purging in the neoplastic cell fraction, b) the quality of the hematopoietic and lymphoid reconstitution after transplantation and c) the clinical outcome. At this time we can report on the first two points of the study. By immunophenotype and PCR analysis performed with consensus oligonucleotide primers for the CDR3 region of rearranged heavy chain alleles we can demonstrate that in all cases the unmanipulated apheresis products contained a heavy plasma cell contamination as opposed to the purified stem cell fraction obtained after in vitro purging which showed a remarkable (> three logs) reduction of tumour cell contamination. Two aphereses were sufficient to meet the required minimum of 5x109 CD34+ cells/kg to support a second transplant and to have a back-up source of unmanipulated stem cells. The haematologic engraftment was rapid and not different in the two arms. The immunologic reconstitution (as determined by enumeration of T, B and NK cells) was comparable in both arms and no transplant related mortality has been seen so far. These results suggest the lack of any significant haematologic and immunologic toxicity associated with transplantation of plasma cell-purged CPC. The clinical benefit of this procedure still remains to be determined.

PO-0135 Involvement of the t(4;14)(p16.3;q32) chromosomal translocation in multiple myeloma

Fabris, S; Zagano, S; Baldini, L; Malgeri, U; Lombardi, M; Maioio, AT; Neri, A
Laboratorio di Ematologia Sperimentale e Genetica Molecolare, Servizio di Ematologia, Università degli Studi, Ospedale Maggiore R. ICCSS, Milano, Italy

Conventional cytogenetics identified a 14q+, the hallmark of translocation involving the IGH locus at 14q32, in about 20% of multiple myeloma (MM). More recently, molecular evidences have indicated that translocations to this locus represent a highly frequent event in MM which involves a large array of novel loci. In particular, we and others have identified a novel, karyotypically not detectable, t(4;14)(p16.3;q32) translocation in MM cell lines and primary tumors. The 4p16.3 breakpoints map scattered over a genomic region of about 4000 bp spanning the breakpoint region of rearranged heavy chain alleles and involved in the 5′ regulatory regions and 5′ introns of the gene WHSC1 (also named MMSET). Interestingly, both genes are overexpressed in cell lines carrying the translocation suggesting that they can be deregulated in MM cell samples. The haematologic engraftment was rapid and not different in the two arms. The immunologic reconstitution (as determined by enumeration of T, B and NK cells) was comparable in both arms and no transplant related mortality has been seen so far. These results suggest the lack of any significant haematologic and immunologic toxicity associated with transplantation of plasma cell-purged CPC. The clinical benefit of this procedure still remains to be determined.

PO-0136 Bendamustine/ prednisone versus melphalan/ prednisone in the treatment of multiple myeloma: a randomised multicentre study

Pönisch, W; Mitrou, PS; Riech, A; Horel, M; Schulte, A; Amsmann, M; Schirmer, V; Wilhelmi, G; Dachet, K; Richter, P; Suber, F; Freund, M; Friedrich, TN; Heilag, W; Niedeierer, W
University of Leipzig, University of Frankfort/Main, rribesopham Muenchen, Hospital Erfurt, Hospital Riesa, Hospital Plauen, Hospital Wernigerode, Hospital Nordhausen, Hospital Zella-Mehlis, Hospital Schweiner, University of Rostock, Germany

Previously untreated patients with multiple myeloma (MM) were randomised in a prospective multicentre study to receive bendamustine/prednisone (BP) or melphalan/prednisone (MP). Between May 1994 and July 1998, 136 MM patients (stage II with tumour progression, n=11; stage III, n=125) were recruited by 31 hospitals in Germany. Fifty-nine patients received BP (bendamustine 150 mg/m2 days 1+2, prednisone 60 mg/m2 and DEX 40 mg/m2 on days 1-4, followed, after 4 weeks pause, by 40 mg DEX on days 1+4-10-13-20-23 and after 4 weeks IFN again for the next 3 months). Results. Complete remission (CR) was achieved only 13% if criteria for CR including immunofixation were used. Progression was achieved in 52% after VAD. Seven-five pts. underwent PBSC and 14% of them reached CR. Overall responses were 72% after VAD and 78% after PBSC in 69 pts evaluated 1 month after PBSC. There were 12% non-responders after the high-dose regimen. Early transplant related mortality was 2.66% (2/75). 65 patients were randomised to the maintenance therapy arm. Median on EFS and OS have not yet been achieved. Details of interim analysis will be presented.

Conclusions. Therapeutic protocol of myeloablative therapy using melphalan 200mg/m2 has acceptable toxicity and good therapeutic effect. More patients and prolonged observation during the maintenance therapy is required for evaluation of two different types of maintenance therapy, which are very well tolerated by patients with MM.
Positive selection of peripheral blood stem cells (PBSC) in multiple myeloma

Patrickia F., Fanini R., Damiani D., Grima S., Silvestri F., Geromin A., Baccarani M.
Division of Haematology and Department of Bone Marrow Transplantation, Udine University Hospital, Udine, Italy.

Positive selection of PBSC was investigated in 23 patients with newly diagnosed advanced multiple myeloma (MM), with the aim of analysing the efficiency of reduction of plasma cell (PC) contamination in the harvests and of evaluating haematologic reconstitution and clinical response after high-dose therapy. All the patients received a 4 cycle induction therapy (VID or VAD) and underwent harvest after high dose cyclophosphamide (7 or 4 g/m²) + G-CSF. The CD34+ stem cells of leukaphereses' products were positively selected with an axid-in-biotin immunomnafinity device (Ceprate, Cell Pro); evaluation of pre- and post-selection PC content was analysed by a biparametric flow cytokmetric technique (CD 138, cytoplasmic light chain).

A median number of 0.83 × 10^10/ kg of PC (range 0.099-7.8) in pre-select- ed samples decreased to 0.005 × 10^10/ kg (range 0.0008-0.049) in post- selection. Production of NTX was often elevated in patients with malignant hypercalcemia (>0.5 × 10^10/ L) and thrombocytopenia (>0.5 × 10^9/ L) were respectively 11 and 21 days. One patient died of cerebral haemorrhage before engraftment. Other adverse effects were 6 cases of Gram-positive septicemia, 9 episodes of FUO and 15 WHO scale III-IV mucositis. At a median follow-up of 12 months (range 3-24), CR or PR was observed in 16 of the 23 patients (70%) and the changes were correlated with the effects of pamidronate treatment on NTx, BAP, OSC, IL-6, and reduction of paraprotein levels (p=0.0281) at 6 months. Bone pains disappeared in all patients of groups I and II with constant levels of paraprotein. Patients of group II also showed significant reductions of NTx (p=0.0077) and IL-6 (p=0.0109) levels from the first month but with a concomitant decrease of paraprotein (p=0.0277) and no change in the other parameters. No changes of NTx were observed in patients of group III (p=0.6), who showed an increase of IL-6 (p=0.0414) and reduction of paraprotein levels (p=0.0281) at 6 months. In multivariate analysis, changes of NTx correlated significantly only with the levels of IL-6 at 6 months. Bone pains disappeared in all patients of groups I and II. It is concluded that pamidronate treatment rapidly reduces NTx and IL-6 values, with concomitant increase of BAP and OSC for group I, insensitive of antimyeloma therapy, resulting in clinical benefit.

Molecular detection of IgH gene rearrangement through PCR of regions CDR1, CDR2 and CDR3 in multiple myeloma

Martínez-López J., Bautista JM, Salama P., Garcia-Fernandez S., Bornstein R., Grande C., Lahuerta JJ.
Servicio de Hematología Hospital Universitario 12 de Octubre and *Departamento de Bioquímica y Biología Molecular IV, Universidad Complutense, Madrid, Spain

In multiple myeloma (MM), the study of immunoglobulin heavy chain gene rearrangements through molecular techniques is difficult. This situation is due to somatic hypermutation in VH region of IgH. We applied different PCR strategies to the IgH gene in 21 MM patients, with the aim of detecting a clonal population in a large number of cases and response following treatment. Design and Methods. Twenty-one patients with MM were enrolled in this study between 1996 and 1998 in our Hospital, later we included them in a double autologous stem cell transplantation (ASCT) protocol. DNA was extracted from bone marrow (BM), peripheral blood (PB) and peripheral blood stem cells (PBSC). We amplified, by PCR, the CDR1, CDR2, and CDR3 regions of IgH gene using consensus primers of FR1, FR2 and FR3 (sense) 25 ng/ml of DNA for two cycles. DNA was run in 2% agarose gel electrophoresis and ethidium bromide dyeing. The technique sensitivity was between 10^2 and 10^4 cells. Results. A clonal population in the CDR3 region was identified in 7/19 cases (37%), CDR2 in 15/19 cases (78%) and CDR1 in 16/21 (76%). Finally, IgH rearrangements could be detected in 19/21 cases (90%). Of 9 BM positive cases at diagnosis, PB was positive in 8 of them. We also analysed 10 patients’ PBSC and 9 were positive. Seven patients were followed between 8 and 30 months after the first ASCT (median 20m). Four cases were in complete remission and 3 in stable partial remission. Twenty-one BM and 24 PB samples were studied, 18 (85%) BM were positive and 17 (73%) PB were positive. Conclusions. In our experience, it is possible to detect a clonal population in a large number of patients with MM by PCR. The detection in PB is comparable to the detection in BM, and may be considered as a simple, economic and sensitive way to follow up MM patients. PBSC are positive in a large number of MM cases. Bearing in mind the sensitivity of the technique, it shows a high degree of tumour contamination.

Markers of bone turnover and Interleukin-6 (IL-6) in multiple myeloma patients treated with pamidronate

Terpos E., Palermos J., Tsonos K., Metelitsa P., Papasavvas P., Loukogolou D., Yataganas X.
*First Dept. of Medicine, University of Athens Medical school, Laiko Hospital; †Haematology and Immunology Dept, 251 Air Force Gen. Hospital, Athens, Greece

Urinary excretion of the cross-linked N-telopeptides of type I collagen (NTx) has been shown in clinical studies to provide a highly specific index of bone resorption. The effect of pamidronate treatment on NTx, BAP, OSC, IL-6, and thrombocytopenia (<0.5 × 10^9/ L) and thrombocytopenia (<0.5 × 10^9/ L) were respectively 11 and 21 days. One patient died of cerebral haemorrhage before engraftment. Other adverse effects were 6 cases of Gram-positive septicemia, 9 episodes of FUO and 15 WHO scale III-IV mucositis. At a median follow-up of 12 months (range 3-24), CR or PR was observed in 16 of the 23 patients (70%) and the changes were correlated with the effects of pamidronate treatment on NTx, BAP, OSC, IL-6, and reduction of paraprotein levels (p=0.0281) at 6 months. Bone pains disappeared in all patients of groups I and II with constant levels of paraprotein. Patients of group II also showed significant reductions of NTx (p=0.0077) and IL-6 (p=0.0109) levels from the first month but with a concomitant decrease of paraprotein (p=0.0277) and no change in the other parameters. No changes of NTx were observed in patients of group III (p=0.6), who showed an increase of IL-6 (p=0.0414) and reduction of paraprotein levels (p=0.0281) at 6 months. In multivariate analysis, changes of NTx correlated significantly only with the levels of IL-6 at 6 months. Bone pains disappeared in all patients of groups I and II. It is concluded that pamidronate treatment rapidly reduces NTx and IL-6 values, with concomitant increase of BAP and OSC for group I, insensitive of antimyeloma therapy, resulting in clinical benefit.

IL-1β and TNFα gene polymorphisms in multiple myeloma and monoclonal gammopathy of undetermined significance

Zhang CY, Huang D, Bengarraint S, Österberg A, Holm G, Yi Q.
Hematology Research Laboratory, Department of Medicine, and *Department of Oncology, Karolinska Hospital, Stockholm, Sweden

Interleukin-1β (IL-1β) and tumour necrosis factor-α (TNFα) may be potential growth factors for myeloma cells, and are responsible for the bone destruction in patients with multiple myeloma (MM). To examine whether the gene polymorphisms in both cytokines could be related to the clinical outcomes in MM patients, we studied the IL-1β Taq1 restriction fragment length polymorphism (RFLP) in exon 5 of the IL-1β gene and a b-allelic polymorphism at position -308 in the promoter region of the TNFα gene. Seventy-two patients with MM, 28 with monoclonal gammopathy of undetermined significance (MGUS) and 131 ethnically matched healthy controls (HC) were included in this study. Our results showed that frequencies of genotypes and alleles of the IL-1β Taq1 and TNFα -308 polymorphisms were not different between patients with MM, with MGUS and HC, respectively. However, the frequency of heterozygotes A1A2 of IL-1β was significantly increased in the MM patients with progressive disease as compared with HC (OR=2.68; p=0.038). Accordingly, the frequency of homozygotes A1A1 of IL-1β were significantly decreased in these patients as compared with HC (OR=0.34; p=0.025). We also compared frequencies of carriers of allele 2 in MM patients and found a correlation between allele 2 carrier- ship and severity of the disease (OR=2.94; p=0.025). Furthermore, the carrier- ship of allele 2 of IL-1β Taq1 RFLP is strongly associated with the disease progression (OR=6.0; p<0.001). Taken together, our results indicate that genetic variation of the IL-1β gene is associated with disease progression and clinical outcome of the patients with MM. Further studies of the polymorphisms, expression and functions of these cytokines in MM are warranted.
PO-0142 \-interferon of human fibroblast on chronic phase chronic myeloid leukemia

Centro Medico Nacional La Raza, Department of Hematology, Mexico

Objectives. To determine the efficiency, tolerance and security of interferon (IFN) treatment in CML patients. Materials and Methods. 11 CML patients aged >16 years with a CML diagnosis confirmed by cytogenetic and RT-PCR analyses were enrolled. The treatment included a cytoreductive phase with busulfan at dose of 0.2 mg/kg/d followed by progressive daily subcutaneous doses of \-interferon for one week at 3-10^6 IV. Six >10^9 IV one week and 9 >10^9 IV for three weeks. The patients with complete haematologic response (CHR) were included in a maintenance phase with 6-10^9 IV daily doses of S. C. or I. M. \-interferon for 3 months. Results. 9 male and 2 female patients were included, M:F ratio 4:1. Median age 42 (±7.7) years, median Hb 12.7 (±2.9) g/dL, median WBC 10^9/1/ L (±2.0) x 10^9/1, median ESR 14 (±6) mm/h, median PB blasts 2, BM blasts 3, median PB basophils 3, BM basophils 5, median LDH 497 (±72) IV. Median splenomegaly 17 (±4) cm; median busulfan 315 mg. Kantarjian’s score: E1 36%, E2 55%, E3 9%. Median survival at diagnosis 13.63 (±6.3) months; 11 (100%) patients have Philadelphia-positive CHI, mosome, 10 (91%) with \-interferon rearrangement by RT-PCR, 9 (82%) with b2a2 fusion transcript, 1 (9%) with b2a2/b2a2 and 1 (9%) \-interferon positive. Response: 9 (82%) patients obtained CHR, 1 (9%) had partial response and 1 (9%) did not respond. One (9%) patients obtained minor cytogenetic response. Toxicity: hepatotoxicity grade III, three (27%) patients. Neurotoxicity grade I and II, five (45%) patients, local toxicity grade I and II, four (45%) patients. Conclusions. These results suggest that \-interferon is an useful therapy for obtaining CHR in CML patients with good tolerance. We need to define whether longer maintenance with \-interferon is similar to \-interferon for improving the molecular and cytogenetic response and increasing a long survival in CML patients.

PO-0143 Spontaneous megakaryocytopenia and platelet hyper-reactivity in thrombocytemia vera: is thrombopoiétin the causative factor?

Michiels JJ, Bellucci S
*Goodheart Institute MPD, Center Europe, Rotterdam, The Netherlands and *Hematology Laboratory, Institut des Vaisseaux et du Sang, Hospital Lambièvre, Paris, France

There is good evidence that the microcirculatory circulation disturbances in essential thrombocytemia (ET) are caused by intravascular activation and aggregation of hypersensitive platelets with sludging or occlusion of the endoarterial microvasculature. In this process the generation of platelet derived products, endothelial cell damage thrombox anal intimal proliferation and platelet thrombi is essential and could be inhibited by a platelet specific regimen of aspirin 50 mg/day plus a rationale for using low-dose aspirin as an antithrombotic agent in thrombocytemia. In contrast, the approach is not to be essential for the formation of platelet thrombi, thereby explaining the inefficacy of coumadin and heparin in the treatment of microvascular disturbances in acquired ET. In acquired ET, the role of TPO is crucial in the observed hypermegakaryopoiesis which is characterised by an increased proliferation of MK progenitors even in conditions of cultures without addition of any known megakaryocyte colony-stimulating factors. The observed increased reactivity of megakaryocyte progenitors to TPO remains to be precisely delineated. A definitive clearance of TPO by megakaryocytes and platelets because of a reduced number of cMpl receptors is possible. TPO is able to enhance megakaryocyte colony-stimulating factors. The observed increased reactivity in the TPO gene as the cause of increased TPO production in hereditary ET can readily explain both spontaneous megakaryopoiesis and platelet hyperreactivity leading to the occurrence of platelet-mediated microvascular disturbances simulating the phenotype of acquired ET. Until now no mutation of TPO structural gene, as shown in two families with hereditary ET, and no mutations in the cMpl receptor could be found in patients with acquired monoclonal and polycyetal ET.

PO-0144 Serum hypercalcaemia is a feature of essential thrombocythaemia

Centro Medico Nacional La Raza, Department of Hematology, Mexico

Hypocalcaemia may lead to an artefactually elevated serum potassium (K) level. It is known that essential thrombocythaemia (ET) with significant thrombo-
between 400×10^3/L and 600×10^3/L. Leukemic transformation was not observed at a median follow-up period of 8.75 years (95% CI, 6.63 to 12.25 years). rIFN appears to be most effective when started at presentation in the early plethoric stage of PV. The required rIFN dose in the early stage of PV is lower, less toxic and does not interfere with working and recreational ability. Reasons for discontinuation of rIFN were side effects in 8 and physician's decision related to patients' age in 8 patients. A randomised trial comparing rIFN with the best conventional therapy consisting of phlebotomy and additional treatment with HU in previously untreated PV patients is indicated. rIFN, as a first line nonleukemogenic treatment option should be offered to all PV patients less than 65 years of age as no randomised study comparing rIFN and HU is available.

**PO-0147** Cardiac involvement in patients with myeloproliferative disorders

Galanopoulou A,* Matakasi E,* Panou F,* Kakkas J,* Grigoraki V,* Patelakis G,* Anagnostopoulou N* \*Dept. of Haematology, and °Dept. of Cardiology, District General Hospital of Athens, Greece

The aim of the study was to evaluate cardiac involvement in myeloproliferative disorders (MPD). This study included 15 patients (pts), 9 male and 6 female with an age range from 41 to 84 years, diagnosed between 1991 and 1998. Five pts had polycythemia vera (PV) and 10 pts had essential thrombocytosis (ET). Diagnostic criteria for PV and ET were those specified by the Polycythemia Vera Study Group. Two dimensional and Doppler echocardiography studies were performed in all MPD pts. Echocardiography revealed valvular lesions in 9/15 pts (60%). Valvular lesions were found in 7/10 pts (70%) with ET and in 2/5 pts (40%) with PV. The mitral valve was the most involved valve and thrombosis and the most common echocardiographic lesion was leaflet thickening, which was found in 8 pts (53%). No valvular vegetations were detected. Pulmonary hypertension was found in one patient with ET. In our past study 9/15 pts had minor or major thrombotic episodes. Six of 9 pts (66%) with valvular lesions had thrombotic episodes compared with 2/6 pts (33%) without evidence of valvular lesions. These findings are consistent with those of other investigators [1]. In conclusion the heart is frequently involved in pts with MPD, particularly when their past history is complicated by a thromboembolic event. A larger number of pts is needed to determine whether the presence of valvular lesions is of diagnostic significance and may herald future thromboembolic events.


**PO-0148** Atypical chronic myelogenous leukaemia following immuno-suppressive therapy for severe aplastic anaemia

Robak T,* Kasznicki M,* Sztelecka B,* Bartkowiak J,° Dziebic-Ryczter M ° *Dept. of Hematology and ° Dept. of Molec. Biology, Medical Univ. of Lodz, Poland

Late clonal complications of aplastic anaemia (AA) such as acute leukaemia, myelodysplastic syndromes or paroxysmal nocturnal haemoglobinuria have been recognised for a long time. To our knowledge chronic myelogenous leukaemia (CML) as a late complication of severe AA has not been reported so far. We report the case of a patient with AA treated successfully with antilymphocyte globulin and cyclosporin in whom Philadelphia chromosome negative (Ph-) BCR-ABL negative (abnormal) CML developed 8 years after diagnosis of AA. 19 year old male patient had a history of viral hepatitis B in October 1988. In February 1989 he developed haemorrhagic diathesis and severe pancytopenia. Bone marrow biopsy was aplastic and severe AA was diagnosed. He was treated with methylprednisolone, antilymphocyte globulin and cyclosporin. The blood counts gradually improved and returned to normal in February 1990. He was well until September 1997 when weakness and chronic abdominal pain developed. On physical examination the patient was pale, spleen was palpable 3 cm and the liver was palpable 3 cm below the costal margins. Blood cell counts during the course of observation were as below:

<table>
<thead>
<tr>
<th>Date</th>
<th>Hb</th>
<th>PLT</th>
<th>WBC</th>
<th>Differential count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb 89</td>
<td>7.5</td>
<td>10</td>
<td>0.3</td>
<td>blasts myelo meta bands neutr mono lymph</td>
</tr>
<tr>
<td>Apr 93</td>
<td>16.2</td>
<td>120</td>
<td>3.5</td>
<td>15% 85%</td>
</tr>
<tr>
<td>Sep 93</td>
<td>13.5</td>
<td>67</td>
<td>77</td>
<td>3% 7% 8% 12% 51% 7% 12%</td>
</tr>
<tr>
<td>Sep 98</td>
<td>29</td>
<td>82</td>
<td>57</td>
<td>2% 12% 27% 1% 51% 2% 4%</td>
</tr>
</tbody>
</table>

The cytogenetic analysis of G-banded chromosomes showed normal karyotype with no evidence of translocation t(9;22). This result was further confirmed by FISH as well as RT-PCR which did not reveal specific the CML transcripts of abl/ab/act fusion gene in patient bone marrow cells. Atypical, sec-

**PO-0149** Additive action of gemcitabine (2',2'-difluorodeoxycytidine) and 2-chlorodeoxadenosine on murine leukemias L1210 or P388 and on human normal and leukaemic haematopoiesis in vitro

Mariana E, Robak T
Dept of Hematology, Medical Univ. of Lodz, Lodz, Poland

Gemcitabine (dFdC) is a new nucleoside antimetabolite of deoxycytosine that resembles cytarabine (Ara-C) in both its structure and metabolism. The aim of our study was to investigate the influence of dFdC used alone and in combination with 2-chlorodeoxadenosine (2-CDA) on the survival time of mice bearing L1210 or P388 leukaemia and on the colony growth of human normal granulocyte-macrophage progenitor cells (CFU-GM) as well as on CFU-GM from patients with chronic myeloid leukaemia (CML) in cultures in vitro. So far, there have been not any studies concerning the interactions between dFdC and 2-CDA. In the in vivo part of our study, the mice were given 2-CDA (20 mg/kg) on days 1-5 after inoculation with leukaemic cells (day 0) i.p. or dFdC (20 mg/kg) on days 1, 4, 7 and 10 i.p. The drugs were administered alone and in combination (sequential therapy). In the in vivo experiment revealed that in both leukaemias tested, combined therapy with dFdC given before 2-CDA was more effective than monotherapy with either dFdC or 2-CDA. Other treatment schedules did not significantly prolong the survival time of the treated mice compared with the treatment with dFdC alone. In the in vitro part of our study normal CFU-GM cells and CML CFU-GM cells were incubated with dFdC or 2-CDA used alone and in combination at the following concentrations: dFdC 0.5 nM, dFdC 1.0 nM, dFdC 2.0 nM, 2-CDA 0.5 nM, 2-CDA 1.0 nM, 2-CDA 2.0 nM. The in vitro experiment revealed that both dFdC and 2-CDA inhibited the growth of colonies formed by normal and CML CFU-GM cells in a dose dependent manner as compared to the control cultures. Moreover, the combined therapy with dFdC and 2-CDA caused statistically significant inhibition of the growth of both normal and CML CFU-GM colonies. In addition, inhibition of the growth of both types of CFU-GM was greater and statistically significant in the case of combined therapy as compared to monotherapy using each drug separately. The results confirmed that dFdC used before 2-CDA has an additive action on murine leukemias L1210 and P388 and dFdC and dFdC act additively on normal and CML CFU-GM cells.

**PO-0150** Chronic neutrophilic leukaemia (CNL): a new classification

Ben-Tal O, Marilus R, Winder A, Eldor A
Institute of Hematology, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel

Chronic neutrophilic leukaemia (CNL) is a rare proliferative disorder characterised by sustained neutrophilic leukaemias in the absence of underlying disease. Typical features are: circulating mature neutrophils without immature cell forms and splenomegaly and skin thickening. Chronic neutrophilic leukaemia can be divided into three subtypes: 1) chronic stable CNL, 45 patients (50%, ages 25-80), with an indolent disease and survival of more than two years; II) CNL accompanied by monoclonal gammopathy of unknown significance (MGUS) or multiple myeloma (MM): 23 patients (25%, with IgG or IgA paraprotein, aged 31-72, median survival 48 months); and III) CNL accompanied by myeloplasia (MDS) and or AML, 24 patients, 25%. This group carried a grave prognosis, with 94% mortality within one year, while 63% of in groups 1 and 2 were alive 9mo-7y after diagnosis. Non-random chromosomal abnormalities were observed in 65% of patients in group 3 CNL, whereas only one patient with CNL group I had abnormalities. This and other observations suggest that CNL group III is definitely a neoplastic disorder. Conclusions: We describe three subgroups of CNL: I) chronic stable phase, II) CNL MGUS-MM and III) CNL MOS-AML. These subgroups have distinct unique features and vary in prognosis and outcome. The appearance of myeloid proliferative features is a fatal leukaemic transformation, while an accompanying plasma cell dyscrasia and gammopathy carries a better prognosis.

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Introduction. Bone marrow necrosis (BMN) is a uncommon complication of Ph+ CML. There are only 10 fully documented cases (7 treated with chemother-apy and 3 with IFN-a). BMN appeared in patients with advanced dis-ease, except in 2 patients (both in early chronic phase and with IFN-a). We report 2 new cases with BM in chronic phase disease and treated with IFN-a. Design and Methods. In the last 4 years we have diagnosed 10 patients with Ph+ CML, 6 treated by IFN-a and 4 treated by other therapies. We observed localized BMN in 2 cases, both treated with IFN-a. Case #1. A 51-year-old man with CML was treated with IFN-a, 3 months later there was sudden onset of severe bone pain in the lumbar area. MRI suggested necrosis in the right iliac bone and bone marrow specimens showed necrosis without any sign of blast crisis. At this time we found a normal level of TNF-a in plasma. We treated the patient with local radiotherapy and the bone pain was relieved. Seven months later the patient remained in chronic phase with hydroxyurea and IFN-a. Case #2. A 55-year-old woman with CML in chronic phase and treated with IFN-a presented 5 months later with recurrent severe bone pain in multiple sites (ribs and left hip). MRI showed BMN in the left femoral head. A bone marrow biopsy performed in the left iliac crest revealed CML in chronic phase disease in plasma was normal. The patient was treated with analgesics and the bone pain improved. Six months later the patient remained in the chronic phase. Conclusions. It has been postulat-ed that IFN-a has a role in the development of BMN likely due to abnormal production of local TNF-a by mononuclear cells. Our short serie indicates a high incidence of localised BMN in CML patients in early chronic phase during treatment with IFN-a. None of the patients showed evidence of acute transformation, DIC, infection or other causes of BMN. Because different series have described bone pain in 10-20% of CML patients in chronic phase during treatment with IFN-a, it is possible to suspect that this bone pain is due to localised BMN. MRI is a useful diagnostic tool in these cases.

PO-0152 Evidence of endothelial cell activation in patients with chronic myeloproliferative disorders

Activated endothelial cells express and release, into circulating blood, a number of relevant adhesion molecules such as intercellular adhesion mol-ecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), endothe-llial-leucocyte adhesion molecule-1 (ELAM-1), fibronectin (Fn) together with von Willebrand factor (vWF), which can in turn elicit inflammatory and thrombotic events. In this context, interactions between endothelial cells and both leucocytes or drug administration have been involved as possi-bile inducers of vascular endothelial activation. We investigated endothel-i-al cell function in 50 patients (27 males and 23 females) affected by chronic myeloproliferative disorders (CMD) (27 CML, 13 ET, 4 PV, 6 IM) since these haematological malignancies are often associated with an increased risk of both vascular inflammation and thrombotic complic-a-tions. All patients were on chronic therapy with either hydroxyurea or interfer-on at the time of the study. Ten normal subjects (age and sex matched) served as controls. Serum levels of the above mentioned adhesion mole-cules were measured by ELISA technique. In addition, phenotypic ICAM-1 and VCAM-1 expression was evaluated by flow cytometry on circulating granuloblastoid cells of all patients.

**Controls**

<table>
<thead>
<tr>
<th>ICAM-1 (µg/mL)</th>
<th>ELAM-1 (µg/mL)</th>
<th>VCAM-1 (µg/mL)</th>
<th>Fn (µg/mL)</th>
<th>s-ELAM-1 (µg/mL)</th>
<th>s-VCAM-1 (µg/mL)</th>
<th>WF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>130±5</td>
<td>1187.71±81.92</td>
<td>3142.83±37.41</td>
<td>144±50.91</td>
<td>319.1±62.92</td>
<td>166.1±62.45</td>
<td>0.7</td>
</tr>
</tbody>
</table>

As compared to controls, our results showed a significant rise in the serum levels of ICAM-1, VCAM-1, ELAM-1 and WF (p<0.0001), whereas no difference was found in Fn levels. On the other hand, flow cytometric analy-sis revealed a very low or absent cellular expression of both ICAM-1 and VCAM-1 on peripheral myeloid cells. In our opinion, the increased serum ICAM-1, VCAM-1, ELAM-1 and vWF concentrations could result from an abnormal release by endothelial cells as an expression of vascular activa-tion or injury. If so, endothelial cell activation could play a pivotal role in both vascular inflammation and thrombogenic risk in CMD patients.

PO-0153 Mini-ICE regimen as mobilisation therapy for chronic myelogenous leukaemia patients at diagnosis
Sureda A, Petit J, Brunet S, Boque C, Aventin A, Martino R, González JR,* Amill B,* Lamba I,* Blanco A,* Martín Henao GA,* Sierra J, Grafena A,* Clinical Hematology Division, Hospital de la Santa Creu i Sant Pau and *Institut Català d’Oncologia; *Criobiology and Cellular Therapy Depart-ment, Institut de Recerca Oncològica, Barcelona, Spain

Between April 1996 and May 1998, 20 consecutive patients with Ph+ chro-mosome-positive chronic myelogenous leukaemia in first chronic phase without an HLA-identical sibling received the mini-ICE regimen shortly after diagnosis with the purpose of mobilising progenitor cells into peripheral blood. Sex distribution was 12 males and 8 females and median (range) age 48.5 (22-62) years. Fourteen patients were in clinical stage I of Kar-tanian’s classification, 5 in stage II and 1 in stage III. Time interval between diagnosis and mobilisation had a median value (range) of 2 (0.5-5) months. Leukaphereses were initiated during recovery from chemotherapy-induced aplasia, when leucocytes reached 1.0×10^9/L and/or CD34+ cells were ≥2.5×10^6/mL. A median number of 3 (1-7) aphereses per patient were performed to collect ≥2.0×10⁶ CD34+ cells/kg, after a median of 19.5 (15-27) days from the start of the mini-ICE regimen. Cytogenetic analysis was performed on the apheresis products of 18 patients: complete cyto-genetic remission was observed in 4 patients, 9 patients had a partial cytogenetic remission, 3 patients a minimal cytogenetic remission and 2 a null cytogenetic remission. Southern blot for bcr-abl was negative in the remain-ing two patients but the polymerase chain reaction analysis was positive in one of both. Severe neutropenia granulocytes <0.5×10^9/L was present during a median of 8.5 (3-19) days and severe thrombocytopoietic platelets <20×10^9/L lasted a median of 8 (3-18) days. Nine patients did not develop neutropenic fever with 4 of them being treated on an outpatient basis. There was no grade 3 gastrointestinal toxicity and treatment related morta lity was not observed. In conclusion, our experience demonstrates thefea-sibility to mobilizing peripheral blood progenitor cells shortly after the diag-nosis of chronic myelogenous leukaemia with a non-risky regimen. Of note, mini-ICE allowed the collection of apheresis products with at least a major cytogenetic remission in almost 75% of the patients.

PO-0154 A prolonged period of prevalent PH-NEG leukaemia can be achieved in CML patients autografted at diagnosis

The aim of this report is to study, in chronic myeloid leukaemia patients, changes occurring after autografting in the percentage of Ph-negative and Ph-positive cells either at mature and at progenitor cells level and their relation-ship with clinical outcome. For this purpose, 13 CML patients, mobilised and autografted early after diagnosis with prevalently Ph-negative progenitors, were studied at a median time of 24 months (range 14-46) from transplant. Transplant modalities produced a reduction in the proportion of Ph-positive progenitors (CFC) from 70-100% to 0-25% in the majority of patients (78%). After autografting, we could recognise two groups of patients: Group A (Ph-positivity <33%) and Group B (Ph-positivity ≥33%). In Group A Ph-positive cells remained at a low level (median: 10%) in fresh bone marrow samples and CFC resulted entirely Ph-negative after the first six months from transplant. In Group B leukaemic cells became quick-ly predominant and at one year from transplant the majority of Ph-positive cells were 100% Ph-positive both in fresh cells and in CFC. Time course analy-sis showed that the clonogenic compartment was less contaminated with the Ph-positive clone than mature cells. We found that detection of Ph-po-itive LTC-IC in the marrow at diagnosis was the only factor significantly asso-ciated with recurrence of the disease (p<0.01); on the other hand, the num-ber of Ph-negative LTC-IC infused showed a significant correlation with a bet-ter outcome (p<0.03). We can conclude that a prolonged period of complete or prevalent Ph-negative haemopoiesis can be achieved in CML patients autografted with Ph-negative progenitors but a longer follow up is required to associate these changes with an improved survival.
PO-0155 Clinical use of arsenic trioxide (As$_2$O$_3$) in the treatment of chronic myelogenous leukemia: results of the first patients treated in the phase I-II trial AS98

Hôpital Saint-Louis, Hôpital Necker, Paris, France

In the 1900s, Fowler’s solution (inorganic potassium arsenite) was used to control elevated leukocyte counts in chronic myelogenous leukemia (CML). In vitro studies were then conducted to delineate the spectrum of activity of this compound. We investigated the effects of As$_2$O$_3$ on various non-APL myeloid cell lines and we found that As$_2$O$_3$ induced a 50% growth inhibition in the K562 myeloid cell line which carries the t(9;22) translocation. CML mononuclear cells from one patient have been exposed in vitro to As$_2$O$_3$ 10–6 M and interferon-α (IFN) 103 IU/mL. At day 7, the combination of As$_2$O$_3$ and IFN induced apoptosis with 69.5% of annexin-positive, P1-negative cells as compared with 2.4%, 40% and 36% in the control, As$_2$O$_3$ and IFN treated cells respectively, indicating a possible synergetic effect of As$_2$O$_3$ and IFN. We have then initiated a phase I-II trial to evaluate the tolerance and the efficacy of arsenic trioxide in CML patients in accelerated or transformed phases. Arsenic trioxide was administrated daily in 3 hour perfusions (0.15 mg/kg/d) for a maximum of 56 days. The first patient included was a 60 year old male with CML in accelerated phase (basophilia 20%). At inclusion, WBC count was 32.4×10$^9$/L (75% myeloblasts including 12% of blasts) with spleen enlargement. The arsenic treatment induced a marked reduction of the WBC count with a nadir of 3.5×10$^9$/L WBC at day 34. The patient developed a grade 3 erythema at day 15 and a grade 2 sensitive neuropathy at day 21. The arsenic concentration was monitored weekly with residual concentrations ranging from 0.517 to 1.214 µmol/L at the week after the first week of treatment. As$_2$O$_3$ was stopped on day 47 because of the occurrence of a pneumopathy. The bone marrow recovery was observed on day 67. No cytoxicogenic response was observed. The patient is actually in second chronic phase (basophilia 7%). A 23 year old female with an acute phase CML is currently under treatment. As$_2$O$_3$ was 0.39 µmol/L at day 30. These results suggest busulfan given as short single course is an effective and well-tolerated drug for the control of thrombosis in patients with CML. Four patients who had relapsed with thrombocytosis 12-170 months after the primary therapy were successfully retreated with the same treatment. There were no significant complications attributable to busulfan. No secondary haematological malignancy was observed. All but one patients are still alive, one dead from stomach ca 32 months after the therapy. Our findings suggest busulfan given as short single course is an effective and well-tolerated drug for the control of thrombosis in patients with CML. Busulfan induces long intervals of remissions with stable blood counts with no need for additional treatment. We believe that the decrease in cumulative dosage may reduce the potential leukemogenic effects of this drug.

PO-0158 Follow-up of GST-p expression in BCR-ABL+ and BCR-ABL- cells from CML patients

Unidad de Hematopoyesis, Instituto Nacional de Cancerología, México

We have recently found that glutathione-sulfhydryl-transferase-p (GST-p) expression in malignant cells, during haematopoesis in liquid cultures of PBSC or BMSC from myeloid leukaemia patients candidates to transplant, might be an earlier than Ph chromosome detection. To confirm this issue, since one year ago we have used FISH to follow-up GSTp-expressión in BCR-ABL+ and BCR-ABL- cells from 20 CML patients in diferent clinical stages: treatment (T), relapse (R), blast crisis (BC) or post-allograft transplant (PT), as well as in PB from 30 blood bank donors. The results obtained (see Table) suggest that GST-p expression might be used for the evaluation of minimal residual disease in CML patients.

<table>
<thead>
<tr>
<th>Clinical status</th>
<th>R</th>
<th>BC</th>
<th>PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML cells (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCR-ABL+ GST-p+</td>
<td>1-67</td>
<td>33-69</td>
<td>90-100</td>
</tr>
<tr>
<td>BCR-ABL- GST-p+</td>
<td>2-31</td>
<td>5-18</td>
<td>0-10</td>
</tr>
<tr>
<td>BCR-ABL+ GST-p-</td>
<td>2-97</td>
<td>13-62</td>
<td>94-96</td>
</tr>
<tr>
<td>BCR-ABL- GST-p-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

GST-p was not expressed in donor cells.

PO-0159 Chronic neutrophilic leukaemia with myelodysplastic features

Herranz ML, Canas MA, Florensia L, Cartany A
*Unitat d’Hematologia, †Servei de Medicina Interna, ‡Servei d’Anatomia Patológica. +Hospital de Valls. Tarragona. ‡Hospital de l’Espanyer. Laboratori de referència de Catalunya, Barcelona, Spain

Objective. To describe a case of chronic neutrophilic leukaemia (CNL) with myelodysplastic features. Case report. An 86-year-old man came to our hospital in [June, 1998] because of fatigue and asthenia. He had a haemoglobin of 9 g/dL, leukocytes of 60,000/mm$^3$ with 90% mature forms, elevated uric acid of 800 µmol/L and an infection of the urinary tract. He was discharged...
with allopurinol and oral antibiotics. Two months later he was admitted to hospital for investigation of persistent anaemia and leukocytosis. On physical examination he was apyretic, had mucous pallor, hepatomegaly of 3 cm below the costal margin and mild splenomegaly. Haemoglobin level was 8.4 g/dL, Htc 26%, MCV 83 fL, platelets 174,000/mm³ and leucocytes 53,400/mm³ with 2% myelocytes, 2% metamyelocytes, 3% bands, 3% monocytes, 3% basophils and 85% mature granulocytes. Dysplastic features could be seen in erythroblasts (anisooxyphilosis, basophilic stippling) and in leucocytes (nuclear clumping, Döhle bodies and abnormal granulation). The leucocyte alkaline phosphatase score was 170 (N20-40). Standard biochemical analyses showed an increase of vitamin B12 level (3000 pg/mL), creatinine of 144 µmol/L, serum alkaline phosphatase of 8.2 µkat/L and a GGT level of 1.31 µkat/L. Serum protein electrophoresis showed a slight polyclonal gammopathy. A cut-off value of 0.5% was positive for E. Coli, although the patient was asymptomatic. Abdominal ultrasonography revealed moderate hepatosplenomegaly, and a stone in the bladder. Bone marrow aspiration and biopsy were extremely hypercellular, with a myeolid erythrodi ratio of 8:6:7. In chromosomes 9 and 22 which appeared normal in standard cytogenetic studies. Some micromegakaryocytes could be seen. Pears reaction showed 52% sideroblasts with 3% ring forms and normal iron stores. No significant fibrosis was observed. Absence of peripheral myeloid progenitors revealed a spontaneous growth of erythroid precursors (BFU-E). Five months later now the patient remains stable, with no further increase of leucocytes or anaemia. He has intermittent asymptomatic bacteriuria. Concluding, the characteristics of this case reinforce the idea suggested by other authors that CML may be related to a myeloidplastic syndrome. The spontaneous growth of BFU-E suggests an underlying myeloproliferative disorder, although there is no evidence of preexisting polycythaemia vera or myelofibrosis.

PO-0160 A Philadelphia negative chronic myelogenous leukaemia with a masked translocation t(2;22) revealed by whole chromo-some painting

Mousa H.*, Jondeau K.*, Jary L.*, Fourcade C.*, Pulik M.*

*Pauirat-Cervera, Cergy Pontoise, France; Hôpital V. Dupuy, Argenteuil, France.

Introduction. We report a case of pH-negative chronic myeloid leukemia (CML) which appeared as a normal male karyotype 46, XY by routine banding analysis. Clinical features included left lateral pleural effusion, ascites and an increase of peripheral blood leucocyte count. The patient was treated by alkylating agents and IFN-α with a complete remission. The patient remains in remission with normal peripheral blood count and no signs of recurrence, 3 years after initiating treatment. In situ hybridisation (FISH) using a whole chromosome paint 22 probe was used to demonstrate the presence of a masked translocation t(2;22)(p24;q11) associated with a sub-microscopic translocation M-bcr/abl fusion in chromosomes 9 and 22 which appeared normal in standard cytogenetic studies. Case report. A 70 year old man presented with a raised blood cell count (40,000/mm³) with normal platelets (316,000/mm³) and haemoglobin level (15 g/dL). Clinical examination was normal. Bone marrow biopsy revealed a myeloid hypoplasia and a provisional diagnosis of CML was made. Design and Methods. Bone marrow cells were cultured and examined using a sequential technique. Prior to FISH, evaluation was first performed on the metaphase spreads. Next, FISH was performed on the bone marrow cells using whole chromosome painting of chromosome 22 to probe metaphases and interphases and metaphases showed that the chimeric fusion gene was localised on chro-

mosome 22q11. Molecular studies (PCR) revealed a rearrangement of the BCR-ABL region and expression of a chimeric bcr/abl mRNA of b3-a2 con-

formation. Molecular studies (PCR) revealed a rearrangement of the translocation between chromosome 2 and 22 t(2;22)(p24;q11). The dual dual

the 9 and 22 chromosomes were normal. Sequential R-banding and FISH

performed on chromosomal metaphases RHG-banding slides, during which

row biopsy revealed a myeloid hyperplasia and a provisional diagnosis of

hemoglobin level was 8.4 g/dL, Htc 26%, MCV 83 fL, platelets 174,000/mm³ and leucocytes 53,400/mm³ with 2% myelocytes, 2% metamyelocytes, 3% bands, 3% monocytes, 3% basophils and 85% mature granulocytes. Dysplastic features could be seen in erythroblasts (anisooxyphilosis, basophilic stippling) and in leucocytes (nuclear clumping, Döhle bodies and abnormal granulation). The leucocyte alkaline phosphatase score was 170 (N20-40). Standard biochemical analyses showed an increase of vitamin B12 level (3000 pg/mL), creatinine of 144 µmol/L, serum alkaline phosphatase of 8.2 µkat/L and a GGT level of 1.31 µkat/L. Serum protein electrophoresis showed a slight polyclonal gammopathy. A cut-off value of 0.5% was positive for E. Coli, although the patient was asymptomatic. Abdominal ultrasonography revealed moderate hepatosplenomegaly, and a stone in the bladder. Bone marrow aspiration and biopsy were extremely hypercellular, with a myeolid erythrodi ratio of 8:6:7. In chromosomes 9 and 22 which appeared normal in standard cytogenetic studies. Some micromegakaryocytes could be seen. Pears reaction showed 52% sideroblasts with 3% ring forms and normal iron stores. No significant fibrosis was observed. Absence of peripheral myeloid progenitors revealed a spontaneous growth of erythroid precursors (BFU-E). Five months later now the patient remains stable, with no further increase of leucocytes or anaemia. He has intermittent asymptomatic bacteriuria. Concluding, the characteristics of this case reinforce the idea suggested by other authors that CML may be related to a myeloidplastic syndrome. The spontaneous growth of BFU-E suggests an underlying myeloproliferative disorder, although there is no evidence of preexisting polycythaemia vera or myelofibrosis.

PO-0160 A Philadelphia negative chronic myelogenous leukaemia with a masked translocation t(2;22) revealed by whole chromo-

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formation. As demonstrated here, the translocation between chromosomes 2 and 22 was discovered only on metaphases using the whole chromosome painting 22 probe. The case reported here should make cytogeneticists aware that it is imperative to have a full understanding of both the capabilities and the limitations of bcr/abl translocation probes and that FISH interphase signals should be confirmed on metaphase spreads at the time of diagnosis. Uncomplete understanding of both the capabilities and the limitations of bcr/abl translocation probes and that FISH interphase signals should be confirmed on metaphase spreads at the time of diagnosis. Uncomplete understanding of both the capabilities and the limitations of bcr/abl translocation probes and that FISH interphase signals should be confirmed on metaphase spreads at the time of diagnosis. Uncomplete understanding of both the capabilities and the limitations of bcr/abl translocation probes and that FISH interphase signals should be confirmed on metaphase spreads at the time of diagnosis.
niosis was 700 -1000/L (range 150-885). Cytogenetic analysis showed a normal 46XX karyotype. All patients showed an unbalanced pattern of clonality of peripheral blood granulocytes. Control tissue from the same patients (T-cells) excluded a constitutive skewed X chromosome inactivation pattern (XCI) or age related acquired skewing of XCI. In two patients clonality was confirmed in the CD34+ cell fraction from bone marrow. In one patient EEC taken right after admission by HUMARA showed a clonal pattern. Clonal hemopoiesis is easily recognised by HUMARA provided that constitutive or age related skewed XCI is excluded. Recently some authors identified monochlonal myelopoiesis in only a minority of ET patients (21.7%) but clonality was associated with a high risk of thrombotic complications. We showed that in female patients with splenomegaly and thrombocytosis and a definite marker for myeloproliferative disorder, clonal hemopoiesis is predominant. Clonality in these patients does not appear to be lineage restricted as documented by CD34+ cells involvement. The increased risk of thrombosis in ET patients with a clonal disorder is not yet understood but if confirmed it might help to allow tailored treatment for both thrombosis prevention and progression to acute myelogenous leukaemia.

PO-0164 The breakpoint cluster region junction site in PH+ CML. A single-centre experience of 100 cases

Department of Clinical Haematology, Institut Català d’Oncologia and *Institut de Recerca Oncològica, Barcelona, Spain

Introduction. The breakpoint cluster region (BCR) on chromosome 22 arises within the major breakpoint cluster region (M-BCR) and generates two different mRNAs designated as b2a2 and b3a2 that encode the protein 210 with elevated tyrosine kinase activity. These two types of junction sites have been correlated with clinical features, chronic phase duration, platelet count and specific responses to therapy, with controversial results. Methods. One hundred consecutive CML patients from 1993 to 1998 were studied at diagnosis. Using the reverse transcription polymerase chain reaction (RT-PCR) we determined the type of chimeric mRNA rearrangement and analysed the relationship of each mRNA group of patients with clinical characteristics (age, Hb level, platelet and leucocyte counts, basophil and blast cell percentage, splenomegaly, bone marrow fibrosis, megakaryocyte (MK) number and morphology, and Sokal index). Statistical methods were used to evaluate the clinical differences in both BCR/ABL transcript types. Results. Thirty one cases were b2a2, 51 were b3a2, 38 had both transcripts, and in 10 cases the RT-PCR was not detectable. The analysis of the b2 patients with b2a2 or b3a2 showed statistical differences in the mean Hb, leucocyte number, basophil and blast percentage, all these parameters except the Hb level being higher in the b3a2 type. Bone marrow characteristics (fibrosis, MK number or morphology) were not related to a definite site of rupture. Sokal risk group was related with the transcript (p of association 0.0047) for trend (0.032); high Sokal index was less frequent in b3a2 (OR-0.15, CI 95%=0.03, 0.73). Conclusions. The type of breakpoint site did not define different CML groups regarding haematological data or bone marrow characteristics at diagnosis in this large series. Sokal risk group and platelet risk were lower in the b3a2 group. Our data are not in concordance with previous reports and reinforce the controversy generated by other studies.

PO-0165 Relevance of histopathological and haematological criteria for the differential diagnosis of thrombocytopenic myeloproliferative disorders

Kvapszicka HM, Thiele J, Beelen D, Schaefer U W, Fischer R
Institute of Pathology, University of Cologne and *Department of BM T, University Hospital of Essen, Germany

Bone marrow morphology in CML is characterised by distinctive changes in megakaryopoiesis which, in particular include a remarkable increase in atypical micro-megakaryocytes. Megakaryocyte-rich subtypes of CML are generally associated with a worse prognosis. However, there is little knowledge concerning the significance of those changes. We performed an retrospective study on 131 patients with 1st chronic phase of Ph+ CML was performed using sequential trephine biopsy specimens to assess changes in megakaryopoiesis and thrombocyte precursors before and after allogeneic BMT including morphometric and immunohistochemical methods. Patients received marrow grafts from HLA-identical family or other donors following standard procedures at a referral center. Reduced frequency of megakaryocytes following marrow-allogeneic therapy and BMT was correlated with a delayed hematopoietic reconstitution. Successful engraftment was characterised by megakaryocyte size increasing to normal. Reappearance of atypical micro-megakaryocytes in the post-transplant period was a definite sign of haematopoietic recovery and 2 alterations of megakaryopoiesis in the post-transplant period facilitate early recognition of relapse.

PO-0167 Real-time quantitative RT-PCR monitoring of interferon-treated or allografted chronic myelogenous leukaemia patients

Bories D, Dumont V, Belhadj K, Bernaudin F, Raffi H, Kuemtz M, Cordonnier C, Tulliez M
Haematologie Biologique et Clinique, Hôpital Henri Mondor, Creteil, France

Bcr-abl mRNA is found in the majority of CML patients allowing minimal residual disease detection by RT-PCR. A non quantitative RT-PCR is difficult more, in this context, the relevance of erythroid precursors has not been completely defined A retrospective study based on 131 patients with 1st chronic phase of Ph+ CML was performed using sequential trephine biopsy specimens to assess changes in megakaryopoiesis and erythroid precursors before and after allogeneic BMT including morphometric and immunohistochemical methods. Patients received marrow grafts from HLA-identical family or other donors following standard procedures at a referral center. Reduced frequency of megakaryocytes following marrow-allogeneic therapy and BMT was correlated with a delayed hematopoietic reconstitution. Successful engraftment was characterised by megakaryocyte size increasing to normal. Reappearance of atypical micro-megakaryocytes in the post-transplant period was a definite sign of haematopoietic recovery and 2 alterations of megakaryopoiesis in the post-transplant period facilitate early recognition of relapse.
A multicentre, immunohistochemical and morphometric study was performed on diagnostic pretreatment bone marrow biopsies in 614 adult patients with Ph+ CML to compare histological features with clinical findings. For identification of megakaryopoesis we used the monoclonal antibody CD61. Labeling of erythroid precursors was carried out by a monoclonal antibody directed against glycophorin C. To selectively stain macrophages and their activated subset we applied CD68 and GSA-I lectin. Density of reticulin and collagen fibers was measured following Gomori’s silver impregnation method. In about 26% of patients early (reticulin) to advanced (collagen) fibrosis was detectable. Significant correlations were calculated between the extent of myelofibrosis with splenomegaly, anaemia and numbers of erythroblasts and myeloblasts in the peripheral blood count. These features were assumed to indicate more advanced stages of disease with ensuing transition into myeloedematosis and unfavorable prognosis. Significant relationships were revealed between the amount of CD61+ megakaryocytes and their precursor fraction with the degree of fibrosis. This result extends previous experimental findings regarding the impact of immature elements of this cell lineage on the generation of myelofibrosis. The significant association of erythroid precursors with the number of mature (resident) macrophages and their activated GSA-I subset sheds some light on their functional involvement in iron turnover and haemoglobin synthesis. A histological classification of predominant bone marrow features is introduced. This synthesis staging system (Cologne Classification) is not only limited to certain sets of laboratory data, but also with different survival patterns.

**PO-0169 Effect of bone marrow fibrosis on outcome of allogeneic BMT in chronic phase Ph+ CML**

Thiele J, Kvasnicka HM, Beelen D, Schaefer UW, Fischer R
Institute of Pathology, University of Cologne and Department of BMT, University Hospital of Essen, Germany

Bone marrow fibrosis (MF) in CML is generally regarded as an independent indicator of poor prognosis. However, the relevance of this risk factor for hematopoietic recovery after BMT is still controversial. Furthermore, dynamics of MF following ablative therapy and BMT have not been completely defined. In a retrospective study based on 160 patients (median age at BMT 36.5 yrs, range 15-57 yrs.) with 1st chronic phase Ph+ CML we investigated the progression and regression of MF in sequential trephine biopsies before and after allogeneic BMT using quantitative morphometric methods. Patients received marrow grafts from HLA-identical family or other donors at a referral transplant center following standard procedures. Pretreatment degree of MF was correlated with engraftment parameters and incidence and evolution of MF in the post-transplant period. Morphological analysis disclosed no significantly different values regarding pre- and posttransplant MF. However, a marked MF before BMT was associated with a initial decrease in fiber density following BMT (mean time 58 days), which was progressively retrieved during the post-transplant period. Regarding engraftment parameters a relevant delay could be observed in the time to reach platelet transfusion independence and granulocyte recovery. Our results suggest that hematopoetic reconstitution depends not only on various clinical and immunological factors, but also on the degree of MF preceding BMT. Moreover, there is no persistent regression of MF in the post-transplant period.

**PO-0170 Venous thromboembolic disease in hospital: correct prophylaxis?**

Cuevas B, Coloma R, González L, Ruiz ML, Cuevas MV, Polo A, Laserra J
Haematology and Pharmacy Services, San Millán Hosp, Logroño; *Divino Valles Hospital, Burgos, Spain

Introduction. In spite of the use of prophylaxis with low molecular weight heparin many patients develop symptoms of venous thromboembolic disease. Design and Methods. The analysis of risk factors in 132 patients (72 medical and 60 surgical) who developed venous thromboembolic disease in hospital enabled us to classify the patients into three risk groups (low, medium and high). We made four groups according to the use or not of prophylaxis (fixed doses of low molecular weight heparin) and, if that was adequate according to the risk group of the patient. Results. The results are shown below:

<table>
<thead>
<tr>
<th>Medical patients</th>
<th>Adequate</th>
<th>Not adequate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis</td>
<td>15.3</td>
<td>15.2</td>
</tr>
<tr>
<td>Without prophylaxis</td>
<td>36.1</td>
<td>33.4</td>
</tr>
<tr>
<td>Total</td>
<td>51.4</td>
<td>48.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surgical patients</th>
<th>Adequate</th>
<th>Not adequate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis</td>
<td>8.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Without prophylaxis</td>
<td>18.4</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>83.4</td>
<td>16.6</td>
</tr>
</tbody>
</table>

Conclusions. The use of prophylaxis should be extended both in medical and surgical patients and be adjusted according to the risk group.

**PO-0171 Monocyte tissue factor activity in controls and patients with breast and colorectal cancer**

Lwaleed BA, Chisholm M, Francis JL
Department of Haematology, Southampton University Hospitals, Southampton, UK and *Haemostasis and Thrombosis Research Unit, Florida Hospital, Altamonte Springs, FL, USA

Objective. In various disease states including cancer, peripheral blood monocytes express increased levels of procoagulant tissue factor (mTF). Such an increase may be clinically important in thromboembolic complications observed in patients with cancer or inflammatory conditions. In the present study we aim 1) to assess mTF activity in controls and patients with breast and colorectal malignancy and 2) to examine whether mTF levels reflect the presence and/or progression of the tumour. Design and Methods. mTF activity was measured using an in-house two stage kinetic chromogenic assay. Its levels were assessed in controls (normal subjects n=60), patients undergoing haemip repair or cholecystectomy (n=60), in patients with benign and malignant disease of the breast (n=63) and of the large bowel (n=62). This was performed under fresh (resting) conditions and after incubation for 6 hours without (unstimulated) and with (stimulated) E. coli endotoxin (lipopolysaccharide; LPS). Results: The malignant groups showed higher mTF levels than each of the three controls for resting (p<0.05 breast, p<0.01 colorectal) unstimulated (p<0.05 colorectal), un-stimulated (p<0.05 colorectal) and stimulated cells (p<0.05 breast, p<0.01 colorectal). There was no significant difference between malignant and benign inflammatory groups for each organ. mTF levels showed an increase corresponding to that of histological tumour progression and were higher in non-surviving patients. Conclusions. mTF levels were significantly raised in malignant and inflammatory disease compared to controls and patients with non-inflamatory conditions. LPS-stimulated monocytes TF activity gave better discrimination between the groups and may be of value in identifying high risk individuals. There was an association between increase mTF activity corresponding to an increased in tumour grade or stage. mTF levels were also higher in patients who subsequently died.
PO-0172 Inherited hypercoagulable states in young patients with venous thrombosis
Haematology Department, Hospital Clinico Universitario, Zaragoza,
*Intensive Care Unit, Hospital San Jorge, Huesca, Spain

Activated protein C resistance (APCR), antithrombin III (AT III), protein C (PC) and protein S (PS) deficiencies are the major causes of inherited thrombophilia. However, the incidence in young populations and the clinical expression of these hypercoagulable states are very heterogeneous. Our objective was the study of the presence of APCR and clinical manifestations of these abnormalities in young patients with venous thrombosis (VT).

Methods and Results. We studied 92 young patients (age 18-50), 48 male and 44 female, diagnosed with VT. The laboratory investigation included functional AT III by chromogenic assay, anticardiolipin by ELISA, functional PC by clotting assay, functional PC by chromogenic assay, antigenic free and total PS by ELISA, plasminogen by chromogenic assay and APCR test by clotting assay. Results. Factors predisposing patients to thrombosis were identified in 47 of them: major surgery (24%), immobilisation (12%), oral contraceptives (6%) and malnourishment (5%). Of the 18,4790 had recurrent thrombosis and 8.6% reported a family history of VT. In 33.86% of the patients some inherited hypercoagulable state was identified. The distribution and main clinical manifestations are shown in the Table:

<table>
<thead>
<tr>
<th>Incidence</th>
<th>1st thromb.</th>
<th>1st thromb.</th>
<th>Recurrent thromb.</th>
<th>Risk factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>APCR</td>
<td>18/92</td>
<td>3/18</td>
<td>9/18</td>
<td>2/18</td>
</tr>
<tr>
<td>(19.56%)</td>
<td>(50%)</td>
<td>(50%)</td>
<td>(50%)</td>
<td>(50%)</td>
</tr>
<tr>
<td>AT III</td>
<td>7/17</td>
<td>5/17</td>
<td>1/7</td>
<td>1/7</td>
</tr>
<tr>
<td>(7.6%)</td>
<td>(71.4%)</td>
<td>(71.4%)</td>
<td>(71.4%)</td>
<td>(71.4%)</td>
</tr>
<tr>
<td>PS</td>
<td>6/10</td>
<td>4/6</td>
<td>1/6</td>
<td>3/6</td>
</tr>
<tr>
<td>(6.5%)</td>
<td>(66.6%)</td>
<td>(66.6%)</td>
<td>(66.6%)</td>
<td>(66.6%)</td>
</tr>
<tr>
<td>AT II</td>
<td>2/24</td>
<td>2/24</td>
<td>1/2</td>
<td>0/2</td>
</tr>
<tr>
<td>(2.17%)</td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
</tr>
</tbody>
</table>

Conclusions. Some inherited hypercoagulable state was confirmed in 33.86% of the patients. AT III deficiency, although less prevalent, was the most thrombotic condition, with severe clinical manifestations (recurrent and spontaneous thromboses, before 30 years old). APCR, although the most frequent condition, gives variable clinical manifestations, with less clinical expression.

PO-0173 Evaluation of pathogenetic prothrombotic factors underlying juvenile deep vein thrombosis in India
Mohanta S, Saxena R, Srivastava A, Choudhry VP*
*Dept. of Haematology; *Dept. of Surgery, All India Institute of Medical Sciences, New Delhi, India

Hereditary prothrombotic protein defects including resistance to activated protein C (APC-R) have emerged as important hereditary causes of juvenile deep vein thrombosis (DVT) in the western world. Since ethnic variations in these are known to exist and no Asian study is available, we evaluated the prevalence of these factors in Indian juvenile DVT patients. Fifty-six young patients (<45 yrs of age) with Doppler proven DVT and no underlying predisposing factors were the subjects of the study. Fifty age and sex matched healthy subjects served as controls. None of the subjects was on anticoagulant therapy. APCR was performed by modified APC test using factor deficient plasma. Activities of protein C, S, and antithrombin III (AT III) & plasminogen activator inhibitor (PAI) were estimated using kits (Diagnostica Stago, France). Lupus anticoagulant (LAC) and anticardiolipin antibody (ACA) were detected by Kaolin clotting time (KCT) and Elisa (ORGen-Tec, Diagnostika GmbH, Germany) respectively. APCR-R was found to be the commonest underlying defect (39.2%) followed by raised ACA (5.3%), deficiency of AT III 2.8%, presence of LAC (2.8%), and elevated PAI (2.8%). Protein C and S were normal in all cases. APCR-R co-existed with ACA in 3 cases, PAI in 2 cases and AT III in 1 case. Nine patients had associated pulmonary thromboembolism. Of these, 2 patients had only APCR-R defect and 5 patients had APCR-R with associated prothrombotic factor defects. This suggests that combined occurrence of prothrombotic factor defects predisposes to more severe thromboembolism than single factor defect. It is thus concluded that in Indian APCR-R is the most common prothrombotic defect underlying juvenile DVT, as seen in the west. Its thrombogenic potential is enhanced by co-existence of other hereditary/acquired defects.

PO-0174 INR and vitamin-K dependent factor levels in frozen plasma and plasma stored for up to 48 hours
Grau E, Tenias JM, Oloso MA, Ferrando I, Juan MT, Pastor E, Real E
Departments of Haematology and Preventive Medicine, Hospital Lluis Alcaniz, Xativa, Spain

Objective. His sometimes difficult to call out of the prothrombin time test on the same day as sample collection in patients receiving oral anticoagulants. The aim of this study was to determine International Normalised Ratio (INR) and vitamin-K dependent factor levels of frozen plasma and plasma stored for up to 48 hours. Design and Methods. The INR of 68 patients receiving acenocoumarol were determined fresh, on samples stored between -2 and +8°C for 24 and 48 hours, and on frozen samples (+40°C) using 3 thromboplastins (Behring Thromborel, ISI 1.07; Dade Thromboplastin ISI, ISI 1.30; Organon Teknika Simiplastin, ISI 1.27). In addition, factors II, VII, IX were determined in 34 of these patients in fresh and frozen samples and after incubation. Results. The following table shows the correlation coefficients of the INR with the different thromboplastins.

<table>
<thead>
<tr>
<th>Factor</th>
<th>24 hours</th>
<th>48 hours</th>
<th>Frozen plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>0.98</td>
<td>0.94</td>
<td>0.98</td>
</tr>
<tr>
<td>Thromborel</td>
<td>0.91</td>
<td>0.55</td>
<td>0.98</td>
</tr>
<tr>
<td>Simplastin</td>
<td>0.95</td>
<td>0.50</td>
<td>0.92</td>
</tr>
</tbody>
</table>

By 24 hours and frozen plasma the activity of vitamin-K dependent factors was slightly reduced (r=0.97 at 24h) and 0.94 with frozen plasma for factor II, r=0.92/0.96 for factor VI, r=0.83/0.98 for factor IX, and r=0.98/0.95 for factor X. By 48 hours however, significant reductions were noted (r=0.94 for factor II, r=0.88 for factor VI, r=0.79 for factor IX, and r=0.98 for factor X). Conclusions. The INR can be reliable determined in frozen plasma and in plasma stored at 2-8°C for up to 24 hours. In these conditions results are similar using different thromboplastins.

PO-0175 Redox integration and regulation of blood coagulation in vitro: evidence from peptide mimics of factor VIII
Bayele HK, Murdock PJ, Perry DJ, Pasi K
Haemophilia Centre and Haemostasis Unit, Royal Free Hospital & School of Medicine, London, UK

Factor VII acts as a cofactor in the activation of factor X by factor IX. Activated factor X, factor Xa, in turn activates prothrombin in a sequence that leads to fibrin clot formation at the site of vascular injury. Although the biochemistry of the cascade has been well studied, the molecular mechanism underlying the cofactor role of factor VIII is not understood. We set out to define the cofactor requirements of the factor IXa/factor X procoagulant complex by random peptide display, and isolated the active site sequence, CGPC, of thioredoxin (TRX) which was able to activate factor X in a factor Xa-dependent manner. Redox catalysts with homologous active site vicinal cysteines could also substitute factor VII cofactor function in factor X activation. These results suggest that kinetically-trapped factor X intermediates may be formed during activation, which may be rescued by thiol-disulphide interchange. Inhibition of this process by changes in redox balance resulted in derangements in blood clotting in vitro. The implication of these findings is that factor VIII probably encodes a redox-active site that may function as a specialised folding catalyst. These data suggest that blood clotting is probably influenced by redox mechanisms and a novel insight into the integration and control of coagulation factors and blood clotting.

PO-0176 Soluble vascular cell adhesion molecule-1 (sVCAM-1): is it a marker of microvascular complications in diabetes?
Aladle DA, Gawisch HE, Awd M
Mansoura Faculty of Medicine, Mansoura, Egypt

Accelerated atherosclerosis and microvascular disease are the major vascular complications of diabetes, and constitute the principal cause of morbidity and mortality in this ubiquitous disorder. Vascular cell adhesion molecule-1 (VCAM-1), is of particular interest, as its expression has been linked to the early phase of experimental hypercholesterolaemia-induced atherosclerosis. Although microalbinuria was a powerful predictor of future overt nephropathy in insulin-dependent diabetes mellitus (IDDM), prospective studies have demonstrated that microalbuminuria markedly increases the risk of death due to cardiovascular disease in NIDDM. Hence, the need for other earlier predictors for vascular dysfunction is mandatory. In view of accelerated vascular disease observed in diabetes, we evaluated the concentration of sVCAM-1 in 81 diabetic patients (type II) and studied its relation to the microalbuminuria, when compared to the normoalbuminuric diabetics (p<0.05). We also found that glycated Hb was positively cor-
Incidence, risk factors and management of recurrent venous thromboembolism in a Spanish population

El-Toukhy HM, Hassanein A, Wassef N, Zouiel M
Evstachevych Y, Lotockyi R, Indenko V
Kobilyanskaya V, Saltykova N, Kargin V, Papayan L, Blinov M

Objectives. The major concern in the management of venous thromboembolism (VTE) is the propagation of thrombus and rethrombosis. The incidence of recurrence varies with the duration of oral anticoagulant therapy in these patients are controversial. The aim of this study was to determine the incidence, timing and outcome of further thrombotic events after an initial episode of VTE. Design and Methods. This was designed as a retrospective analysis of all patients admitted to our Centre with an episode of deep vein thrombosis (DVT) and/or pulmonary embolism between 1986 and 1996. The patients included in the study had to be treated with unfractionated heparin or low molecular weight heparin, followed by at least three months of oral anticoagulants. Natural and acquired haemostasis inhibitors were assayed in patients aged less than 50. Results. A total of 290 patients with a first episode of VTE were included in the study. Of these patients 33 (11.9%) (95% CI 7.4-14.6%) had one or more recurrent VTE. The cumulative incidence of recurrent VTE after two, five and ten years was 7.68%, 10% and 12.4%, respectively. Each DVT was the most frequent localisation of rethrombosis. The incidence of idiopathic VTE was significantly higher in patients with recurrent VTE than in patients with a single VTE. Abnormalities of haemostasis were found in 54.5% (95% CI 37.6-71.4%) of the patients. Recurrent VTE and aged under 50. Three of 7 patients who stopped anticoagulant therapy after the second episode had a third event. Conclusions. In our population, patients with idiopathic DVT seem to have an increased risk of recurrent VTE. The second thrombotic episode occurs more frequently during the following two years after cessation of anticoagulant therapy. Our data do not provide evidence that a high risk for recurrence persists for many years. We strongly support the use of long-term anticoagulant therapy in patients with recurrent VTE.

Incidence, risk factors and management of recurrent venous thromboembolism in a Spanish population

PO-0178 Fasting total homocysteine blood level, an independent risk factor for vascular disease

Mahmoud MY, El-Toukhy HM, Hassanein A, Wassef N, Zouiel M
Drs. Solomon Fakheet Hospital, Jordan, Saudi Arabia

Objective. To evaluate fasting total homocysteine blood (F.t.Hcy.bl.) level as an independent risk factor in patients with acute myocardium infarction or recurrent CVA. Methods. F.t.Hcy.bl. level was measured, using high performance liquid chromatography separation an flourescence detection, in 156 patients with either AMI (n=96) or recent CVA (n=60). The results were compared with values obtained from a control group (n=150) matched for age, sex, body mass index, smoking habit and serum cholesterol with the patient group. Patients suffering from concomitant illnesses (e.g. diabetes mellitus and or taking drugs e.g. multivitamins, known to affect F.t. Hcy.bl. level were excluded. Results. Mean F.t.Hcy.bl.level in patients with AMI (11.9 umol/L 95% CI 11.7-12.1) and patients with recent CVA (12.2 umol/L 95% CI 11.7-12.6) is significantly higher than in the control group (9.8 umol/L 95% CI 9.3-10.6). Also the mean value of the combined patient group (12.0 umol/L 95% CI 11.3-12.6) is significantly higher than that in the control group. Moreover, 8.7% (n=13/150) of controls have F.t.Hcy.bl.level ≥10.3 umol/L (upper normal) as compared to 84.6% (n=132/156) of patients. Conclusions. Our results support the hypothesis that raised F.t.Hcy.bl.level is an independent risk factor for vascular disease.

Application of cytapheresis and plasmapheresis for the prevention of intravascular coagulation in leukaemia, haemolytic anaemia and thrombocytopenia

Kurban M, Evtachchevych Y, Lobotsky R, Indenko V
Research Institute of Haematology, Lviv, Ukraine

Patients with chemotherapy-resistant leukaemia (L), haemolytic anaemia (HA) and thrombocytopenia (TR) develop generalised cell destruction causing thromboplastinemia and intravascular coagulation (IC). We studied application of cytapheresis (CP) and plasmapheresis (PP) in these cases as a way of possibly preventing IC. Refractory cases to chemotherapy, IC features, TR, haemolysis, anaemia, cytosis were among the indications for this treatment option. Twelve L patients underwent CP on fractionators. Treatment course included 3-5 operations (OP). Removal of leucocytes and plasma pool reached 0.3-0.51 pro OP with a cell titer 280-300109/L. Three TP patients and 5 HA patients underwent PP on fractionators. Treatment course included 3-6 OP with the plasma removal of 0.8-1.0 pro OP. Plasmapheresis and CP restored susceptibility to chemotherapy, decreased cytosis, bilirubinemia, normalised haemocoaagulation, increased haemoglobin and platelet count in all cases. Lasting (4-7 months) recoveries were reported in TP and HA patients. When necessary, repeated courses were applied. Six TP patients and 9 HA patients underwent combined PP treatment with CP on blood bags. As a result, the obligatory number of OP for the achievement of treatment efficacy decreased (by 1-2) and the duration of recoveries increased (extra 1-3 months).

Creation of the technology of purification of coagulative and fibrinolytic proteins

Maevskyvy T, Danysh T
Research Institute of Blood Pathology and Transfusional Medicine, Lviv, Ukraine

Over the last years our laboratory has been engaged in the important task of obtaining protein preparations for subsequent use in both diagnostic and medical settings. For this purpose affinity sorbents on a basis of modified silica (silochrome) with use of various ligands (antibiotics-polypeptides, amino acids residues, and others) were synthesized. As a result of numerous experiments, in the beginning analytical, and then preparative, we managed to purify the following proteins: thrombin (solvent-bacitracin- silochrome, initial raw material- III Kohn fraction from human blood plasma), plasminogen (L-Lysine-silochrome; II+III or III Kohn fraction). Urokinase from urine of humans with use of granimycin-silochrome was obtained. The activation of plasminogen with the help of urokinase or streptokinase with subsequent chromatography purification on fractionators L-Lys-silochrome of plasmin, plasin-streptokinase and plasminogen-streptokinase complexes were performed. These preparations become a basis for production of a new generation of fibrinolytic-acylated plasmin, plasmin- and plasminogen-streptokinase complexes. Using methods of fractionation, we have produced pure preparations of thrombolisin, fibrinogen and aprotinin. For an estimation of activity of fibrinolytic preparations synthesised in our laboratory, chromogenic protein substrate azofibrin (human fibrin labeled by pedia-benzensulfonylic acid) is used. The preparations have enabled us to create a lot of diagnostic kits for assay of coagulation and fibrinolytic activity in blood plasma. They may also become the basis for new medicinals.

Anti-β2-glycoprotein-1 and anti-prothrombin antibodies in sickle cell patients with high antiphospholipid levels

Siri B, *Ghazouani E, Chabik C, Bayoudh F, Gritli N, Machghoul S
Laboratory of Haematology-Immunology and Biochemistry, Military Hospital of Tunis, Tunisia; *Paediatrics Clinic, Military Hospital of Tunis, Tunisia

Background and Purpose. In a previous report (1) we showed that high levels of antiphospholipid antibodies were commonly associated with sickle cell disease. The purpose of our present study was to quantify the anti-β2-glycoprotein I (anti-β2, GPI) and anti-prothrombin (anti-PT) antibodies in sickle cell patients with high antiphospholipid levels, and to evaluate the relationship between these two antigentic targets and the major sickle cell disease related complications. Study population and Methods. Thirty seven sickle cell patients with high levels of an antiphospholipid antibodies and 22 healthy individuals were studied. IgG and IgM anti-β2, GPI and anti-PT were measured using an Eisa method. Results. Because of their lack of standardization, anti-β2, GFI values were comparable to those in controls, while the mean anti-PT antibodies were increased in 5 and 2 cases, respectively. Conclusions. Despite their high frequency in sickle cell patients, neither anti-β2, GPI nor anti-PT antibodies were significantly associated with sickle cell disease related complications. 1. Nsiri et al. Hematol Cell Ther 1998; 40:107-12.

Genetic determinants of deep-vein thrombosis and its complications in North-Western Russia

Kapustina S, Kobilyanskaya V, Saltykova N, Kargin V, Papayan L, Blinov M
Research Institute of Haematology and Transfusional medicine, St Petersburg, Russia

Factor V (FV) Leiden, prothrombin (Ptr) G20210-A and methylenetetrahydrofolate reductase (MTHFR) C677-T mutations have been recently identified and found to be associated with thrombotic complications of thrombophilia state. The aim of the study was to assess the risk of these genetic defects for the development of deep vein thrombosis (DVT) and its complications in the population of North-Western Russia. Seventy-six DVT patients were genotyped for FV Leiden, P2-G20210-A and MTHFR C677-T mutations.
PO-0183 Effects of administration of rFVIIa in the management of severe bleeding in cardiac surgery

Pathology Department, Riyadh Armed Forces Hospital, Department of Pathology, Cardiac Anaesthesia and Cardiac Surgery, Riyadh Armed Forces Hospital & Cardiac Center, & Novo Nordisk Company, Saudi Arabia

Recombinant activated factor VII (rFVIIa) is being used increasingly to secure haemostasis in those difficult clinical situations in which conventional treatment with blood products has failed to achieve the desired effect. Patients undergoing open heart surgery for valvular heart disease (replacement or repair) or other associated complex procedures can be prone to bleeding due to a variety of causes and in some of these cases haemostasis may be difficult to secure. It is in these clinical situations that we are evaluating the role of rFVIIa in an open pilot study. To date we have treated 5 such patients (1 boy 2 and 1/2 years and 4 adults). Amongst the four adults, two were male and 2 females with a mean age of 59 years (46-73).

The surgical procedures included arterial switch closure, closure of atrial septal defect and left Blalock Tausig shunt and Le Compte manoeuvre (child). Ban-Tal Lion (adult). We will report our results and our experience, since we have not found other published studies on this issue. The preliminary results indicate that the use of rFVIIa in these cases is effective and well-tolerated for serious bleeding episodes pre- and post-operatively in cardiac surgery.

PO-0184 Activation of coagulation in cancer patients correlated with activation of peripheral blood monocytes

Nani Z, van Oene R, van Pampus ECM, Hamulyak K
Department of Haematology, University Hospital Maastricht, the Netherlands

Introduction. Peripheral blood monocytes can be stimulated to express procoagulant activity. This is supposed to play a role in hypercoagulability in cancer. Activation of coagulation in relation to the expression of procoagulant monocyte membrane proteins was studied in cancer patients and controls. Materials and Methods. Twenty-five patients with disseminated cancer (breast 11, digestive tract 9, other 5) and 24 sex matched healthy volunteers were studied. All coagulation parameters were measured in citrated platelet poor plasma. Monocytes on CD14 positive monocytes were measured in whole EDTA blood using a FACSCalibur flow cytometer and CELLQuest software (Becton Dickinson). The negative and positive delineator was positioned by determining 5% background staining on the isotype control fluorescent resonance. Statistical analysis was done using a one-way ANOVA, an ANOVA with age as co-variant, an independent samples T-test and Pearson correlation coefficients. Results. In the patient group compared to the controls there was a significant increase of fibrinogen (3.9 vs. 3.1 µg/L; p=0.003), d-DIMERS (3.3 VS. 0.3 µg/mL; p=0.005) and WFPII (22% vs. 94%; p=0.005) and a significant decrease of antithrombin (86 vs. 99%; p<0.01). A trend to increased levels was found for TFP, PAI and t-PA complex.

We suppose that FV Leiden is the strongest risk factor for DVT and associated PTS. In conclusion, rFVII a represents an important new adjunct for the management of bleeding in cancer patients undergoing open heart surgery for valvular heart disease (replacement or repair) or other associated complex procedures can be prone to bleeding due to a variety of causes and in some of these cases haemostasis may be difficult to secure. It is in these clinical situations that we are evaluating the role of rFVIIa in an open pilot study. To date we have treated 5 such patients (1 boy 2 and 1/2 years and 4 adults). Amongst the four adults, two were male and 2 females with a mean age of 59 years (46-73).

The surgical procedures included arterial switch closure, closure of atrial septal defect and left Blalock Tausig shunt and Le Compte manoeuvre (child). Ban-Tal Lion (adult). We will report our results and our experience, since we have not found other published studies on this issue. The preliminary results indicate that the use of rFVIIa in these cases is effective and well-tolerated for serious bleeding episodes pre- and post-operatively in cardiac surgery.

PO-0185 Coagulation inhibitors and fibrinolytic potential evaluation in Behçet’s disease patients

Nisit B, Othmani S, Chazouali E, Bahri M, Aziz Ch, Mostedek F, Doghri A, Mahjoubi W, Otri N, Machtouph S, Bahri M
Laboratory of Haematology-Immunology and Biochemistry, Internal Medicine Clinic, Military Hospital of Tunis, Tunisia

Although arterial and venous thromboses are a major complication of Behçet disease, the pathogenic mechanisms of the thrombotic event is still poorly understood. The aim of the present study was to evaluate the coagulation inhibitors and the fibrinolytic potential in patients with Behçet’s disease. Functional activities of protein C, protein S and antithrombin III were measured. The main finding was that there was a significant difference between antithrombin and the expression of aPC (r=0.49; p=0.015). Conclusions. Activation of coagulation in cancer patients is correlated with increased expression of aPC, CD16 and uPA-R on CD14 positive monocytes. In contrast to other reports we found no difference in plasma levels of factor VII and basal monocyte membrane tissue factor expression factor.
or with cigarette smoking. uTf levels were not significantly influenced by storage of urine samples prior to assay. Patients with malignant (p<0.001) and inflammatory (p<0.001) disease had significantly higher uTf levels than normal controls or subjects with benign non-inflammatory conditions. Conclusions. The new uTf method may have clinical applications in investigating haemarthrosis abnormalities in patients with malignant and inflammatory disease. Further study on this assay is cost effective, easy to perform and can be adapted for use in clinical laboratories.

**PO-0187 Preparation of monoclonal antibodies specific for D-dimer**

Kogan A, Berezinikova A, Katurka A, Bulargina T

Dept. Biochemistry, Biological faculty, Moscow State University, Russia

D-dimer is a main final product of fibrin degradation. It is believed to be a reliable marker of many thrombotic diseases such as disseminated intravascular coagulation of different etiology, deep vein thrombosis, pulmonary embolism, etc. In our work, fibrinogen was purified from human plasma, clotted with thrombin, and lyzed using streptokinase. The resulting D-dimer was purified by affinity chromatography on Protein A-Sepharose and gel filtration on Sephadex G-200. To raise antibodies against the D-dimer, male BALB/c mice were immunised using a usual protocol. Then mice were sacrificed, and B lymphocytes were isolated and fused with SP-2 myeloma cells. Resulting hybrids were screened on D-dimer, and positive ones also tested on D-monomer and fibrinogen. From about 1000 selected monoclones, 70% produced antibodies that also reacted with D-monomer and fibrinogen. About 20% of clones produced antibodies against D-dimer and D-monomer but not against fibrinogen, but only one of them did not stain fibrinogen in Western blotting. 10% of clones produced antibodies that react with D-dimer and fibrinogen and did not react with D-monomer. Eleven clones produced antibodies against D-dimer only when tested in ELISA, but only one of them did not stain D-monomer and fibrinogen in Western blotting. From all antibodies tested in blotting, only one stained D-dimer (both α- and γ-chains) subjected to the reduction of disulfide bonds by mercaptoethanol.

**PO-0188 The PFA-100® may not be suitable for monitoring the therapeutic efficiency of von Willebrand concentrate in type III von Willebrand disease**

Krogan A, Berezinskova A, Katurka A, Bulargina T

Dept. Biochemistry, Biological faculty, Moscow State University, Russia

We describe the case of a type III von Willebrand patient who was admitted to the hospital with severe deformity and functional deficit of the left knee joint due to recurrent haemarthrosis. Orthopaedic intervention was necessary. To prevent bleeding episodes, von Willebrand factor (vWF) replacement therapy was given during surgery. APTT, plasma FVIII activity (FVIII c), vWF antigen (vWF Ag) and vWF ristocetin cofactor (vWF Rco) and FVIIIc levels. These observations suggest that intraplatelet monitoring of disulfide bonds by mercaptoethanol.

**PO-0189 Plasminogen activator inhibitor-1 4G/5G polymorphism in thrombotic patients with and without FV 1691 G-A**

Akar E,* Akar E,* Avcu F,* Spahi T,*° Yalcin A,° Cin S,*°

*Departments of Pediatric Molecular Pathology and Neurology, University of Ankara; †Simli Ulus Children’s Hospital, Ankara, Turkey

Inherited gene defects related to the coagulation system have been reported as risk factors for stroke. These gene defects are FV 1691 G-A gene causing APC resistance; the PT 20210 G-A which is associated with increased levels of thromboplastin activity and methylenetetrahydrofolate reductase (MTHFR) 677 C-T causing increased levels of homocysteine. We carried out a case-control study including 28 patients with cerebral infarct all below the age of 18 (10 months - 18 years). Of the 28 patients, seven were heterozygotes for FV 1691 mutation (25.2%). Five of the patients carried the PT 20210 A mutation (17.8%). Two of the patients carried both mutations (7.1%). When compared to controls the difference was significant for both mutations (p: 0.007; p: 0.04 respectively). The frequency of the risk allele “T” of MTHFR 667 was found to be 0.23 which was not significantly different from that in controls (0.23) (p: 0.3). Of the 28 patients, 12 (42.8%) had mutations (FV 1691 G-A and PT 20210 G-A) either alone or together. From our data, it is apparent that FV 1691 G-A and PT 20210 G-A are independently associated with the risk of cerebral infarct. Risk assessment of double prothrombotic gene alterations did not reveal a synergy between these mutations. In conclusion, we found a strong correlation between cerebral infarcts and the presence of FV 1691 A and PT 20210A mutations.

**PO-0190 Effect of methylenetetrahydrofolate reductase 677 C-T and 1298 A-C on factor V 1691 G-A mutation in Turkish deep vein thrombosis patients**

Akar E,* Akar E,* Avcu F,* Yalcin A,° Cin S,*°

*Ankara University, Dept. of Pediatric Molecular Genetics; G‘ilahane Military Faculty of Medicine, Hematology Department, Ankara, Turkey

Possible interaction of two common mutations in MTHFR 677 C-T, 1298 A-C and FV 1691 G-A mutation, was studied in Turkish patients with thrombosis and compared to that in normal controls. The case-control study included 68 patients with the diagnosis of deep vein thrombosis and 66 controls consecutively selected among subjects without personal or familial history of atherothrombosis. Patients with DVT were selected if Doppler ultrasonography was positive. Only the comparison of FV 1691 G-A mutation was statistically significantly different between the control (6.06%) and DVT (23.5%) groups. Risk assessment of double prothrombotic gene alterations revealed only FV 1691 G-A mutation as an independent risk factor for thrombosis (OR 5.4 [2.1-12.7]) but our data suggested that MTHFR 677 T has an effect on its own (OR 1.97 [0.6-5.7]), but it may have synergy with FV 1691 G-A [OR 8.12 [2.0-35.3]]. However, MTHFR 1298 C does not have any effect [OR 1.2 [0.46-3.5]]. Further, being heterozygote at two different loci or homozygote at least in a locus for 677 T and 1298 C revealed a significant thrombotic risk increase [OR 9 and 24 (1.3-59.3 and 2.3-240.3)] between these two groups in our population.
Cerebral venous thrombosis (CVT) is an infrequent thrombotic event with a variable of neurological symptoms. Headaches, seizures and paroxysms sometimes developing over a period of days to weeks characterise this serious thrombotic event. Oral contraceptive (OC), pregnancy and puerperium antithrombotic syndrome and deficiencies of the natural anticoagulants protein C and protein S have been implicated in the pathogenesis of CVT. Factor V Leiden mutation was recently described as a causative factor for CVT. Recently, a mutation in the 20210 A allele of the prothrombin gene was discovered. Heterozygosity for this mutation increases the risk of venous thromboembolism 3-5 fold. The association with CVT, which is a major thrombotic event, has not been fully investigated. Here we report the cases of 4 individuals among 14 consecutive patients with CVT diagnosed between 1988–1997 in Rambam Medical Center; three out of four patients were females and one male. All were found to be heterozygous for the prothrombin 20210 A mutation. Two out of the three women were on OC just before the cerebral thrombotic event. One of them was homozygous for the thrombophilic methylene-tetra-hydrofolate reductase mutation with a high plasma homocysteine level and the other had acute promyelocytic leukaemia (APL) that was treated with ATRA. The male patient (fourth case) was heterozygous for factor V Leiden mutation. Homozygous MTHFR gene mutation especially with mild hyperhomocysteinemia is associated with a 2-4 fold increase in venous thrombus. Likewise, heterozygosity for prothrombin 20210 A mutation is associated with a 3-5 fold increase in VTE. Conclusions. 1. To our knowledge this is the only report in literature describing the association of MTHFR and hyperhomocysteinemia with CVT. 2. In patients presenting with CVT an effort should be made to detect possible thrombophilic patterns (family history of thrombosis and acquired risk factors—mostly OC usage) including the recently discovered prothrombin gene mutation, as it may affect the duration of anticoagulation treatment.

PO-0193 Hyperhomocysteinemia is a common finding in patients with antithrombophilic syndrome
Brenner B,* Avivi I
Thrombosis and Haemostasis Unit, Department of Hematology, Rambam Medical Center, Haifa, Israel

The pathogenesis of thrombosis in antithrombophilic syndrome, a common acquired thrombophilic disorder, has not yet been fully elucidated. Hyperhomocysteinemia is a common thrombotic risk factor for venous and arterial thrombosis and has recently been associated with recurrence of deep vein thrombosis. Prevalence of hyperhomocysteinemia has recently been described to be higher in patients with systemic lupus erythematosus. We have evaluated the prevalence of hyperhomocysteinemia in 53 patients with antithrombophilic syndrome who presented with recurrent abortions and/or thromboembolism. Plasma homocysteine levels determined by HPLC, were higher than 15 µmol/L in 18 of the 53 patients (34%) (range 15-67 µmol/L). Levels higher than 10 µmol/L but lower than 15 were found in another 16 patients (30%). Hyperhomocysteinemia is common in antithrombophilic syndrome and may contribute to a thrombotic tendency in these patients. The pathogenetic mechanisms leading to hyperhomocysteinemia in patients with antithrombophilic syndrome remain to be determined.

PO-0194 Factor V Leiden, factor II mutation and factor XII deficiency in women with complicated pregnancy

van Oostveen JW,* van Leerdam ME,* Bockx-Maat VMJ,* Tol CAM,* Huijgens PC,* de Vries JJP,*
*Department of Haematology and &Obstetrics, University Hospital Vrije University, Amsterdam, The Netherlands

Background and Objective. The cause of uteroplacental insufficiency has been mainly attributed to fetomaternal maladaptation. However, early and severe forms seem to be related to thrombotic risk factors such as protein S deficiency, factor V Leiden and hyperhomocysteinemia (HHC). Recently, factor II mutation and factor XI deficiency were also found to induce thrombotic risk. We evaluated the occurrence of factor II (20210 A) mutation and factor XII deficiency in women ten weeks after pregnancy complicated by severe and/or early placental insufficiency. Patients and methods. In this study 83 women were included (median age 32 years, range 23-44). The median duration of gestation was 212 days (range 112-287), median birth weight of their neonates was 1270 grams (range 117-3734). Their

pregnancies were complicated by pre-eclampsia (n=22), HELLP syndrome (n=26), small-for-gestational age neonates (n=10), abruptio placentae (n=5), intrauterine fetal death (n=10) or a combination of these items (n=10). Results. In 33 women abnormalities were found: 4 factor II mutation, 7 factor XII deficiency, 17 activated protein C resistance, 13 factor V Leiden, 5 protein S deficiency and 8 HHC. Of these six women had more than one abnormality: three APC Resistance with factor V Leiden and factor II mutation or protein S deficiency or HHC, two factor XII deficiency with HHC and one APC resistance with protein S deficiency. No abnormal antithrombin III or protein C levels were found. Conclusions. In 40% of this population with severe uteroplacental insufficiency a coagulation abnormality was found. Factor II mutation occurred in a low number of women and once in combination with factor V Leiden, indicating that simultaneous presence of both factor V Leiden and factor II mutation does not increase thrombotic risk factor in this number of women studied. A relative high occurrence of Factor XII deficiency was found and needs to be investigated further in this patient group. A study with randomised treatment during pregnancy with or without low molecular weight heparin is needed to determine whether treatment of these coagulation abnormalities benefits fetal and maternal outcome.

PO-0195 Variations in coagulation factors in women: effects of age, ethnicity, menstrual cycle and combined oral contraceptive
Kadir RA,* Economidou DL, Sabin CA, O’wens D, Lee CA
The Royal Free Hospital, Hampstead, London, UK

Objective. To assess variations of coagulation factors in women. Design and Methods. One hundred and twenty-three women were included in a cross-sectional study to assess the effect of age, ethnic origin, blood group and menstrual cycle on activated partial thromboplastin time, thromboplastin time and plasma levels of Factor VIII clotting assay, von Willebrand factor antigen, von Willebrand factor activity and factor XI. The effect of menstrual cycle was further assessed in a longitudinal study including 39 Caucasian women, 20 of whom were using combined oral contraceptives. Results. Activated partial thromboplastin time was longer in women with blood groups B or 0, and plasma levels of factor VIII clotting assay and von Willebrand factor antigen and activity in women taking the combined oral contraceptive. Fibrinogen, von Willebrand factor antigen and von Willebrand factor activity concentration showed a strong cyclic variation with peak values in the luteal phase. This pattern was dampened for von Willebrand factor antigen and activity but completely disappeared for fibrinogen with the use of combined oral contraceptives. There was a cyclical pattern for factor VIII clotting assay in pill users, which was not evident in non-pill users. There were strong associations between the levels of von Willebrand factor antigen and activity and levels of fibrinogen, von Willebrand factor antigen and activity respectively, with increasing age. However, there were no significant associations between coagulation markers and weight, alcohol consumption or smoking status.

PO-0196 Reproductive choices of women in families with haemophilia
Kadir RA,* Sabin CA, Goldman E, Pollard D, Economidou DL, Lee CA
The Royal Free Hospital, Hampstead, London, UK

Objective. To assess the women’s experience in pregnancy and attitudes towards their reproductive choices in families with haemophilia. Design and Methods. A structured questionnaire was sent to all obligate and poten-

tial carriers of haemophilia (A and B), aged 14-60 years (n=454), regis-
tered with our haemophilia centre. Of these 197 (36.1%) completed and returned it. Clinical details including type and severity of the disease in the family, results of DNA analysis for carrier detection and history of HIV infec-
tion in the family were obtained from Haemophilia Centre records. Results. 43.7% (86/197) did not want to know foetal gender during pregnancy. Of the women who had given birth 22.5% (36/160) had a paternal diagnostic test in at least one of their pregnancies. Of the total 41 pregnancy terminations performed, only in 26.8% (11/41) was haemophilia the reason for the termination of pregnancy. Women’s religion and results of DNA studies had some influence in this decision. Living close to a haemophilia centre, proper counselling at the centre and awareness of the availability of prenatal diagnostic tests influenced women’s decision to become pregnant in 13.8% (22/160) and 9.8% (13/132) of first and subsequent preg-
nancies, respectively. These factors were more likely considered in women with severe haemophilia in the family (p=0.002) and those for whom DNA studies confirmed carriership or were inconclusive (p=0.04). When these women made a conscious decision not to have children, the reasons were fear of passing haemophilia 44.3% (47/106), previous experience with haemophilia 5.7% (6/107) and stress of going through parental tests or
would not have termination of pregnancy even if the baby was affected 6.6% (7/107). Seventy of the disease in the family, type of haemophilia (A or B), results of DNA studies, religion and year of birth had no effect on this decision. The most useful information was provided by the 72 (75-197) of the women by the staff at the haemophilia centre. Therefore, 19.3% 38 (197) did not receive useful information from any source. Conclusions. Our data show that haemophilia and its related factors in the family influence a women's reproductive choices.

PO-0197 Antiphospholipid antibody syndrome in a pediatric case


Department of Pediatrics, Faculty of Medicine, Ankara University, Ankara, Turkey

Antiphospholipid antibody syndrome (APS) associated with thrombosis is reported quite rarely in children. However it is expected to have a high incidence among pediatric thrombotic cases due to the lack of other precipitating events such as atherosclerosis and smoking in children. Here we report the cases of an 8 year old girl with primary APS. The patient was first admitted with blurred vision followed by complete loss of sight in the right eye. Retinal artery thrombosis was diagnosed and mannitol was started at the Department of Ophthalmology. Her physical examination revealed macular lesions on abdomen and lower extremities suggesting livedo reticularis. Her haematological investigation revealed iron deficiency. Prothrombin time was normal, APTT was prolonged. D-dimers were elevated. Protein C, protein S, antithrombin levels were normal. There was no factor V Leiden or prothrombin 20210 A mutation. ANA and anti-DNA were negative. Anticardiolipin antibodies (ACA) and anti-β2-Glycoprotein I (β2-GPI) were elevated. She was heterozygous for PT 20210 mutation. Protein C, protein S, antithrombin levels were normal. Her INR was 2.3 she had one more attack of left hemiparesis which also finally resolved. Her MR angiography did not reveal any thrombus, infarct or aneurysm. PANT was excluded by normal coeliac and renal angiography. She is currently under warfarin in treatment to keep INR between 3.0-4.5. The clinical findings of livedo reticularis, retinal vein thrombosis and hemiparesis together with ACA positivity confirm the diagnosis of primary APS.

PO-0198 Protein C, protein S and factor V Leiden as causative factors in childhood stroke

Deda G, Akar N, Kemahlı S, Uysal S, Olivia U, Karagöl U

Departments of Pediatric Neurology and Pediatric Hematology, Faculty of Medicine, Ankara University, Ankara, Turkey

Cerebrovascular accidents (CVA) constitute a far smaller proportion of neurologic diseases in childhood than in adulthood. The incidence is 2.52 per 100,000 people. Many etiologic factors mostly inherited disorders are considered in childhood CVA. Protein C, protein S, antithrombin levels and factor V Leiden and prothrombin 20210 mutations were studied in pediatric patients with CVA. Over a 5-year period 14 children (7 males, 7 females) aged from 13 months-15 years admitted for ischaemic stroke were enrolled in the study. The diagnosis was based on clinical neurologic deficits, computed tomography, magnetic resonance imaging and angiography in some cases. Protein C levels were low in 3 patients and they were between 27 and 42%; two of these patients were male. Activated protein C resistance was found in one patient and he was negative for factor V Leiden mutation. One female patient was heterozygous for that mutation; her protein C, protein S, antithrombin levels were normal. One other patient was heterozygous for PT 20210 mutation, Protein C, protein S and factor V Leiden mutation seem to play an important role in childhood stroke and they should be evaluated in every case.
Conclusions. The PFA-100™ cannot discriminate between type 1 and type 2 MWD. However, the sensitivity of the CT (94% for each cartridge) was superior to the BT (67%). These results confirm that the PFA-100™ is a sensitive and reliable diagnostic tool for detecting congenital MWD and more effective than the BT.

**PO-0202** Determination of the level of thrombosis precursor protein (TTP™) in orthopaedic patients. Preliminary results


*Transfusion Service and *Orthopaedic Department of Athens University, General Hospital KAT, Athens, Greece

Patients undergoing surgery are at major risk of thromboembolism and it has been shown that surgery induces a hypercoagulable state. The utility of blood tests to predict the development of perioperative venous thromboembolism (VT) is still undefined. TTP™ has been shown to measure accurately soluble fibrin polymers, the ultimate soluble precursors of fibrin. The aim of this study was to determine soluble fibrin levels pre- and post-surgery in patients undergoing total hip replacement (THR). Three males and five females (average age 63.6 y) who underwent THR and received exeariparin 40 mg twice prophylaxis of VT, were evaluated for clot- rate plasma levels of TTP™, PT, aPTT, fibrinogen and D-Dimers. Plasma samples were drawn pre- immediately after the surgery, on the 2nd, 4th, and 6th day post-procedure. All the patients were also evaluated by Colour Triplex for the presence of deep vein thrombosis (DVT) of the lower extremities and were negative. The level of TTP™ peaked immediately following the surgery and remained elevated until 48-72 hours. This is suggested to be in consequence of normal physiological wound healing. The TTP™ level in healthy volunteers is in the range of 0.7-6.1 µg/mL (mean 3.9 µg/mL; n=40). The level of D-dimers increased after the surgery and returned to normal the 4th day after surgery.

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These preliminary data suggest that (TTP™) has a role in determining patients at risk of developing thrombotic complications post operatively. Further study is ongoing.

**PO-0203** Activation of the protein C anticoagulant system during cardiopulmonary bypass and the influence of aprotinin


Hospital La Fe, Valencia, Spain; Onze Lieve Vrouwe Gasthuis, Amsterdam and Academisch Ziekenhuis, Leiden, The Netherlands

Objective. The protein C system is important in the regulation of haemostasis. We studied its behaviour during cardiopulmonary bypass (CPB) procedures with or without aprotinin treatment using assays sensitive for activation of the protein C system. Design and Methods. In a prospective, double blind, randomised study we investigated the levels of proteins C and S and the complexes between activated protein C (APC) with its two major plasma inhibitors, protein C inhibitor (PCI) (APC-PCI complex) and α1-antitrypsin (α1AT) (APC:α1AT complex), in patients treated with placebo, low dose and high dose aprotinin during elective CPB. A total of 48 patients, 17, 15 and 16 respectively were included. Results. The levels of protein C and S showed a rapid decrease after heparinisation, dropped significantly after start of CPB and remained stable during CPB. APC:α1AT decreased significantly after the start of CPB and remained stable during CPB. A significant peak was observed in the ICU. APC:PCI levels showed a peak after heparinisation in accordance with the accelerating effect of heparin on complex formation but decreased thereafter. Treatment with aprotinin did not significantly alter any of the measured patterns. Conclusions. In this study no evidence was found for increased activation of the protein C system during CPB. Administration of aprotinin did not result in different patterns of activation of the protein C system. (MEC, PM-07-0024).

**PO-0204** Infusion trial with desmopressin (DDAVP) in patients with severe hereditary von Willebrand disease

*Nitu-Whalley J,* Owens D, Riddell A, Jenkins PV, Lee CA

Apheresis Centre and Haemostasis Unit, Royal Free and University College Medical School, London, UK

Objective. DDAVP is considered effective in the majority of patients with type 1 and in some with type 2 WD, but response can not be predicted. As part of a multicenter trial, we report preliminary results in evaluating the effectiveness of DDAVP (0.3 µg/kg, iv) in severe WD-defined by severe bleeding history and a BT >15 min or Wf:Rco <10 U/DL or Fv:C <20 U/DL. Methods. Thirteen patients were enrolled. BT was recorded pre and at 2 h and blood was collected pre and 30 min, 1, 2 and 4 h post-DDAVP for Fv: II:C, Wf-Rco and Wf-Ag. secondary parameters (multimers, proteolytic fragments, Wf-Ag) and genotytype. Patients were responsive if both BT was normal at 2 h and Wf-Rco remained normal at 4 h and unresponsive if only one or none normalised. Results.

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<th>Wf-Rco Pre</th>
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Conclusions. One out of 9 patient with type 1 WD was responsive to DDAVP and all type 2 WD patients were unresponsive. However, 519 patients with type 1 had normal Wf:Rco at 4 h and 1/3 patients with type 2A had normal BT at 2 h. Further work is required to assess whether response to DDAVP is related to specific mutations in the Wf gene and the role of the secondary parameters.

**PO-0205** Haematological and lipid parameters and homocysteine derangement in atherosclerosis. Relationship with the MTHFR gene


Hematology Laboratory and 5th Medical Dept, Evangelismos Hospital, Athens, 1st Cardiology Dept, Nasisos Cardiovascular Surgery Center, Athens, Greece; Center for Cardiovascular Genetics, The Rayne Institute, University College, London Medical School, London, UK

Hyperhomocysteinemia is widely recognised as a risk factor associated with atherosclerosis and coronary artery disease (CAD), and its genetic background has been recently studied. Objective. The aim of this study was to compare haematological-haemostasis parameters, lipids, folate acid and homocysteine levels of patients (pts) with CAD and healthy individuals (H) and to associate them with the 677C-T genotype in the methylene tetrahydrofolate reductase (MTHFR) gene. Design and Methods. Thirty-seven H (aged 44±16 years, 20 females) and 54 CAD pts (aged 58±11 years, 6 females) were studied. In all individuals the following were determined to compare haematological-haemostasis parameters, lipids, folic acid and homocysteine levels of patients (pts) with CAD and healthy individuals (H) and to associate them with the 677C-T genotype in the methylene tetrahydrofolate reductase (MTHFR) gene. The TTP™ level in healthy volunteers is in the range of 0.7-6.1 µg/mL (mean 3.9 µg/mL; n=40). The level of D-dimers increased after the surgery and returned to normal the 4th day after surgery.

*Haematologica* vol. 84 (EHA-4 Abstract Book); June 1999
revealed 37 CC, 35 CT and 6 TT allele carriers. Conclusions. Hyperhomo-
cysteinemia seems to be an additional risk factor for CAD especially in pts
with triple vessel disease. PS increase might be attributed to its property
of acting as an acute phase protein. The distribution of MTBFR gene alle-
les (47% for CC and 7.6% for TT homozygotes) was in accordance with the
known figures. It was not possible to correlate HC levels and allele distrib-
ution probably due to the small number of cases studied so far.

PO-0206 Heparin needs and calcium blood levels in a patient with
depth vein thrombosis, hypercalcaemia and lung tumour
Cannazz1 A, Girolami A
Clinica Medica II, Azienda Ospedalire di Padova, Padua, Italy
Case report. An 82 year-old male with lung tumour was admitted to our
institution for swelling of the left leg. Venous ecography demonstrated
thrombosis of the left common femoral vein. Blood tests revealed severe
hypercalcaemia (3.37 mmol/L). The treatment of hypercalcaemia was start-
ed, and heparin was given: in the first four days as i.v. sodium salt, and
then as subcutaneous Ca-heparin. Interestingly, the dose of heparin need-
ed daily, adjusted according to PTT, decreased in parallel with blood calci-
um levels (see the Figure). This observation, previously unreported, suggests
several considerations: i) hypercalcaemia may affect in vivo the solubility of
Ca-heparin; ii) it may take part in the tumoral hypercoaplable state; and
iii) it could alter in vitro the tests utilised to monitor heparin treatment. This
topic seem to us to merit further investigation.

PO-0207 Cytokines and microthrombosis in acute pancreatitis
Pereslavets A, Chookhin S, Detko Y
Medical University, Lviv, Ukraine
Objective. Imbalance in the system of coagulation-fibrinolysis has an impor-
tant place in the pathogenesis of numerous diseases including acute pan-
creatitis. An analysis of parameters of the coagulation system showed dis-
seminated intravascular coagulopathy during the first days of necrotising
pancreatitis. It is shown that proinflammatory cytokines such as IL-1 and
TNF have obvious procoagulative effects. Design and Methods. The level
of the IL-1, TNF, fibrinogen and antithrombin-III was studied in 35 patients
with acute pancreatitis. Blood was sampled at the first, third and seventh
day after admission. The cytokine level were determined by ELISA. Fibrin-
gen was measured colorimetrically. Antithrombin-III was analyzed by an enzy-
mimunoassay using specific antisera. Results. Already at the first day, increased
levels of both cytokines were noted. The elevation was more sig-
nificant in patients with necrotising pancreatitis (IL-1 8.5±12.76 pg/mL vs.
3.2±7.44 pg/mL; TNF 138.9±6.09 pg/mL vs. 9.3±5.28, p<0.05). IL-
1 stimulate the synthesis of tissue factors of coagulation, platelet activat-
ed factor and inhibitor of plasminogen activation. Fibrinogen reached its
peak on the third day but there was no significant difference between patients
with necrotising or interstitial pancreatitis (p=0.08). On this back-
ground the levels of antithrombin-III were decreased. In patients with a
favorable outcome, a gradually decrease of cytokine and fibrinogen levels
with simultaneous increase in antithrombin-III was noted in patients
with a fatal outcome, these indices were stably increased. Conclusions.
Thus, an increase of proinflammatory cytokines with a simultaneous ele-
vation of procoagulant activity was observed in patients with several pan-
creatitis. Explaining drugs with anticytokine effects and which improve
microcirculation, such as pentoxiphilline, might inhibit the negative effects of
cytokines.

PO-0208 Effects of cyclosporin-A treatment on haemostatic parameters
Ozcebe O, Bujovsky G, Gurcan M, Kolar A, Uskudar O, Ozatli D,
Hazardoglu A, Sayini N, Dunder S, Kirazi S
Hacettepe University Medical School, Department of Haematology,
Ankara, Turkey
Use of cyclosporin-A has been suggested to cause predisposition to throm-
bus in organ transplant patients. However, because many risk factors for
thrombosis generally co-exist in such cases, this suggestion has not yet
be confirmed. In this study, we evaluated the effect of cyclosporin-A on
fibrinogen, factor VIII:C (FVIII:C), thrombin-antithrombin III complex (TAT)
and prothrombin fragment 1+2 (F 1+2) levels; levels of antithrombinulin (TM),
ATIII antigen, protein C (PC), protein S (FIS) and antithrombin III (ATIII)
activities; plasminogen activator inhibitor (PAI) and tissue plasminogen
activator (t-PA) activities, t-PA and PAI-1 antigens, plasminogen and D-
dimer levels. The study population consisted of 43 psoriatic patients. 22
of them were on cyclosporin-A treatment (group I) and 21 on topical drugs
(group II). Severity of the disease was equal in both groups. Nineteen
healthy subjects served as the control group (group III). PFI+2, TAT, TPA
activity, PAI-1 activity, D-dimer and TM levels were significantly increased
in groups I and II compared to group III. FVIII:C was elevated in group I
compared to group II. ATIII and PC activities were decreased in group I
in comparison with group II. Plasminogen activity slightly different in
each group, highest in group I and lowest in group III. Prothrombin time,
activated partial thromboplastin time, platelet count, fibrinogen, ATIII anti-
ogen, t-PA antigen, PAI-1 antigen and PS activity levels were not statisti-
cally different in any group. These results indicate presence of global
haemostatic activation in patients with psoriasis. Cyclosporin-A treatment
was associated with decreased activities of the natural anticoagulants pro-
tein C and ATIII.

PO-0209 High plasma caeruloplasmin level is not responsible for
acquired activated protein C resistance in pregnancy
Hung A, Walker AT, Cumming AM, Tait RC
Haematology Departments, Manchester Royal Infirmary & Southern
General Hospital, Glasgow, UK
Activated Protein C resistance is associated with an increased incidence
of venous thrombosis. While the inheritance of Factor V gene Leiden muta-
tion accounts for most APC resistance (APCR) phenotypes, acquired APCR
is not uncommon. We have previously reported acquired APCR developing
in approximately 50% of women during pregnancy. The mechanism under-
lying this acquired APCR remains unclear. An inverse correlation between
APC sensitivity ratio (APC:SR) and plasma caeruloplasmin (CP) was sug-
gested in a recent small study (Ripoll et al., Thromb Haemostas 1998;
79:449-501). CP is a plasma copper-binding glycoprotein which increases
during inflammation, oestrogen therapy and pregnancy. It contains sig-
nificant sequence homology to terminal A domains of FVa and FVIIIA and
could compete with their binding to activated Protein C. This may inhibit
inactivation of FVa and FVIIa and induce an acquired APCR phenotype. In
order to assess the influence of rising CP on APC:SR in pregnancy we
measured these parameters in 19 Factor V Leiden negative females seri-
ally throughout pregnancy (14 wk, 28 wk, 36 wk) and at 8 wk post par-
mum. APC:SR and plasma CP showed a modest inverse correlation at 8 wk
post partum (mean APC:SR = 2.83, mean CP = 75 mg/dL; r = 0.60, p
<0.01). During pregnancy mean plasma CP increased in the first trimester
as APC:SR fell (mean APC:SR = 2.1, mean CP = 73 mg/dL). However cor-
relation between individual CP and APC:SR results became weaker as preg-
nancy progressed (at 14 wk, r = -0.49, p = 0.04 at 28 wk, r = -0.26, p
= 0.28; at 36 wk, r = -0.2; p = 0.46). This small study would suggest that
acquired APC in pregnancy is not due to elevated CP, but more likely to be
related to the multifactorial changes of coagulation proteins.

PO-0210 Sensitivity and specificity of antiphospholipid syndrome 1 year
after diagnosis of deep vein thrombosis
Polistena P, Cagnin G, Radin E, Vespiagnani M, Chinellin M, Polacco A,
Sariceta R, Chiesi T
Hematology Dept, General Hospital, Venice-Mestre, Italy
The antiphospholipid syndrome (APS) is mainly characterised by the presence
of venous and arterial thromboses, recurrent fetal losses and thrombo-
cytopenia, associated with the presence of antiphospholipid antibodies
(aPL). We present the case of a patient with seronegativity for aPL at the
time of the thrombotic event, but in whom these antibodies were detect-
ed 1 year later. A 47 year old woman, with a history of migraine since
1994, was admitted to our hospital unit in October 1997 complaining of
back pain and swelling of both legs. Physical examination revealed multi-
petechiae and haematomas. Laboratory investigation showed leuco-
cytes 11,880/m3, Hb 10.6 g/dL, plt 42,000/m3, prothrombin time, acti-
vated partial thromboplastin time in the normal range, normal liver and kid-
ney functionality. An ultrasound test showed superficial and deep femoral
vein thromboses in both legs and an abdominal tomography the extension to
iliac and inferior cava vein as far as the hepatic one, with involvement of the
renal vein. The thrombosis started with no congenital or acquired risk
factors (Hgb aCh, IgM ACL and lupus anticoagulant were absent) and the
immunologic tests showed only a weak positivity for antinuclear antibod-
ies [1-40]. The anticoagulant therapy did not modify the thrombocyto-
penia. One month later she presented with a recurrence of thrombosis with
extension to the left popliteal vein although treatment with warfarin. Screen-
ing for antiphospholipid antibodies was performed several times and was
always negative. Eleven months later she returned to our hospital comp-
aining of spontaneous brushing and hypertension: haematologic tests showed
the following results: pt. 60.000/m³, creatinine 1.4 mg/dL, AST 859 U/L, ALT 526 U/L, LDH 964 U/L, 11.91 NR, hepatitis markers were
absent, the negative, anticoagulant test positive. An abdominal
tomography demonstrated progressive extension of thrombosis to renal
and suprahepatic veins and several infarcts of spleen and kidneys. We
started treatment with heparin, aspirin, cyclophosphamide as for cata-
strophic antiphospholipid syndrome. Two months later she had normal
blood pressure, normal liver and kidney function tests and platelet count.
This case, together with the others already present in literature, could cor-
robore the existence of a serious antiphospholipid syndrome.

Poster discussions Red cells and related disorders, anaemia, iron metabolism I

PO-0211 Non transferrin-bound iron and ineffective erythropoiesis in
thalassaemia intermedia and myelodysplastic syndromes

Capogellini M.D., Cattaneo C., Cortellezi A., Sarina B., Duca L, Cristiani
S., Balatti E.T., Lamberti G. Delilliers G., Fiorelli G.
Centro Alminio Congenita, *Servizio Autonomo di Ematologia, Ospedale
Maggiore, IRCCS, Milan, Italy.

Non transferrin-bound iron (NTBI) is a form of iron capable of generating
hydroxyl-radicals and promoting lipid peroxidation. Very weak correlation
between NTBI and markers of iron overload has been reported, suggesting
that NTBI might be influenced by other factors such as the degree of ery-
thropoiesis. Thalassaemia intermedia (TI) and myelodysplastic syndromes
(MDS), particularly low risk ones, are characterised by ineffective erythro-
poiesis. A hallmark of MDS erythropoiesis is an increased apoptotic rate,
depending on amounts of Epo still acting on cells, but further stud-
ies are needed for confirmation of this hypothesis.

PO-0213 Cell cycle analysis in the diagnosis of Fanconi anaemia (FA)

Timeu F, Crescenzi N, Leone L, Cerchio R, Bermond S, Farinasso L
Department of Paediatrics, University of Turin, Italy.

Previous studies demonstrated a cell cycle disturbance with an increase of
cells in G2/M phase in FA. However, experimental conditions, specificity
and sensitivity of the test are not clearly defined. We evaluated cell cycle
distribution by flow cytometric analysis in peripheral blood
mononuclear cells after exposure to PHA 100 µg/mL and melphalan (0.01,
0.05, 0.1, 0.5, 1.0 µg/L). The diepoxybutane test was also performed.
Forty-nine controls, 13 FA, 12 FA parents, 15 other children for anaemia
were found in extensive workups. All patients had severe
anaemia, iron metabolism I

Dep. Ped. Henn, Oncol. and Endocrinol, Children's Hospital, University of
Essen, Germany.

In previous studies and case reports the IGS presented as a heterogeneous
clinical picture. Children with deficiency of vitamin B12 usually present with

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Red cells and related disorders, anaemia, iron metabolism I

Seifer D, Bogdanovic AD, Suzadzic D, Djurdjevic V, Colovic M
Institute of Haematology, Clinical Center of Serbia, Belgrade, Yugoslavia

It is known that spontaneous growth of committed progenitors is one of
the hallmarks of myeloproliferative syndrome, especially PRV. Patients
with sideropenic anaemia are considered normal and are included in many
studies as a control group. We have evaluated growth pattern of
hematopoietic committed progenitors by in vitro cultures on methylcellu-
lose according to method described by Iscove (1974). Bone marrow MNC
were separated on Ficoll gradient, and grown in culture dishes (final
2×10³ cells/dish). Epo (IU) was used for CFU-E/BFU-E. We analysed 7 patients,
5/2M. All patients were referred due to severe anaemia. Women had
prolonged menstrual blood loss, and males had GIT bleeding. No other
results for anaemia were found in extensive workups. All patients had severe
sideropenic, microcytic anaemia [Hb 70 628.2 g/L, MCV 61±4 µL, serum
Fe 3.13±1.22 µmol/L with TIBC 65 815.6 µmol/L and saturation 4.7±1.53%.
FM in normal WBC and platelet counts. Fetuin was low in all of
them. Erythropoietin level in sera was elevated in all patients, 69±28 U/L
with median (Me) 57.4 U/L. In in vitro cultures without Epo, in all patients
spontaneous growth of erythroid progenitors was observed. CFU-E medi-
ated 75 colonies/dish (range 7-560) and BFU-E mediated 97 colonies/dish
(10-284). Also, there was marked increase in proliferation of erythroid
progenitors after addition of exogenous Epo (IU). CFU-E rose to a median
of 416 colonies/dish and BFU-E to a median of 209 (range 12-622,
p<0.05. Wilcoxon matched pair test). Growth of stimulated CFU-E and BFU-
E were above laboratory control values. Also, there was spontaneous pro-
fileration of erythroid progenitors from mononuclear cells in peripheral
blood. Conclusions. Our findings are puzzling because it is believed that
spontaneous growth of committed progenitors is connected only with
myeloproliferative disorders. We have shown that in a benign, de facto,
reactive condition such as sideropenic anaemia, there is a remarkable
degree of spontaneous growth of erythroid progenitors, with further increase
lig by stimulation with endogenous erythropoietin. Our findings may be
explained with the hypothesis that erythropoietin acts with an increase
in bone marrow of sideropenic patients that they preserve substantial burst
growth depending on amounts of Epo still acting on cells, but further stud-
ies are needed for confirmation of this hypothesis.
ly that the C282Y mutation of the HFE gene accounts for the iron alterations related to HCV infection. Nevertheless, the role of the H63D mutation in the iron abnormalities warrants further studies.

PO-0217 FY has increased cell surface expression on reticulocytes when compared with other circulating red blood cells

Divisions of Geographic Medicine and Infectious Diseases, and the Cancer Research Center, University Hospitals of Cleveland/Case Western Reserve University, Cleveland, USA

Objective. To compare reticulocyte (R) and more mature erythrocyte (RBC) cell surface expression of the Duffy (Fy) antigen and the receptor for Plasmodium vivax reticulocyte invasion and to correlate expression with promoter and open reading frame (FyA* FY B) genotype. Design and Methods. Blood samples from 12 Caucasian (C), 12 African American (AA) and one from another race (O), tested serologically for FyA and FYB antigen, were sequentially incubated with a FYA mouse-derived monoclonal antibody and a second goat anti-mouse immunoglobulin antibody conjugated with R-phycocerythrin (PE). Blood samples were then incubated with thiazole orange (TO) to distinguish reticulocytes. Immunofluorescence was then measured using a flow cytometer, using washes of known fluorescence. Mean levels were calculated for TO+ and TO− populations. Samples were also genotyped for the QA1−1 promoter and FY A FY B polymorphisms (PCR-ARFLP using restriction endonucleases St I−1 Ban I−1 respectively). Results. The mean fluorescence of the reticulocytes was increased relative to the mean value of other red blood cells in all individuals (p<0.001). The range of increase was 23−59%. The mean increase was 43%. The percentage increase did not differ significantly by race (AA=42.7% vs C=37.4%). Mean fluorescence for those identified as heterozygous for the promoter mutation was 42% of that of those homozygous for the wild type promoter on RBC and 31% of that on R (p<0.001). Conclusions. Reticulocyte expression of FY antigen was significantly increased when compared to that on other circulating red blood cells as measured by two color flow cytometry. A gene dosage effect was demonstrated for FY expression on R and RBC surfaces.

PO-0218 Arginine 490 is a hot spot for mutation in the band 3 gene in hereditary spherocytosis

Lima PRM, Sales TD, Costa FF, Saxe STO
*Department of Biochemistry, Institute of Biology, Hematology and Hemotherapy Center, Faculty of Medical Sciences, State University of Campinas-UNICAMP, Campinas São Paulo, Brazil

Hereditary spherocytosis (HS) is common inherited haemolytic anaemia. The molecular defects reside the red cell membrane proteins. The erythrocyte protein band 3 (EPB3) is the predominant integral membrane protein, totalling about 30% of the membrane proteins. Several mutations in band 3 deficient HS patients have been found in the band 3 (AEI) gene. We studied five patients presenting HS and (EPB3) deficiency characterised by the presence of spherocytes or pincered cells on peripheral blood smear and confirmed by SDS-PAGE of membrane proteins. The molecular study was based on genomic DNA extraction, exon amplification by PCR, non-radioactive single strand conformation polymorphism analysis−SSCP, and DNA sequencing of abnormal SSCP patterns. The study revealed one new mutation present in three patients, Arg 490−Hs. The red cells' sensitivity to the anion transport inhibitor H-2-DIDS was increased in the (EPB3) deficient patients. We conclude that there is a hot spot for mutation in the band 3 gene located at exon 13. Arg 490−Hs is the fourth mutation described in this exon, and the second mutation changing CG (1582-1583) dinu- leotide, altering the normal conserved Arg 490. The altered protein probably cannot insert in the red cell membrane or does not have transport activity, as shown by DIDS-sensitive sulphate self exchange quantification.

PO-0219 Detection and quantification of foetal in maternal blood by laser scanning cytometry

Vandekerckhove P, Boeckx N, Claeyes R, Brusselmans C, Goossens W
Laboratory of Hematology, Dept. Clinical Pathology, University Hospital Leuven, Belgium

The detection and quantification of foetal erythrocytes in maternal blood is widely carried out by the Kleihauer-Betke test (KBT). This test is an important clinical indicator of foetal-maternal haemorrhage (FMH), and in many countries is routinely undertaken for all RH-negative mothers with RH-positive children. For mothers with detectable FMH, prophylactic anti-D gamma globulin is given to prevent maternal immunisation with foetal D-positive erythrocytes and the risk of Rh haemolytic disease in the subsequent pregnancies. The KBT method is based on acid elution of adult Hgb, eosin staining of residual foetal Hgb and subsequent microscopic
evaluation. The method is, however, labour-intensive, highly subjective, and poorly reproducible with reported inter-institutional variation approaching 500% (Br. Obstet Gynaecol 1997; 104:845). Biometric Imaging Inc. has developed an automated method in the United States using the IMAGN Microvolume Fluorimeter. The assay involves preliminary fixation with a dried (no-wash) fixative, membrane permeabilization, and subsequent staining with a fluorochrome-labeled monoclonal antibody to Hgb gamma (γ) chain. Methodologically, 4 laser-scanning approaches is faster and not subject to observer interference. A comparison between the manual KBT and Microvolume Fluorimetry was undertaken using 60 blood samples from pregnant women. This study indicated (a) a good correlation between the two methods, (b) a significantly low variability with laser scanning cytometry, and (c) a better lower limit sensitivity for laser scanning cytometry than for the classical KBT method.

PO-0220 Sequences, evolution and ligand binding properties of mammalian Duffy antigen/receptor for chemokines


The Duffy blood group antigens are carried by a 7 transmembrane domains glycoprotein, DARC, which acts as a widely expressed promiscuous chemokine receptor. DARC is also the erythrocyte receptor for Plasmodium vivax and Plasmodium knowlesi malaria parasites. We have previously shown that the functional activation of the first and second extracellular domains of DARC is required for chemokine binding to human red cells (1). Conversely, sequence analyses of the DARC gene homologues in non-human primates (chimpanzees, rhesus monkeys) show that the 3rd and 4th extracellular domains (2) as well as inhibition of parasite binding, by DARC synthetic peptides (3) pointed out the critical role of the NHE2 extracellular domain for the interaction with parasite merozoites. To gain further insight into the evolution and structure/function relations of DARC, we analysed the binding of chemokines and anti-Fy monoclonal antibodies (MoAb) to RBCs from non-human primates and non-primate mammals and elucidated the structure of DARC homologues from gorilla, baboon, orangutan, chimpanzee, capucin monkey and cow. Human IL8 efficiently bound to RBCs samples of 11 non-human primate species investigated and of cow but, as previously shown, not of mouse. Among three anti-Fy6 used in flow cytometry analysis, one confirmed expression of Fy6 by chimpanzee, baboon and squirrel monkey, whereas the two others revealed expression only by human and chimpanzee RBCs. In addition to human and chimpanzee, Fy6 was also found to be expressed, albeit poorly, by RBCs from gorilla, baboon and the sus monkey but not from New World monkeys. Considering mouse and catte sequences as archetypes of non-primate inanimate, alignment of all DARC homologous sequences allowed us to construct a phylogenetic tree in which all branchings were in accordance with current knowledge of phylotype phylogeny. Analysis of the deduced amino acids sequences highlighted the conservation of some amino acids residues which would prove to be critical for the structural and functional properties of DARC. Finally, the amino acid variability of DARC like polypeptides was found to be well correlated with the hydrophilicity indices, with the highest divergency and the worst soluble properties at the terminal extracellular domain.

2. Chandhuri et al, Blood 1995; 83:615-21;

PO-0221 A study of the coregulation and tissue specificity of XG and MIC2 gene expression in eukaryotic cells


Institut National de la Transfusion Sanguine, Paris, France; Institut Pasteur, Paris, France.

XG, the product of the N/MC2 gene, exhibits an erythroid-specific quantitatitive polymorphism coregulated with the polymorphism of the XG blood group gene. The expression of the two genes, tightly linked on the human X chromosome, is thought to be controlled at the transcriptional level (1). As a preliminary study of this phenomenon, the human XG and MIC2 CDNAs were expressed in mouse RAG2/2 cell lines or together in double transfected, followed by characterisation of the recombinant proteins (blood group Xg and CD99 antigen) by flow cytometry, immunoprecipitation and Western blot analysis. Both proteins were surface independently and at a similar level in single and double transfected. Specific proteins of 26 kD (Xg) and 32 kD (CD99) were characterized. A putative 26-kD intracellular precursor of CD99 was also detected, as well as a 26-kD species after neuraminidase digestion. The expression of CD99 was found to be cell line-dependent. No evidence of association or complex formation between XG and CD99 proteins could be proven, either on transfected cells or in erythro-cyes. These results were confirmed by somatic hybrids between single transfectants. Altogether these findings suggest that the phenotypic relationships between XG and CD99 is regulated at the transcriptional but not at the post-translational level. Such a difference in the tissue specificity of XG expression showed that surface production of the XG protein could not be restored in somatic hybrids between B-lymphoblastoid cell lines from Xg(a+)-individuals and fibroblasts (RAG2/2) cells. RT-PCR analysis of the transcripts revealed the existence of a XG MRNA for each cell line, suggesting that the tissue-specific regulation of XG expression either occurs at a quantitative transcriptional level or is a post-transcriptional event. Northern blot analysis also showed that two major XG transcripts of 2.3 and 1.0 kb and one minor species of 3.8 kb were detected in erythroid tissues and several nonerythoid tissues.


PO-0222 Binding sites of leucocyte integrins (LFA-1, MAC-1) on the ICAM-4/LW blood group protein


*Inserm U 76, INS, CNRS U 660-1P, Paris, France; *Univ. of Helsinki, Finland.

The red cell ICAM-4/LW blood group glycoprotein which belongs to the family of intercellular adhesion molecules (ICAMs) and is encoded by the MIC2 gene is expressed on all human tissues and has developed an automated method for the enumeration of foetal RBCs using the IMAGN Microvolume Fluorimeter. The assay procedure involves preliminary fixation with a dried (no-wash) fixative, membrane permeabilization, and subsequent staining with a fluorochrome-labelled monoclonal antibody to Hgb gamma (γ) chain. Methodologically, 4 laser-scanning approaches is faster and not subject to observer interference. A comparison between the manual KBT and Microvolume Fluorimetry was undertaken using 60 blood samples from pregnant women. This study indicated (a) a good correlation between the two methods, (b) a significantly low variability with laser scanning cytometry, and (c) a better lower limit sensitivity for laser scanning cytometry than for the classical KBT method.

2. Chandhuri et al, Blood 1995; 83:615-21;

PO-0223 Leukocyte activation in hereditary spherocytosis pre and post splenectomy


Hereditary spherocytosis (HS), the most common haemolytic anaemia in Europe, presents a broad spectrum of clinical manifestations, which range from mild to extremely severe, and is responsive to haemoinitiative therapy. The haemolytic events are usually associated with inflammatory and infectious processes, and this is probably due to the destabilised membrane structure and to an accelerated red blood cell (RBC) aging process. The enhanced splenic removal of main RBC after transfusion or in the absence of splenic stress, allow a continuous removal of defective RBC by imposing a continuous, stressful condition may induce continuous enhanced leucocyte activation, as well as continuous proliferation of lymphocytes, plasma cells and macrophages. It is well known that activated leucocytes can release oxygen metabolises, cationic protein. To test this hypothesis we evaluated the total and differential count of white blood cells (WBC), as well as leucocyte activation products, namely elastase and lactoferrin, and the granulocyte-macrophage colony-stimulating factor (GM-CSF), in 16 HS patients (HS), in 18 HS patients who had undergone splenectomy more than six months before (HS-SPL) and in 28 healthy individuals. We found...
that both HS and HS-SPL patients had higher values than controls of all the studied parameters except for elastase in HS-SPL patients. These ris-es were significant (p<0.05) for total WBC, granulocytes, lymphocytes and lactoferrin. In HS-SPL patients the rise in elastase was also significant. Splenectomy was accompanied by a reduction in the WBC activation prod-ucts, and products in the GM-CSF, but no improvement parameters stud-ied. Our data seem to support the hypothesis that the enhanced RBC aging and removal triggers WBC activation proliferation in HS patients. It also sug-gests that this continuous stressful condition may favor the development of immune disturbance. And that splenectomy, imposing a prediction in WBC activation and proliferation, may inhibit these disturbances.

PO-0224 Frequency of the HFE hereditary haemochromatosis associated genes in Greece

Papanikolaou G, Politou M, Terpos E, Fourlemadis S, Konstantopoulos K, Sakellaropoulou N, Loukopoulos D, First Dept. of Medicine, University of Athens, Greece

Hereditary haemochromatosis (HH) is a common disease among Cau-casians. Reported frequencies vary from 0.3 to 0.8%. Recently a candidate gene HFE was identified and two ancestral mutations were described: the Cys282Tyr and His63Asp mutations. The prevalence of HH in Greece is unknown. Also unknown is the carrier frequen-cy of the two mutant alleles. This communication reports the results of our research for the above genes in 125 healthy male blood donors of Greek origin and 8 unrelated hemochromatosis patients. The latter complied with the following criteria: iron overload detected by liver biopsy, >4 g of iron removed by phlebotomy, elevated serum ferritin and absence of underly-ing causes of secondary iron overload. Gene analysis was carried out by amplification of the fragments of the gene containing the two mutations by PCR and digestion of the PCR products with Rsal and MboI which may detect the C282Y and H63D mutations respectively. Results.

<table>
<thead>
<tr>
<th>C282Y</th>
<th>C282Y</th>
<th>C282Y</th>
<th>C282Y</th>
<th>H63D</th>
<th>H63D</th>
<th>H63D</th>
<th>H63D</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/-</td>
<td>+/-</td>
<td>+/+</td>
<td>+/-</td>
<td>-/-.</td>
<td>-/-.</td>
<td>-/-.</td>
<td>-/-.</td>
</tr>
</tbody>
</table>

HH patients 2 0 2 0 0 4
Controls n=125 0 1 0 3 5 86

Conclusions. (a) The C282Y allele was detected in 50% of the HH patients. This is considerably lower than the frequencies reported for HH patients in USA (82%) and France (91%) and closer to that reported in Italy (64%). These data fit with the hypothesis that a significant number of HH Mediterranean HH cases are due to other, not-as-yet identified mutation(s). (b) The C282Y mutation was found in 1 out of 125 individuals (0.8%) with no evi-dence of iron overload. Although the number of persons examined is still low to draw a conclusion, the estimated frequency of C282Y homozygos-i ty is 0.16%. According to this frequency a high number of HH patients should be expected which is not the case. Therefore, the possibility that C282Y homozygosity is not always clinically expressed must be considered; (c) the frequency of the H63D mutation was 16.4% and maybe correlates with HH since two compound heterozygotes were found to express HH phe-notype.

PO-0225 Value of various laboratory parameters for diagnosing iron-deficiency anaemia in hospitalised patients

Van Tellingen A, Kuiken JC, De Kleewit W, Van Tinteren H, Vasmel WLE Sint Lucas Andreas Ziekenhuis WLE, Department of Internal Medicine; Amsterdam, the Netherlands

Patients on a general, internal medicine ward form an heterogeneous pop-ulation in whom various factors complicate the diagnostic evaluation of anaemia. Diagnostic iron deficiency or sufficient iron deficiency has impor-tant clinical implications. The real value of various laboratory parameters (other than bone marrow) for the individual patient in this population is not clear. The aim of our prospective study was to test the predictive value of a set of laboratory parameters, including plasma transferrin receptor (Pfr) and zinc protoporphyrin (ZPP), for diagnosing iron deficiency in hospi-talised patients with microcytic or normocytic anaemia. Furthermore we tried to support the hypothesis that the enhanced RBC aging and removal triggers WBC activation proliferation in HS patients. It also sug-gests that this continuous stressful condition may favor the development of immune disturbance. And that splenectomy, imposing a prediction in WBC activation and proliferation, may inhibit these disturbances.

PO-0226 Clinical outcome of ten Chinese patients with severe aplastic anaemia

Chan JCW, Liu HSY, Bho BCS, Pamela Tounde Nethersole Eastern Hospital, Hong Kong, China

Between 11/93 and 10/98, ten Chinese patients with severe aplastic anaemia (SAA) were diagnosed in a regional hospital in Hong Kong accord-ing to the criteria - bone marrow hypoplasia, ineffective haemopoiesis, (a) The C282Y allele was detected in 50% of the HH patients. This is considerably lower than the frequencies reported for HH patients in USA (82%) and France (91%) and closer to that reported in Italy (64%). These data fit with the hypothesis that a significant number of HH Mediterranean HH cases are due to other, not-as-yet identified mutation(s); (b) The C282Y mutation was found in 1 out of 125 individuals (0.8%) with no evi-dence of iron overload. Although the number of persons examined is still low to draw a conclusion, the estimated frequency of C282Y homozygos-i ty is 0.16%. According to this frequency a high number of HH patients should be expected which is not the case. Therefore, the possibility that C282Y homozygosity is not always clinically expressed must be considered; (c) the frequency of the H63D mutation was 16.4% and maybe correlates with HH since two compound heterozygotes were found to express HH phe-notype.

PO-0227 Ribavirin-induced haemolytic anaemia in chronic hepatitis C

Tavazzi D, Duca L, De Feo TM, Mattioli M, Molteni V, Di Maio V, Cappellini MD, Fargion S, Fiorelli G Dipartimento di Medicina Interna, Ospedale Maggiore Policlinico IRCCS, University of Milan, Italy

Reversible haemolytic anaemia is the major side effect following ribavirin (RIBV) treatment of chronic hepatitis C but the mechanism responsible for haemolysis is far from clear: RBC antioxidant defences and the role of iron status in patients with HCV-related chronic liver disease have been studied in six relapsers or non-responders to interferon (IFN) and hepatitis C virus (HCV) infection. SAA should remain the treatment of choice for SAA especially when HLA-identical SibBMT is not available. Longer follow-up is needed to detect clonal dis-ease.

PO-0228 Red cells and related disorders, anaemia, iron metabolism I

Red cells and related disorders, anaemia, iron metabolism I
normal GSH stability (<20%) was apparent. Further GSH reduction and more pronounced instability (40±10%) were observed in haemolytic patients, and halving RIBV dosage led to pretreatment values. Over-normal NTB values (1.87±0.80 vs. -0.48±0.64 µM controls) were associated with haemolysis despite the absence of iron overload and decreased (0.55±0.33 µM) after RIBV reduction. In a mildly haemolytic patient, HPMS showed progressive overstimulation (3.1 to 6.2 molCO₂/cell × 10^-11) after 2-weeks administration, indicating ongoing oxidative stress. No significant band 3 increase in membrane protein aggregates was detected (1.12 to 2.21%), while increased methbin deposition (zero to 3.1 nmol/mL RBC) was observed. During antiviral RIBV therapy, RBC antioxidant defences are soon enhanced before any sign of irreversible membrane damage in terms of band 3 clustering, a signal for RBC removal from peripheral circulation. RBC antioxidant status could be an early predictive risk factor for haemolytic anaemia in patients eligible for RIBV treatment.

PO-0228 Sensitivity of red cell osmotic fragility tests in hereditary spherocytosis patients with various biochemical defects

Zaapa M, Marzani M, Vercellati C, Pelissero G, Bianchi P, Boschemi C, Zamella A
Division of Haematology, Ospedale Maggiore, IRCCS, Milan, Italy

Hereditary spherocytosis (HS), a highly heterogeneous syndrome, is caused by abnormalities of red cell cytoskeletal proteins: spectrin (Sp), ankyrin (Ank), band 3 (B3) and protein 4.2. The laboratory diagnosis of HS is based on the observation of red cell concentric fragility, which is typically increased in this disease. The osmotic fragility test in NACI (OF) and acidified glycerol lysis test (AGLT) are considered the most sensitive methods, but their sensitivity has so far been evaluated on a limited number of cases. The aim of the present study was to compare the sensitivity of OF on fresh and incubated blood and AGLT on 123 HS cases and to determine whether the tests' sensitivity is influenced by the presence or absence of spleen and by the type of biochemical defect. The results are presented in the following tables:

<table>
<thead>
<tr>
<th>Test</th>
<th>Tot. cases</th>
<th>No. Positives/Total (%)</th>
<th>Spnucleotized</th>
<th>Not spnucleotized</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGLT</td>
<td>115/123 (93)</td>
<td>23/25 (92)</td>
<td>92/98 (94)</td>
<td></td>
</tr>
<tr>
<td>OF fresh</td>
<td>98/123 (80)</td>
<td>22/25 (88)</td>
<td>76/98 (77)</td>
<td></td>
</tr>
<tr>
<td>OF inc.</td>
<td>113/123 (92)</td>
<td>22/25 (88)</td>
<td>91/98 (93)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test</th>
<th>Tot. cases</th>
<th>No. Positives/Total (%)</th>
<th>Sp</th>
<th>Ank</th>
<th>B3</th>
<th>4.2</th>
<th>Undetected</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGLT</td>
<td>42/47(89)</td>
<td>15/16 (94)</td>
<td>4/4(100)</td>
<td>12/13(92)</td>
<td>6/7(86)</td>
<td>6/7(86)</td>
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</tr>
<tr>
<td>OF fresh</td>
<td>37/47(79)</td>
<td>11/16 (69)</td>
<td>4/4(100)</td>
<td>12/13(92)</td>
<td>7/1(100)</td>
<td>4/7(57)</td>
<td></td>
</tr>
<tr>
<td>OF inc.</td>
<td>42/47(89)</td>
<td>13/16 (61)</td>
<td>4/4(100)</td>
<td>13/13(100)</td>
<td>7/1(100)</td>
<td>6/7(86)</td>
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</tbody>
</table>

AGLT and OF inc. displayed the same sensitivity, both in splenectomised and not splenectomised patients. Although the number of cases which underwent SDD analysis of membrane proteins was small, OF inc. seems to be less sensitive than AGLT (81% vs. 94%) in Sp defects and more sensitive (100% vs. 92%) in B3 deficiency. In conclusion, overall AGLT and OF inc. have a comparable sensitivity. However, AGLT is easier and faster than OF inc. and does not need sterile blood samples.

PO-0229 Fanconi anaemia group a mutations in Israeli patients

Hematol/Oncol, Schneider Children’s Medical Center of Israel, Petah Tikva, Sackler Faculty of Medicine, Tel Aviv University, Ped Hematol/Oncol, Rambam Medical Center, Ped Hematol/Oncol Kaplan Hospital, Rehovot, Israel and Laboratory of Human Genetics and Hematology, The Rockefeller University, New York, USA

Defects in the Fanconi anaemia A (FAA) gene are estimated to cause 60-65% of FA cases. More than 70 FAA mutations, none of which predominates were identified in 7 FA allees of Moroccan-Jewish origin in codon 724/725 was found. Further characterisation of FAA defects will enable carrier detection, prenatal diagnosis and gene therapy, as well as provide insight into the FAA protein structure-function relationships.

PO-0230 Serum erythropoietin level during the treatment of deficiency anemias

Betul B, Konieczna M, Sulek IC, Klos M
Central Clinical Hospital Military School of Medicine, Warsaw, Poland

Objective. The aim of the study was to assess the trends in erythropoietin (Epo) concentration during the treatment of anaemia. Design and Methods. The study group consisted of 78 patients suffering from severe anaemia of different origin: group 1-45 patients with iron deficiency anaemia, group 2-33 patients with vit. B12 deficiency anaemia. All the above patients have undergone four examinations during the course of anaemia treatment: 1st before the treatment, 2nd between 8th and 10th day of treatment, 3rd between 16th and 18th and 4th between 24th and 26th day. The following parameters were examined: smw Epo concentration, blood morphology, reticulocyte number (relative and absolute), serum iron and ferritin level. During the 1st examination serum vit. B12 and folinic acid concentration were also analysed. Bone marrow cytology was evaluated at the 1st and 2nd examinations. Results. Statistically significant differences were found in smw Epo levels during the treatment process within both groups of patients. Median of Epo concentration showed a gradual decrease in consecutive examinations: 3.6±2.06 4.33±1.71 3.1±1.52 2.7±2.14 mg/mL in vit. B12 deficiency anaemia respectively. In the second part of the study the correlation coefficients between Epo concentration and several haematological parameters were analysed. Significant negative correlations between Epo concentration and haemoglobin level and hematocrit were observed. Conclusions. 1. Effective treatment of anaemia is associated with a gradual decrease in crythropoietin concentration; 2. hematocrit and haemoglobin concentration are the main factors associated with Epo level; biological reasons for correlations of Epo with other indices require further investigations.

PO-0231 Concentration of soluble transferrin receptors in first time blood donors and autologous donors

Klos M, Korsak J, Gaweda J, Sulek K
Central Clinical Hospital Military School of Medicine, Warsaw, Poland

Objective. The aim of the study was to determine the influence of blood donations on soluble transferrin receptors (STR) and ferritin concentration. Design and Methods. The study, groups consisted of 59 blood donors (18-22 years of age) before donation and 3 months after; 22 healthy people aged 40-50 years and donors of autologous blood, who donate 1 or 2 units in intervals of 7 days, without iron supplementation. The concentration of STR was measured with IEMA IDEA STR (Onion Diagnostica) and ferritin concentration was measured with Coat-A-Count IRMA (Diagnostic Product Corporation). Results. Blood donation did not influence soluble transferrin receptors and the ferritin concentration. The soluble transferrin receptor concentrations at first donation and 3 months later were 2.97±1.24 mg/L and 2.59±0.61 mg/L respectively. Similarly, no differences were observed in ferritin concentration in this group of blood donors: 106.1±63.9 µg/L and 91.1±43.9 µg/L respectively. In the group aged 40-50 years the soluble transferrin receptor concentration was 2.34±0.9 mg/L and ferritin concentration 193±146 µg/L in patients who donated blood for autologous purposes the soluble transferrin receptor concentrations before first donation and after second donation were 2.86±1.06 mg/L and 3.71±1.59 mg/L respectively and ferritin concentrations were 215.2±138.1 µg/L and 221.9±172.1 µg/L respectively. Conclusions. These preliminary studies indicate that soluble transferrin receptor concentration determination can be helpful for early diagnosis of iron deficiency.

PO-0232 Iron overload response to highly active antiretroviral therapy (HAART) in HIV infection

Terrile A, Cassola G, Lorusso C, Penco G, Giuliano C, Camparena A, Ternile A
Galilea Hospital, Genoa, Italy

Objective. To monitor, through serial serum ferritin determinations, iron overload in HIV+ve patients treated with HAART including one protease inhibitor. Methods. Ferritin, CD4 cell counts and FHV viral load (VL) were
measured at 3–4 month intervals in 62 severely compromised HIV pts (45 M, 17 F, mean age 37.2, mean follow-up 20 ms, range 12-28) after the onset of HAART. Results. Mean values at onset (O) and end (E) of follow-up are reported in the table for both responders (R) (nv 64 pts = 71%) and non responders (NR) (18 pts = 29%), as defined by evaluation of clinical, immunological and virological response:

<table>
<thead>
<tr>
<th></th>
<th>R/O</th>
<th>R/E</th>
<th>NR/O</th>
<th>NR/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (nv 10-250 ng/mL)</td>
<td>375</td>
<td>137</td>
<td>470</td>
<td>207</td>
</tr>
<tr>
<td>CD4+ cells (nv 489-2200/m3)</td>
<td>101</td>
<td>284</td>
<td>104</td>
<td>233</td>
</tr>
<tr>
<td>HIV viral load (copies/mL)</td>
<td>338,114</td>
<td>4,255</td>
<td>134,417</td>
<td>150,797</td>
</tr>
</tbody>
</table>

HAART induced a statistically significant (p<0.001) improvement of all parameters in R; in NR both ferritin and CD4 counts improved (p<0.005 and <0.001, respectively), while viral load increased. Conclusions. The consistency of CD4 count decrease in both R and NR pts, in the face of virological failure in NR, shows that even minor benefits in in HAART-treated pts are translated into a significant reduction of iron overload, in itself a precious achievement.

PO-0233 Very severe aplastic anaemia (VSA) following resection of lymphatic thymoma: effectiveness of antilymphocyte globulin (ALG), cyclosporin A (CyA) and granulocyte colony-stimulating factor (G-CSF)

Dincel G, Saka B, Akta M, Na飆aci M, Keskin H, Dincel K
Division of Hematology, Department of Internal Medicine, Istanbul Medical School, University of Istanbul, çapa, Istanbul, Turkey

Aplastic anaemia is a rare complication of thymoma. A patient who developed a VSA 3 months after the resection of a thymoma is presented in this report. The patient was a 38-year-old male in whom widening of the mediastinum was detected by an X-ray film of the chest, performed during an upper respiratory tract infection in February 1998, and a mass was demonstrated in the anterior mediastinum by a computerised tomographic study of compatible with a thymoma the thorax. An initial work-up then, including routine haematological examinations and a bone marrow sample, revealed no abnormalities. The post-operative course was uneventful, but the patient began to complain of weakness and high fever 3 months after the surgical intervention. An immunologic basis for the hematopoietic suppression in our patient was considered. The reduced ratio of CD4+ to CD8+ T cells suggested an immune mechanism. Another important finding in our patient was the complete response obtained with intensive immunosuppressive treatment which indicates that this may have abolished the myelosuppressive mechanism, hence stimulating the pluripotent stem cells.

PO-0234 Haematopoiesis, normal and neoplastic stem cells

Monacca B, Chiariotti A, Breccia M, Bongarzoni V, Caminoso I, Antocci Borza P, Di Bartolomeo E, D’Ambrosio E* Pascale E*, Mascelli F
Dept. of Biotechnology Cellular and Hematology, University “La Sapienza” of Rome; *CNR: Institute of Experimental Medicine, Italy

From April 1996 to November 1998 at our Institution, we observed 60 patients with high serum ferritin. Forty-Three (71%) were males with a median age of 54 years (range 28–74 years) and 17 (29%) females with a median age of 49 years (range 18–71 years); no patient had a cause of secondary iron overload. Screening for hereditary haemochromatosis (HH) was based on: absence of known causes of secondary iron overload, transferrin satu-
plus Cy administration. The three patients with severe aplastic anaemia who had been unresponsive 6 months after antithymocyte globulin plus Cy therapy underwent treatment with fludarabine without discontinuation of oral Cy administration. Three months later, patients became progressively transfusion independent and showed a significant improvement in peripheral blood cell counts. These good results persisted 6, 18, and 29 months for the 3 patients without severe side effects. There are several reports on the effect of androgens in the management of AAM but no experiences have been reported before for patients treated in second line therapy by cyclosporine plus androgen after failure of ALG plus Cy. Androgens plus cyclosporine should be administered after failure of front line therapy for patients not eligible for bone marrow transplantation and should also be considered for others, particularly those with unrelated donors. However, it is necessary to assess the risk of fatal infections during the post-transplant period delayed efficacy of androgen treatment and the risk of allogenic bone marrow transplantation.

PO-0237 Clinical and biological study in cyclic neutropias
Burgos-C. Lopez Rubio M, Heranz N
Service Hematology, Departament of Medicine, Hospital Principe de Asturias, Universidad de Alcalá de Henares, Madrid, Spain

Introduction and Objective. Cyclic neutropenia (CN) is a rare disease manifested by transient severe neutropenia that recurs approximately every 21 days. Autosomal dominant transmission is found in some families and pathogenesis and treatment are under study. We describe clinical and biological characteristics of patients with CN. Patients. Review of 53 patients previously diagnosed an having chronic neutropenia was performed. CN was found in 9 cases. Their age ranged from 3 to 24 years old. Familial history was obtained from 6 patients from two families. In both families CN was documented in members from two generations. Results. History of infections was reported in the father and relatives from the previous generation. All the patients had chronic neutropenia with regular oscillations in the neutrophil nadir were present and included fever, mucosal ulcers. Monocytosis was always present, with regular cycling. HB and platelets were normal. The majority showed partial depletion of neutrophilelyte level, at the time of nadir. No chromosomal abnormalities were found. Granulopoietic precursors (CFU-GM) were determined in bone marrow agu culture. colonies were present in 8-66 colonies, (n=10). Decreased numbers of CFU-GM with respect to control values were found in 6 patients. In the presence of patients serum the number of BFU-GM ranged from 12 to 66 with moderate improvement in 4 patients. Recent clinical manifestations during the neutrophil nadir were present and included fever, mucosal ulceration, lymphadenopathy and respiratory infections. Pneumonia and otitis were present in the propositus from the 2 families with several members affected. None of these patients was treated with haemopoietic growth factors. Follow-up to ten years in these patients showed a lower number of clinical infections with age. Conclusions. Defective haemopoiesis and autosomal dominant transmission is found in some patients with CN. Genetic counselling should be considered.

PO-0238 Adhesion of megakaryocytic progenitors to fibroblasts in an in vitro stromal model: role of TPO, VLA-4 and VLA-5
Zweegman S, Veerhof MA, Huijgens PC, Drijger AM
University Hospital Vrije Universiteit, Amsterdam, The Netherlands

Objective. Interactions between human progenitor cells (HPC) on the one hand and marrow stromal cells, cell-adhesion molecules (CAM) or extracellular matrix proteins on the other hand have been found to be critical for the regulation of haemopoiesis. However, the understanding of the influence of cell adhesion interactions on megakaryopoiesis remains poor. Methods. Therefore, we studied megakaryocytic development of HPC in an in vitro stromal model. Purified CD34+ cells (>90%), isolated from bone marrow (BM) and from BM mobilised by TPO plus Cy, were cultured for 10-12 days. The presence of CFU-GM and BFU-MK, as well as of CFU-MK and BFU-MK, were determined. Results. The adherent cell fraction harvested after 24 hours of culture, gave rise not only to a higher early (BFU-Mk), but also to later (CFU-MK and BFU-MK) colony formation, compared to the non-adherent cell fraction. Ecarin-activated prothrombin induced membrane association of the PKC isoforms a, b, and c, in CD34 + cells. Further, these K+ channel inhibitors inactivate PKC, which is confirmed by Western blotting. Conclusions. Thrombopoietin (TPO)-induced ERK activation in CMK cells mobilised ex vivo by cytokines and stroma is inhibited partly by BIM. In addition, BIM also decreased MAP kinase activity in TPO-stimulated CMK cells. Conclusions. TPO-activated PKC could be targeted for prodrug activation. Further, PKC inhibitors can be important for the development and the treatment of megakaryocytic disorders.

PO-0239 Involvement of protein kinase C- and PI3-kinase-activity in thrombopoietin-induced map kinase signaling in CMK cells
*Division of Pediatric Oncology and Hematology and §Research Group Pharamacological Oncology and Haematoology, Medical Faculty at the Friedrich Schiller University Jena, Germany

Objective. Extracellular signal-regulated kinases (ERKs) are involved in thrombopoietin (TPO)-induced megakaryocytic differentiation. However, the signaling cascades contributing to this mitogen-activated protein kinase (MAP kinase) pathway in human megakaryocytes are poorly defined. Methods. Using the human megakaryocyte cell line CMK, the effect of TPO on activation of protein kinase C (PKC) and ERK was estimated by Western blotting and immunoprecipitation. Moreover, the effect of the PKC inhibitor bisindolylmaleimide I (BIM), and the phosphatidylinositol-3-kinase (PI3K) inhibitor wortmannin on MAP kinase activation was investigated in CMK cells stimulated With TPO. Results. Treatment of CMK cells with TPO for 10 minutes increased tyrosine membrane association of the PKC isoforms a, b, and c, and MAP kinase activation. TPO-induced ERK activation was inhibited partly by BIM. In addition, wortmannin also decreased MAP kinase activity in TPO-stimulated CMK cells. Conclusions. TPO-triggered MAP kinase signaling in CMK cells is dependent on PKC and PI3K activation. These studies show that in the presence of stroma the majority of megakaryocytic progenitors adhere to fibroblasts, whereby their maturation is inhibited. These adhesive interactions are probably not mediated by TPO, VLA-4 or VLA-5. This finding has implications for the early development of HPC, as for preservation of BFU-MK and CFU-MK, contact with stroma may be necessary, whereas for obtaining, mature megakaryocytes non contact cultures are preferable.

PO-0240 Interactions of prothrombin cleavage induce (CA)+ mobilisation in CD34+ haematopoietic progenitor cells
Kaufmann B*, Sauer M, Tauch S, Zieger M*, Zindl F, Nowak G
*Research Group Pharmacological Haematoology and §Division of Paudiatic Oncological and Haematoology, Medical Faculty at the Friedrich Schiller University Jena, Germany

Objective. Since meizothrombin/meizothrombin-desF5 (MTIMT-desF5), catalytically active intermediates of prothrombin activation, have been shown to activate rat aortic smooth muscle cells by interaction with thrombin receptors their function in other cell types responding to alpha-thrombin may be supposed. Methods. CD34+ hematopoietic progenitor cells fresh isolated and frozen were newly thawed and cultured for 10-12 days. The effect of MTIMT-desF5 on free intracellular calcium was investigated in single CD34+ cells using confocal laser CML fluorescence microscopy with fluo-4 as calcium-sensitive probe. Results. Ecarin-activated prothrombin induced very rapidly mobilisation of free intracellular calcium in CD34+ cells with a potency comparable to that observed with alpha-thrombin. This effect could be partly blocked with the anti PAR-1 antibody Mab 61-1. Conclusions. MTIMT-desF5 are able to activate CD34+ cells. This effect is at least partly mediated by PAR-1-type thrombin receptors. Since increase in cytosolic calcium initiates a variety of cellular responses including hematopoietic progenitor cell proliferation and maturation a role of intermediates of prothrombin activation in these physiological processes might be suggested.

PO-0241 Gu/Gi cell cycle differences of bone marrow versus mobilised peripheral blood CD34+ cells: possible explanation for differences in post-transplant engraftment kinetics?
Scheding S, Bergmann M, Becker EW, Buhring HG, Kanz L, Brugger W
Department of Hematology/Oncology, University of Tübingen, Germany

Objective. Transplantation (Tx) of mobilised peripheral blood progenitor cells (PBPC) is characterised by a considerably faster hematopoietic recovery than bone marrow (BM) Tx. This might be due to differences in G0/G1 cell cycle distribution of CD34+ cells. Therefore, we aimed to characterise the cell cycle distribution of CD34+ cells in BM and in CD34+ cells in PBPC.
cycle characteristics of standard BM (n=10) and PBPC (n=12) grafts used for allogeneic Tx. Methods. Following Ficoll density centrifugation, 200,000 cells were analyzed by multi-parameter flow-cytometry utilising TAAO, Ki-67-FITC, and PBPC-conjugated antibodies for simultaneous cell cycle (G0, G1, S/G2/M) and two-color surface marker analysis. Results. (mean±SEM): A lower fraction of CD34+PBPC (5.2±0.71%) was in S/G2/M when compared to BM CD34+ cells (14±2.0%). No significant differences were observed for total cell numbers in BM (G0 45±2.2% vs. PBPC 36±3.9%) and G0 (BM 50±2.5% vs. PBPC 50±4.0%). However, CD34+ subset analyses revealed that a significantly smaller fraction of G0 cells and a higher fraction of G1 cells was found in bone marrow when compared to PBPC (G0 55±3% vs. PBPC 40±2%).

PO-0242 Cytokine-mediated proliferation of AC133+ cord blood cells and selective differentiation to megakaryocytic and erythroid lineages
BMT Unit, “Agia Sophia” Children’s Hospital, Athens, Greece
AC133 is a novel 5 domain transmembrane protein, expressed on a population of stem and progenitor cells that contains all the CD34+CD38- progenitors, as well as the CD34+CD38+ cells committed to the granulocytic-monocytic pathway we investigated the proliferative and differentiative potential of AC133+ cells isolated from human cord blood using immunomagnetic depletion (MACS, Miltenyi Biotec). Purified AC133+ cells from 80-98% with a medium value of 92%, (n=4). We studied the proliferative potential of purified AC133+ cells in culture containing serum-free Iscoves medium supplemented with FLT3 ligand 100 ng/ml, SCF 100 ng/ml, TPO 20 U/ml, and IL-6 20 ng/ml for two weeks. In addition, we investigated the differentiative potential of AC133+ cells in serum-free liquid cultures supplemented with cytokine combinations that favor either erythropoiesis or the megakaryocytic lineage. The proliferative response was at day 7 and then AC133+ cells were reselected, further expanded under the same conditions and assessed again on day 14. We evaluated the following parameters: nucleated cells (NC), committed progenitors (CFU-C), AC133+ and CD34+ cells, and non cycling-stem/progenitor cells HPP-Q (High Proliferative Potential-Questinent). The results of the expansion experiments are shown in the following table.

<table>
<thead>
<tr>
<th>Fold-Expansion of Stem/Progenitor Cells (mean, n=4)</th>
<th>AC133-cells</th>
<th>CFU-C</th>
<th>HPP-Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td>82</td>
<td>26.5</td>
<td>9.8</td>
</tr>
<tr>
<td>Day 14</td>
<td>367</td>
<td>35.2</td>
<td>31.8</td>
</tr>
</tbody>
</table>

AC133+ cells showed an efficient differentiation capacity in response to selected cytokines for either erythroid or megakaryocytic commitment, as was proven by morphological and immunological studies. These results suggest that: (1) the AC133+ cell population is enriched for stem/early progenitor cells as manifested by an increased expansion potential; (2) selected cytokine combinations are able to modulate differentiation of AC133+ cells to megakaryocytic and erythroid lineages.

PO-0243 A novel human multipotential progenitor assay and its application in myelodysplasia
Heasman MJ, Djin B, O’Brien NJ, Pippard MJ
Department of Molecualr and Cellular Pathology, Ninewells Hospital and Medical School, University of Dundee, Dundee, UK

The CFU-A assay detects human CD34+ multipotential progenitor cells with a low cycling status and a high replating efficiency. The aim of this study was to modify culture conditions in an attempt to alter the cellular composition of the CFU-A colonies towards the erythroid lineage at the expense of the granulocyte-macrophage (GM) lineage, and to use this to investigate erythroid differentiation in myelodysplasia. Multiple combination of recombinant growth factors in place of Mia PaCa-2 conditioned medium (CM), and sequential exposure to different growth factors were used to define a CFU-A assay in which myeloid progenitor differentiation produced high proportions of haemoglobinised erythroid cells, suggesting derivation from a multipotential progenitor. Addition of Epo to standard CFU-A cultures (which contained SDF, GM-CSF, and Mia PaCa-2 CM), however, even with a later addition of Epo Epo to standard CFU-A cultures (which contained SDF, GM-CSF, and Mia PaCa-2 CM) showed no significant change in the composition of the BFU-E colonies. Addition of IL-3, a multipotential growth factor, had no notable effect on the composition of the BFU-E colonies, but by omitting GM-CSF and Mia PaCa-2 CM the proportion of the large colonies (>3 mm) which were of the GM lineage decreased as mixed colonies appeared. Furthermore, a later addition of Epo to haemarin did not affect growth of CFU-A (mix) colonies, though the number of BFU-E in the same culture plate was markedly reduced: such CFU-A (mix) are thus likely to have arisen from single pluripotential progenitors and not overlap of erythroid and GM colonies. These studies suggest that Epo was not required for the early growth of CFU-A (mix) but was needed for the maturation of erythroid progeny within such colonies. CFU-A (mix) colonies also grew in a serum-deprived system. All but 2 patients with myelodysplasia (n=14) showed no CFU-A (mix) growth, suggesting a delayed a percentage of heterozygosity of 82%. PCR reaction erythroid and GM development of an early progenitor in the CFU-A (mix) assay provides a potential new approach to the investigation of defective erythropoiesis, and may help in defining prognosis and therapeutic strategies in myelodysplastic syndromes.

PO-0244 Detection of clonality in acute and chronic myeloid leukaemic colonies by HUMARA
Mavrogianni D, Akel S, Mleites J, Yataganas X, Terpos E, Loukopoulos D, Viniou N
First Dept of Medicine, Laikon General Hospital, University of Athens, Greece

The detection of clonal population in patients with acute myeloid leukaemia (AML) and chronic myeloid leukaemia (CMN) after treatment and before autologous transplantation is essential for the prognosis of the disease. Clonality may be evaluated by using the HUMARA (Human Androgen Receptor). A non-random inactivation of this X-linked gene is associated with the presence of a monoclonal population. The aim of this study was the detection and quantification of clonal population in stem cell colonies in patients before autologous transplantation. DNA is first digested with HhaI, a methyl-sensitive enzyme, which will create a restriction site only if the correspondent gene is unmethylated. In this way only methylated alleles are amplified. DNA was extracted from peripheral blood. We applied the polymerase chain reaction (PCR) in colonies from 1 patient with CML and 3 patients with AML. A polymorphic study of 30 women revealed a percentage of heterozygosity of 82%. PCR reaction erythroid and GM development of an early progenitor in the CFU-A (mix) assay provides a potential new approach to the investigation of defective erythropoiesis, and may help in defining prognosis and therapeutic strategies in myelodysplastic syndromes.

PO-0245 Telomere shortening in peripheral blood neutrophils and T cells with aging in healthy individuals
Robertson ID, Gale R, Wynn RF, Robinson SA, Groussakis S, Dougal M, Linch DC, Boyle J, Testa NG, Chopra R
*Paterson Institute for Cancer Research, Manchester; *University College, London; Royal Manchester Children’s Hospital, Manchester, UK

The incidence of haematological malignancies and myelodysplasia increases with age. Telomere shortening, a marker of the aging process, is associated with genetic instability which may predispose to malignancy. The kinetics and regulation of telomere length in normal haemopoiesis remain obscure. Our objective was to define the role of telomere shortening in healthy individuals and to ascertain potential mechanisms for this Telomere shortening and expression were measured in neutrophils and CD3+ T cells isolated from samples of cord blood (n=11), blood from healthy women aged 25-45 years (n=11) and blood from healthy elderly women over 75 years (n=10). Telomere length of peripheral blood leucocytes was also measured in the following age groups: ML cord blood (n=23), 1-9 yrs (n=25), 10-20 yrs (n=13), 21-31 yrs (n=8) and 75+ yrs (n=29). Telomere length was measured by in-gel hybridisation to a P-labelled oligonucleotide probe (C2TA). Telomerase expression was detected using the TRAP assay. The rate of mean telomere length (MTL) shortening in peripheral blood leukocytes was 0.031 Kb/year. The rate of MTL shortening was highest in the first year of life (0.74 Kb/year). A high variation was noted in MTLs of cord blood samples (9.8-13.6Kb) compared to that of the older age groups. Neutrophil mean MTLs were significantly shorter than that of the older age groups. Neutrophil mean MTLs were significantly shorter than that of the older age groups.
ly shorter than T cell mean MTls in 29/31 cases; 0.34KB, 0.36KB and 0.23KB for cord blood, young and elderly samples respectively (p=0.003 for all age groups). This has not been previously described and suggests that the internal divisions between stem and progenitor cells and mature leucocytes are different for T-cells and neutrophils. Neutrophil and T-cell MTls decreased with age and this occurred at the same rate (0.018 Kb/yr (neutrophils)) and (0.036 Kb/yr (T cells)). Either population is therefore suitable for the study of telomere length kinetics. The rate of T cell and neutrophils shortening is the same despite the expression of telomerase by T-cells and no detectable enzyme in neutrophils. Therefore telomerase is not the rate limiting step in the kinetics of telomere shortening and this may explain why the low level of telomerase expression in haemopoietic stem cells is insufficient to prevent telomere shortening.

PO-0246 Isolation and characterisation of a bipotent (megakaryoctic and erythroid) precursor from the spleen of phenylhydrazine-treated mice


*Div. of Hematology, University of Florence, Florence and Lab. of Cell Biology, Istituto Superiore di Sanità, Rome, Italy

To clarify up to what stage haemopoietic cells remain bipotent for megakaryocytic and erythroid lineages, we analyzed (by RT-PCR) the expression of erythroid (β-globin, EpoR) and megakaryocytic (Acte, GPIIb, Myl-) specific genes in single colonies derived from early (BFU-E and day 7 CFU-Meg) and late (CFU-E) erythroid and megakaryocytic progenitors. All the day 7 erythroid bursts and megakaryocytic colonies (out of a total of 73 single colonies investigated) and none of the CFU-E-derived colonies (out of 30 single colonies analyzed) expressed both erythroid and megakaryocytic markers. As expected, GM colonies, analyzed as control, did not express either erythroid- or megaspecific genes. These data suggest that, in vitro, the bipotent cell type is intermediate between BFU-E and CFU-E. In an attempt to identify the bipotent precursor in vivo, we have measured (by FACs) the frequency of cells expressing 4A5 and Ter-119 (megas- and erythroid specific markers, respectively) in the bone marrow and in the spleen of normal mice and of mice recovering from anaemia induced by phenyl-hydrazine (PHZ). A 4A5+ Ter-119+ double positive cell population was recognized in the bone marrow of normal mice. This cell population represented only 3.4±6.0% of normal marrow cells and was undetectable in normal spleen. Their frequency increased to 3.8±0.8 and 1.2±0.6% among PHZ-treated marrow and spleen cells, respectively. 4A5+ Ter-119+ cells have a blast-like morphology, are benzidine- and do not express β globin by RT-PCR. They are distributed throughout all the phases of the cell cycle and, when exposed to either erythropoietin (Epo) or thrombopoietin (Tpo), progress through the cycle to accumulate in Go/G1, within 24 hrs without further proliferation. Stimulation with either Epo or Tpo, induces 4A5+ Ter-119+ cells to express both β globin and GPIIIb within 2 hrs and to accumulate as 4A5+ and Ter-119+ single positive cells with a clear erythroid and megakaryocytic morphology in 24 hrs. In conclusion, we have identified in vivo a late 4A5+ Ter-119+ double positive cell population which is bipotent for the erythroid and the megakaryocytic pathways and may be an important role in the recovery from anaemia in PHZ-treated animals.

PO-0247 AC133 expression on mobilised peripheral blood progenitor cells

Scott MA, Bloxham DM, Jeddice HK, Marcus RE, Craig J

Stemm Cell Laboratory, Addenbrooke’s Hospital, Cambridge, UK

AC 33 is a recently described antigen which is expressed on a subset of CD34+ cells from bone marrow, blood and foetal liver. In this study, we determined the expression of AC133 on CD34+ PBPC from patients undergoing stem cell mobilisation for haematological and solid tumours and from normal donors donating G-CSF-mobilised PBPC for allogeneic transplant. Furthermore, we assessed the potential of the CD34+AC133+ and CD34+AC133− subpopulations to generate chimeric progeny in culture. Flow cytometry, AC133 was found to be expressed on 82% of CD34+ cells from mobilised blood as compared with 55% of CD34+ cells from adult bone marrow (p<0.05). There was no significant difference in the proportion of AC133 expression on CD34+ cells between patients with malignancy mobilised with cytokines and non-mobilised with cytokines and normal donors mobilised with G-CSF alone. Preliminary data suggest that CD34+AC133+ cells express low levels of the apoptotic marker Annexin V. The majority of CFU-GM (80%) were contained in the CD34+AC133+ fraction while erythroid progenitor (BFU-E) were present in both the CD34+AC133+ (49%) and CD34+AC133− (51%) populations. All patients transplanted to date have engrafted rapidly. In the alogeneic setting, days to neutrophils >0.5 and platelets >50 were 13 and 20 (p=0.28) days, respectively. In conclusion, the AC133 antigen may be useful in assessing the quality of mobilised stem cell harvests.

PO-0248 Identification of the new genes involved in human embryonic haematoipoiesis

Pratt S, Teyssier-Le Discorde M, Kinzenbaum M

CDR - DRM - SRHI - Groupe de Recherche sur la Regulaton et l’Expresson des Gene, Hôpital St-Louis, Paris, France

Objective. Haematoipoiesis is the cascade of molecular events by which the blood cells are formed from undifferentiated, pluripotential haematoipoietic stem cells (HSCs). In order to understand the molecular mechanisms that control early human haematoipoiesis, we cloned and identified the genes differentially expressed in haematoipoietic tissues during human embryogenesis. Design and Methods. We compared the expression of mRNAs from the tissues presenting different levels of haematoipoietic differentiation as YS (week 4), AGM region (w3.5-4.5), liver (w7-14), and umbilical cord blood (CB) using RNA Differential Display (RDD) technique. Results and Conclusions. We cloned three CDNAs differentially expressed in early haematoipoiesis. The first, p36-10, is highly expressed in AGM region and early liver (w5) then its expression decreases; its expression in YS and CB is very low. The sequence of p36-10 clone is highly homologous to the DNA break repair genes family. The differential expression of the genes involved in DNA break repair may be relevant in early human haematoipoiesis and in haematoipoietic disorders. The second clone, p36-16, is highly expressed in YS and early liver then rapidly decreases; its expression in AGM as in CB is low. The complete p36-16 CDNA, cloned from human embryonic CDNA library, did not present any homology with genes in the GenBank database. Molecular analysis revealed that p36-16 CDNA may encode an extracellular protein composed of an extracellular domain, transmembrane helices, and a cytoplasmic domain, thus it may be a new marker of HSCs. Moreover, the simultaneous expression of p36-10 mRNA in AGM and early liver may indicate the migration of HSCs from AGM; nevertheless the other HSCs sub-population from YS expressing p36-16 mRNA may also colonise the liver. The third clone, pV18, which did not have any homologues in the GenBank database. Several mammalian Notch molecules and ligands are coexpressed in many differentiating tissues including, haematoipoietic cells. However, it is still unclear the unique role that each homologue may have in the process of controlling cell differentiation in the different contexts. We have previously demonstrated that truncated Notch1 and Notch2 specifically inhibit myeloid differentiation in response to G-CSF and GM-CSF respectively, indicating a connection between Notch function and the cytokine network in haematoipoietic differentiation. We formerly described the Notch Cytokine Regulatory (NCR) domain as being responsible for the specific response of these molecules to G-CSF and GM-CSF. We have now studied proliferation and differentiation of 32D cells expressing Notch2 NCR mutant molecules. Deletions in the NCR region eliminate Notch2 specificity, resulting in inhibition of differentiation by GCSF and GM-CSF; and enhanced proliferation in the presence of IL-3. This deletion contains 2 putative CKI phosphorylation sites (SXXS) which are not present in any other Notch homolog. The truncated Notch2 molecule is highly phosphorylated when expressed in 32D cells. In addition, recombinant CKI is able to phosphorylate the truncated Notch2-GST fusion protein in vitro. We are currently investigating further connections between Notch2 regulation and CKI phosphorylation. These results suggest that regulation of Notch2 function in haematoipoietic differentiation may involve phosphorylation by CKI. This research was supported by ICYTI (SAF98-0052).

PO-0249 Phosphorylation of Notch2 by casein kinase I (CKI): possible implications in haematopoietic differentiation

Ingles-Esteve J, Espinosa LI, Garcia J, Binades A

Departament de Terapia Cellular, lnstitut de Recerca Oncológica, Barcelona, Spain

Several mammalian Notch molecules and ligands are coexpressed in many differentiating tissues including, haematoipoietic cells. However, it is still unclear the unique role that each homologue may have in the process of controlling cell differentiation in the different contexts. We have previously demonstrated that truncated Notch1 and Notch2 specifically inhibit myeloid differentiation in response to G-CSF and GM-CSF respectively, indicating a connection between Notch function and the cytokine network in haematoipoietic differentiation. We formerly described the Notch Cytokine Regulatory (NCR) domain as being responsible for the specific response of these molecules to G-CSF and GM-CSF. We have now studied proliferation and differentiation of 32D cells expressing Notch2 NCR mutant molecules. Deletions in the NCR region eliminate Notch2 specificity, resulting in inhibition of differentiation by G-CSF and GM-CSF; and enhanced proliferation in the presence of IL-3. This deletion contains 2 putative CKI phosphorylation sites (SXXS) which are not present in any other Notch homolog. The truncated Notch2 molecule is highly phosphorylated when expressed in 32D cells. In addition, recombinant CKI is able to phosphorylate the truncated Notch2-GST fusion protein in vitro. We are currently investigating further connections between Notch2 regulation and CKI phosphorylation. These results suggest that regulation of Notch2 function in haematopoietic differentiation may involve phosphorylation by CKI. This research was supported by ICYTI (SAF98-0052).
Poster discussions: Cytokines and growth factors: experimental

**PO-0250** Differentiation of megakaryocytic cell lines and expression of platelet factor 4 and CC chemokines

Fouse A,* Sato T,* Mourit-ronue N, *Golenaker G,* Van Damme J,*
*National Institute of Infectious Diseases, Tokyo, *School of Medicine, Chiba University, Chiba, Japan; *Rega Institute, University of Leuven, Leuven, Belgium

Objective. Beside positive effectors, it is possible that megakaryocytocpoiesis is regulated by negative effectors. It has been reported that several chemokines inhibit in vitro megakaryocytocpoiesis. In this study we assessed the possible role of TPO in the negative regulation of megakaryocytocpoiesis through the induction of chemokines in megakaryocytic cell lines. Design and Methods. The four different human megakaryocytic cell lines at different stages of differentiation were established from the peripheral blood of different individuals. Expression of CD41, a megakaryocyte-associated marker, differed CML considerably between CMK (high), CMY and CMS (medium) and CTS (low). The time of chemokines production was assayed using sandwich EIA systems. TPO and PMA were used as inducers for chemokine production. Results. CMK and CMS (more differentiated cell line) produced platelet factor 4 (pF4) and its production was stimulated by thrombomodulin (TPO). The four cell lines except CMS produced MCP-1 spontaneously. TPO increased the production of MCP-1 in CMK and CMY cells but not in CMS and CTS cells (poorly differentiated cell lines). Production of pF4, MCP-1 and MCP-1α and β 1 and MIP-1b was also induced by PMA in CMK, CMS, CMS and CMS cells, but the rate of induction was higher in more differentiated cell lines. Induction of RANTES and IL-8 by PMA was found in all four cell lines but the amount was relatively low. Another negative regulator of cell growth, TFβ, was produced in all four cell lines non-specifically. MCP-1 and PAF added extracellularly suppressed the growth of CMK and CMY cells but not CMS and CTS cells. Conclusions. TPO induces pF4 and MCP-1 which may function as a negative autocrine regulator of human megakaryocytocpoiesis. These results raise the possibility that megakaryocytocpoiesis may be under the control of positive and negative effects of TPO.

**PO-0251** Vitamin D3 interacts with erythropoietin in the TF1 stem cell line

Ben Alon D,* Nathan H,* Vatner A,* Doudnova A,* Shany S,* Chaimovitz C,*
*Clinical Biochemistry Department Hematology Unit; *Tor Institute. Faculty of Health Sciences, Ben Gurion University of Negev, Beer Sheva, Israel

The effect of vitamin D3 (1,25(OH)2D3) and erythropoietin (Epo) on the TF-1 cell line with stem cell characteristics was investigated. The cells responded to Epo by increased proliferation in a dose-dependent manner. 1,25(OH)2D3, slightly enhanced TF-1 cell proliferation. Simultaneous addition of Epo and 1,25(OH)2D3 had a synergistic effect on cell proliferation as measured by the XTT method, [3H] thymidine incorporation, and trypan blue exclusion. The effect of Ca2+ modulators on TF-1 cells was assessed. The most prominent effect was seen when thapsigargin (10nM), an inhibitor of the Ca2+/ATPase pump of the endoplasmic reticulum, potentiated the proliferative effect of 1,25(OH)2D3 to a level close to that obtained by the combination of Epo and 1,25(OH)2D3. Epo did not potentiate the effect further. The results suggest a role for [Ca2+] in mediating the proliferative effect of 1,25(OH)2D3. However, there was no indication of a role for intracellular Ca2+ in the synergistic interaction between 1,25(OH)2D3 and Epo. The expression of Epo receptor numbers was increased by 1,25(OH)2D3. Up regulation of Epo receptor numbers was invoked by 1.25(OH)2D3. The increase in expression of Epo receptors on the cells induced by 1.25(OH)2D3 and lonomycin behaved in a similar way, thus increasing the proliferative effect of 1.25(OH)2D3 to a level close to that obtained by the combination of Epo and 1.25(OH)2D3. The results point to a novel interaction between 1,25(OH)2D3 and Epo at the level of blood progenitors.

**PO-0252** Serum of healthy donors receiving rhG-CSF induces T-cell unresponsiveness

Rutella S, Rumia C, Sica S, Leone G
Dept. of Hematology, Catholic University School of Medicine, Rome, Italy

The effects of serum from healthy donors receiving rhG-CSF (G-serum) on blast transformation, expression of activation-related antigens, secretion of IL-2 and proliferation were evaluated on allogeneic lymphocytes stimulated with phytohemagglutinin (PHA). Escalating concentrations of G-serum induced 27%, 47% and 70% suppression of lymphocyte proliferation; interstrainly enough, CD4+ and CD8+ cells underwent blast transformation and up-regulated early (CD25, HLA-DR) and late (CD5, CD71, HLA-DR) activation-related antigens. Negligible fractions of apoptotic cells were found after mito- genic challenge, suggesting that the strongly inhibited proliferation was not attributable to extensive activation-induced programmed cell death of responding T-cells. The levels of interleukin-2 (IL-2) in cultures containing G-serum were comparable to those in cultures performed without G-serum; however, high concentrations of exogenous IL-2 restored lymphocyte mito- genesis irrespective of G-serum concentration these findings, i.e. cell enlargement, up-regulation of activation-related antigens, inability to prolif- erate upon mitogenic stimulus and restoration of cell division by exogenous IL-2, resembled lymphocyte partial activation, a fundamental control mechanism for tolerance induction in T cell clones. Soluble immunoregulatory mediators infused with allogeneic haemopoietic progenitor products collected after rhG-CSF administration could induce T-cell unresponsiveness in vivo, thus preventing clonal expansion and amplification of immune respons- es, and could account for the unexpectedly reduced incidence and severity of GVHD compared to that occurring after allogeneic marrow infusion.

**PO-0253** In vitro and in vivo effects of leptin on haematopoiesis in humans

Laheurque P, Fontainville AM, Pénicaud L, Corberand J, Castella L, Laboratoire d’Hématologie et UPRERA CN-IR 5018, CHU Rangueil, Toulouse, France

We previously demonstrated that leptin is secreted at a high level by human bone marrow (BM) adipocytes in culture (Rangueil FASEB J. 1998; 12:747-752). The characterization of a leptin receptor on human haematopoietic stem cells CD34+, and the results obtained in vitro with rodents suggest a putative role of leptin on haematopoiesis. Using a BM colony-forming assay, we showed in the present study that human recombinant leptin treatment of CD34+ haematopoietic progenitor cells from adult BM - dose-dependent- stimulated the appearance of a granulocyte-macrophage colony with 50 (P<.05) and 100 (P<.01) ng/mL leptin. - in presence of 2 U/mL ery- thropoietin, significantly inhibited erythroid colony development, with 100 ng/mL leptin (P<.005). Examination of paraffin blocks of BM biopsies revealed the close association of unilocular adipocytes with haemopoietic ind- icators. Moreover, an immunohistochemical study performed with anti- CD34 antibody indicated that about one third of stained cells were localised at the periphery of adipocytes. The simultaneous determination of BM and plasma leptin concentrations in patients indicated that mean concentra- tion of leptin in BM supernatant (15.6±10.5 ng/mL) was not significantly different from that in plasma (21.6±11.6 ng/mL), but these parameters were strongly correlated (r= -0.946, P<0.001). The concentration of leptin required for an effect on haematopoiesis could be reached through a paracrine mechanism (locally-released leptin from bone marrow adipocytes complemented by an endocrine effect of circulating leptin produced from extramedullary tissues). Thus in conditions with high circulating leptin lev- els such as obesity, inflammation, or chronic infection, an effect of leptin on haematopoiesis could be expected. The recent report of a relationship between leptin concentration and leucocyte count in constitutionally-obese children and adults such as obesity, inflammation, or chronic infection, an effect of leptin on haematopoiesis could be expected. Thus, in conditions with high circulating leptin lev- els such as obesity, inflammation, or chronic infection, an effect of leptin on haematopoiesis could be expected. In addition, in an important role in the regulation of body energy balance, these studies demonstrate a potential role for leptin in the regula- tion of haematopoiesis in humans. These results provide supporting evi- dence that leptin belongs to the family of factors able to modulate the development of the haematopoietic system.

**PO-0254** Mechanism of action studies on myelopoietins: a family of dual IL-3 and G-CSF receptor agonists


IL-3 is an early acting multi-lineage growth and differentiation factor while G-CSF induces proliferation and differentiation of cells committed to the granulocytic lineage. Myelopoietins (MPO) are a family of chimaeric proteins which contain both IL-3 receptor agonist (IL-3RA) and G-CSF receptor ago- nist (G-CSF) activity. MPO chimeras possess in vivo haemopoietic activi- ty which is superior to the co-addition of the individual receptor agonists. The lead MPO is currently in late stage clinical trials for myelo-restoration during standard-dose chemotherapy. The present study serves to define the role of the IL-3RA within MPO by comparing three chimaeras composed of structurally and functionally diverse IL-3 variants and a common G-CSFRA.
The IL-3 receptor agonists include a high affinity, high efficacy agonist (IL-3f15-12550D), a low affinity, high efficacy agonist (IL-3f15-12543N), and an agonist (IL-3f15-12522A) that binds, but does not efficiently activate, the IL-3 receptor. Each IL-3 agonist both activates the G-CSFR with potency similar to native G-CSF. Binding of MPO to both IL-3R and the G-CSFR was demonstrated using surface plasmon resonance analysis. Affinity of MPO for the IL-3R was found to mirror the order of IL-3 activity in the chimaeras: IL-3f15-12550D (Kd=240nM) > IL-3f15-12522A (Kd=350nM) > IL-3f15-12543N (Kd=9000 nM). It was further determined that MPO binding both IL-3R and G-CSF receptors, simultaneously, intercellular signaling induced by the various IL-3R agonists, with activation of both receptors. Despite the diversity of IL-3R activity, these MPO chimaeras demonstrated increased potency compared with the individual receptor agonists or the co-addition of agonists in the colony forming unit assay. These results add insight into the mechanism underlying the biological activity of MPO.

PO-0255 The synergistic effect of thrombopoietin in erythropoiesis and myelopoiesis from human bone marrow cells

Liang D-C,* Shih L-Y,* Kuo M-C,* Chen S-H, * Liu H-C,* Shimosa A,* * Mackay Memorial Hospital; *Chang Gung Memorial Hospital, *Taipei, Taiwan; *Kirkby, Tokyo, Japan

Objective. To determine whether recombinant human thrombopoietin (TPO) acts synergistically with recombinant human erythropoietin (EPO) and/or recombinant human interleukin-3 (IL-3) on erythroid burst formation and granulocyte-macrophage colony formation from human bone marrow (BM). Design and Methods. BM cells from 3 adults and 13 children, who had undergone a BM examination because of a clinical suspicion of malignancy but whose BM as well as complete blood counts were normal, were cultured in a methylcellulose system. Non-adherent BM MNCs (10,000/mL) were plated in TPO 400 ng/mL, EPO 2 U/mL, IL-3 50 ng/mL or G-CSF 50 ng/mL was used. Results. The addition of TPO to EPO significantly gave rise to 20 samples were examined more erythroid bursts (means±SEM 158±28 vs 151±27). The addition of BM MNCs to IL-3 and EPO yielded more than 2-fold increase in the growth of BM progenitors and their activity on AML blasts. Blasts from 17 cases of human primary AML (FAB: 2 M1, 6 M2, 6 M4, 3 M5) were purified of lymphocytes and GM-CSF was the most potent inducer of TNF production in AML M4-5 cases.

PO-0256 TNF-α induced maturation and apoptosis are exclusively mediated by CD120a in primary acute myeloid leukemia blasts

Santini V, Gozzini A, Scapini B, Rossi Fentini P

Cattedra e U.O. di Ematologia, Policlinico di Careggi, Università di Firenze, Italy

Acute myeloid leukemia (AML) is characterised by a blockage in maturation, which is sometimes partially overcome by stimulation with differentiating agents and cytokines, indicating that the maturation potential of AML cells is to some extent conserved. It has been demonstrated that TNF-α may induce monocytic maturation in normal hematopoietic precursors and in AML cell lines. AML cells express on their surface two different receptors for TNF-α: p55 TNF-receptor type I CD 120a and p75 TNF-R type II CD120b. These receptors are expressed at different densities and numbers, which may vary by TNF-α subtype, can respond to TNF-α with monocytopoiesis and subsequent apoptosis. Both effects appear to be mediated exclusively by TNF-R I (p55).

PO-0257 Haematopoietic expression of an interferon-inducible gene

Bond HM,* Bonelli P,* Stefanianni P,* Agpolo V,* Frigeri F,* Luciano L,* Mesuraca M,* Lamberti A,* Montonaro D,* Tuccillo F,* Cerra M,* Cecco L,* Morone G,* Venuta S,* *Dept of Experimental Haematology, National Cancer Institute, Fondazione G. Pascale, Naples; *Dept of Haematology, University Federico II, Naples; *Dept of Experimental and Clinical Medicine, University of Catanzano, Catanzano, †CEMGE Advanced Biotechnology, Naples, Italy

The technical representation of differential analysis (RDA) was applied to identify genes whose expression was regulated during in vitro differentiation of the immature myeloid cell line KG-1 in response to TPA and ionomycin. One of the cDNAs thus obtained corresponded to a transcript not detectable in untreated KG-1 and termed KIG-8, which had a high degree of homology with two recently described genes that are induced by all-trans retinoic acid (ATRA) or interferon-γ (IFN-γ) and mediate the initiation of the myeloid and lymphoid cell lines tested. In fresh peripheral blood leukocytes, a strong induction of KIG-8 in response to IFN-α was observed in purified granulocytes (4-fold), whereas a milder induction occurred in T-lymphocytes. In chronic myeloid leukaemia (CML) cells, in vitro treatment with IFN-α resulted in accumulation of KIG-8 mRNA, and detectable levels of the transcript were observed in cells from the majority of patients treated with IFN-α. The further characterisation of the expression and of the biological properties of KIG-8 may provide insight into the functional role of this protein and its involvement in interferon-mediated growth regulation and its potential usefulness as an indicator of responsiveness to interferon.

Support from AIRC, CNR, FSN, FSR, MURST (40 and 60%) grants.

PO-0258 Influence of rhGM-CSF on TNF and IFNs production by peripheral blood mononuclear cells of patients with acute leukemias

Husli S,* Kaminska T,* Dmowskiinska A,* Kandefer-Szerszen A,* Wailer-Croneck A,* Koltysz M,* Cloch M,* W Wojtaszek M,* *Department of Haematology, “Wojciech Gotlib” School of Medicine; °Department of Virology and Immunology, M. Curie University, Lublin, Poland

The aim of this study was to evaluate the influence of rhGM-CSF on stimulated TNF and IFNs secretion by PBMC of 23 patients with acute leukemias (ALL 17 AML, 6 ALL). We studied cytokine production in supernatants from PBMC short-term cultures. NDV, LPS, PHA and ConA were used as cytokine inducers. Before each experiment, samples were incubated with GM-CSF (50ng/mL). TNF activity was measured by biologic assay and expressed as a percentage of cytokotoxicity in L929 cell line and IFN activity by inhibition of cytopathic effect assay. Mean values of TNF levels were: 64.8±10.3 pg/mL vs 95.6±11.8 pg/mL (p<0.05). Mean values of IFN levels were: 40.5±2.6 U/mL vs 62.3±1.7 U/mL (p<0.02). The further characterisation of the expression and of the biological properties of KIG-8 may provide insight into the functional role of this protein and its involvement in interferon-mediated growth regulation and its potential usefulness as an indicator of responsiveness to interferon.

Support from AIRC, CNR, FSN, FSR, MURST (40 and 60%) grants.
Experimental transplantation

PO-0259 Th1 and Th2 cytokine production by peripheral blood mononuclear cells of patients with acute leukemias in short-term culture

Huska J, Dmoszynska A, Kaminiska T, Kanderer-Szwerska A, Waler-Cronneck A, Koztyn M, Ciacho M, Wolozacki M.

Objective. The aim of this study was to determine the differences in spontaneous and stimulated secretion of TNF and IFNs by various types of leukaemic blasts in PBMC short-term cultures. We studied cytokine production in culture supernatants of 57 patients with AL (47 AML, 10 ALL) vs 25 healthy controls. This technique defines the frequency of Th 1 and Th2 populations. We compared cytokine produced by blasts of patients with ALL and AML M0-M3 and M4-M5. Tand the subsequent IL chronic phase is characterised by Th2 type cytokines (e.g. IFN-γ) reduced compared to BMT. This has been attributed to the reduced immunosuppressive activity of cytokines of Th2 type. The table shows that patient's adherence cells (mean value x10^4 and samples examined in brackets).

<table>
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<th>Cell type</th>
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<th>Coll IV</th>
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<td>27.7 (23)</td>
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<td>PB</td>
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<td>43.4 (19)</td>
<td>57.6 (22)</td>
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Statistical analysis (Student's t-test) did not show any significant difference between PB and CB leukocyte adhesion to ECM components. Conclusions. CB and PB-recovered leukocytes showed a similar capacity to bind ECM proteins, thus rendering it unlikely that adhesion properties are directly implicated in the mild GVHD observed after CB transplantation.

PO-0260 CD31+CD45ra+ naive lymphocytes can achieve similar levels of IFN-γ and IL-4 production as CD3+CD45ro+ memory cells

Perez-Cruz I, Fallen P, Madrigal JA, Cohen SBA.

Objective. CD31+CD45ra+ naive lymphocytes can achieve similar levels of IFN-γ and IL-4 production as CD3+CD45ro+ memory cells. This technique has since been used as an alternative to bone marrow transplantation (BMT). The most common complication of BMT is graft-versus-host disease (GVHD), which mediates a Th1 response characterized by IFN-γ production. The table shows that patient's adherence cells (mean value x10^4 and samples examined in brackets).

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**PO-0263** Neutrophils are essential mediators in interleukin-8-induced mobilisation of hematopoietic progenitor cells (HPC)

Prolli et al.,

*Department of Hematology, Usc University Medical Center, The Netherlands; **Department of Immunology and Cell Biology, Mario Negri Institute, Milan, Italy*

Previously, we have shown that the rapid (20 min) IL-8-induced mobilisation of HPC could be completely prevented by antibodies against 12/interleukin-8 receptor (LFA-1/CD11a) as well as by antibodies against metalloproteinase gelatinase A (MMP-9). We then hypothesised that neutrophils (PMN), which express LFA-1, and high affinity IL-8 receptors, and release MMP-9 upon activation by IL-8 would serve as key regulators in IL-8-induced stem cell mobilization. To study this, Balb/c mice were treated with a single dose of 250 μg anti-Gr-1 mAb (RB6-8C5). The number of PMN in the blood was assessed by differential counts and the % of PMN in the BM by FACS analysis. Absolute neutropenia was induced up to 5 days after injection. Then a profound neutropenia was observed at day 7 followed by return to baseline levels at day 14. Antibody treatment reduced the proportion of PMN in the BM and increased the proportion of immature myeloid cells without affecting the number of HPC. Groups of mice were then treated with a single dose of 30 μg IL-8 or saline at day 0 or day 1 or 7 after injection of anti-Gr-1 mAb. After 20 min, the mice were killed and blood and BM was obtained to assess the number of HPC in semi-solid cultures containing MFG-CSF (1.25 ng/mL). Addition of the anti-Gr-1 mAb to colony cultures had no inhibitory activity. The IL-8-induced mobilising capacity was completely absent during the neutropenic phase and was increased during the neutrophilic phase.

**PO-0264** Analysis of CD34 positive cells for the bcr/abl gene fusion in mobilised peripheral blood stem cells and bone marrow

Irving JE,

*Department of Haematology, Newcastle, UK*

Objective. To investigate the Ph status of CD34+ cells in mobilised PBSC harvests and to compare it with that of CD34+ cells isolated from chronic phase BM harvests, taken several weeks prior to mobilising chemotherapy. Methods. CD34+ cells were purified from 1x10^6 mononuclear cells from BM or PBSC harvests using MiniMACS columns. Isolated cells were assessed for purity by flow cytometry and prepared for FISH by standard cytogenetic methods. FISH was performed with directly labelled fluorochrome probes to bcr and abl (Vysis) and examined using a standard fluorescent microscope. Results. Ph positivity in PBSC CD34+ populations from 14 different patients was always detectable either by FISH or one round PCR methods. The median number of Ph negative cells in these populations was 79.05% compared to 14.95% in BM harvests (p=0.0163, Mann Whitney). Evaluation of paired PBSC and BM harvests were performed in 11 individuals; higher levels of Ph negative CD34+ cells (median 14%) were isolated from BM harvests correlated with higher levels of Ph negative CD34+ cells (median 76.8%) in autologous PBSC harvests (Spearman correlation, p<0.0008). Irregular correlations between Sokal scores and levels of CD34+ Ph negative cells in both BM and PBSC harvests were also found (Spearman correlation, p=0.006 and 0.036, respectively). Conclusions. Levels of Ph negative haemopoiesis after autotransplant have been shown to correlate with numbers of Ph negative cells in CD34+ grafts (1), thus a more accurate assessment of malignant contamination of autograft material requires investigation of the Ph status of the CD34+ population. In our series of patients, mobilised PBSC harvests contained significantly higher levels of Ph negative CD34+ cells compared to chronic phase BM harvests, but malignant contamination was always detectable in these isolated populations. Estimation of Ph negative CD34+ cells in BM, prior to mobilising chemotherapy may be useful for excluding those patients for whom the risk, morbidity and expense of PBSC harvesting may have no apparent benefit over a chronic phase BM harvest.


**PO-0265** The GvH reaction: the “cytokine storm” and its effect on lymphocyte alloreactive responses

Koh BC,

*Department of Haematology, Royal Free & University College Medical School RF Campus, London, UK*

Graft versus host disease (GvHD) remains one of the major complications of allogeneic bone marrow transplantation and the “cytokine storm” which occurs during conditioning is intimately involved in its pathogenesis. We have investigated the effects of various cytokines on the cell surface expression of major histocompatibility antigens and adhesion molecules on T lymphocytes. Peripheral blood mononuclear cells (PBMCs) were obtained from 9 healthy normal volunteers. TNF-α, γ-IFN and IL-6 were added in increasing concentrations, alone and in combination to the PBMCs. We looked at W632 (MHC Class I) and HLA-DR expression (MHC Class II) as well as the following adhesion and costimulatory molecules; CD54 (ICAM-1), CD11a (LFA-1), CD80 (B7.1), CD86 (B7.2) and CD49d (VLA-4). The cells were incubated with the cytokines for 24 hours and analysed via flow cytometry using 3 colour dye combinations. We compared the median fluorescent intensity of the various cell surface molecules against that of the untreated control as well as the percentage of cells that were positive. IL-6 alone had no discernible effect on the incidence or level of expression of any of the above molecules. γ-IFN produced a four-fold increase in the cell surface expression of Class I, 25% increase in Class II and a small increase (<50%) in the numbers of cells expressing CD54 although the median fluorescent intensity remained the same. γ-IFN produced similar changes as TNF-α but the increases were substantially larger: up to 2 log increase in Class I expression and up to 3 times the number of cells expressing CD54. Unlike TNF-α, γ-IFN had no effect on HLA-DR expression on T lymphocytes although it is a potent stimulator in other tissues such as skin. Significantly, when combinations of TNF-α and γ-IFN were used they displayed synergistic effects. We also noticed a saturating effect of the cytokines in that higher concentrations of the cytokines did not produce corresponding increases in surface molecule expression. CD80, CD86, CD11a and CD49d expression were not affected by any of the cytokines tested, either singly or in combination to the PBMCs. We therefore conclude that TNF-α and γ-IFN affect the level of expression of various cell surface molecules integral for antigen recognition and cell-cell contact. This would influence the alloreactive responses seen between donor and recipient, leading to varying degrees of the GvH reaction.

**PO-0266** Interleukin-12 (IL-12) enhances differentially both graft-versus-host (GvH) and graft-versus-leukaemia (GvL) reactions

Schmitt M,

*University of Ulm, Germany*

In a bone marrow transplantation (BMT) model of DBA/2 x BALB/c mice (major histocompatibility complex matched, but minor histocompatibility antigen mismatched), GvH and GvL reactions were studied. As T lymphocytes and natural killer (NK) cells are considered to be effector cells for the GvH reaction, and IL-12 is known to be a potent inducer of both cell populations, we examined whether the administration of IL-12 results in the aggravation of GvH. Simultaneously, we assessed the outcome of the GvL reaction by using the syngeneic cell line RL-1. A dose of 200 ng of IL-12, when administered subcutaneously to the recipient mice at the time of BMT and two days later, was lethal in all of the mice receiving allogeneic transplants, but in none of the recipients of syngeneic BMT. Histopathological analysis showed lymphocyte infiltrations and apoptotic bodies in the liver and the colon. In contrast, administration of only 25 ng of IL-12 did not result in the aggravation of GvH, but in a ten-fold increase of the GvL reaction. Effector cells of GvH were activated CD8+ T lymphocytes, whereas GvL was mediated by CD8+ T cells. NK cells were not involved in any reaction. As shown by antibody in vivo blocking experiments, the effect of IL-12 on GvH was mediated by interferon-γ (IFN-γ) whereas its effect on GvL was IFN-α independent. In summary, low-dose IL-12 was revealed to be an important tool to enhance the GvL reaction without aggravation of the GvH reaction, opening new immunotherapeutic options for BMT strategies.
Poster discussions  Clinical transplantation I

PO-0267 Results of high-dose therapy and autologous peripheral blood stem cell transplantation in patients with lymphoma

Varo MJ, Palomera L, Azaceta G, Cajal R, Moreno JA, Mayordomo JL, Tubero A, Olaye T, Fuertes MA, Arruza MA, Sola C, Tres A, Gutierrez M, Department of Haematology and *Medical Oncology, University Hospital, Zaragoza, Spain

Background. High dose chemotherapy (HDC) and autologous peripheral blood stem cell transplantation (PBSCT) is a promising treatment for non-Hodgkin’s lymphoma (NHL) and Hodgkin’s disease (HD). Methods. From Oct/93 to Feb/98, 27 consecutive patients (pts) with NHL (N=22) and HD (N=5) underwent PBSCT at our institution. There were 17 males and 10 females. Median age was 46 years (range 22-63). Disease status: 16 pts (13 high grade NHL and 3 HD) were in 1st complete or partial remission; 9 pts were in 1st relapse (7 NHL 6 high grade and 1 low grade- and 2 HD) and 2 NHL in 2nd or further relapse. Induction therapy for NHL was CHOP (± escalated dose) for newly diagnosed pts. and MINE or E-ESHAP for relapse. HD pts received ABVD or hybrid MOPP/ABV (first line) or MOPP for CNS disease. The conditioning regimen for patients: the group of autologous patients utilized Cyclophosphamide, BCNU and etoposide (CBV) for 15 pts (10 NHL and 5 HD) and BEAC or BEAM in 12 NHL pts. Results. Median number of CD34+ cells infused was 3.7 ± 1.0 10^6 (1.4-8.8). Engraftment was prompt and predictable with a median 10 days (9-25) to reach ANC >0.5 10^9/L and 11 days (8-34) to platelet independence. All pts had neutropenic fever, mucositis and diarrhea (WHO grade 2-3). No grade 3-4 hepatic and renal toxicity were noted. One patient developed P. Carinii pneumonitis. Four pts died with progression at day +42, +62, +90 and +101 and one died of urinary sepsis at day +70. Twenty pts achieved CR and remain disease-free at a median follow-up of 23 months (range 2-35). Two pts are alive with relapse. Actuarial 3-year disease free survival and total survival (DFS/TS) are 62.8/81.5%. Three-years DFS/TS according to disease status for NHL is 92/39,2% for first line and 31/55% for first or further relapse. For HD, actuarial 3-year DFS/TS is 100/100%. Conclusions. HDC with PBSCT is highly active in this setting of unfavorable NHL and HD pts with acceptable toxicity. Although results of further randomised trials are needed, early HD with PBSCT for poor prognosis lymphoma is safe and effective.

PO-0268 Detection of residual leukaemic cells in PBSCT from patients with AML and trisomy 8 predicts early relapse after autologous transplantation


The impact of minimal residual disease (MRD) analysis in peripheral blood stem cell transplantation (PBSCT) apheresis and marrow graft for autologous transplant varies between the different types of leukaemia. In AML-M3 the persistence of PMU RARAα transcript in the pretransplant analysis is predictive of relapse in virtually all cases. In AML-M2 with a t(8;21) translocation no clearcut relationship has been reported between the number of AML/E TO transcripts and the incidence of leukaemic post-autotransplant relapses. In AMLs characterised by trisomy 8, low levels of MRD have been found, with FISH analysis, in patients who do not subsequently relapse; however, little is known about the clinical outcome of patients undergoing autologous transplant with respect to the MRD status of the engrafted stem cells. We report two cases of AML-M4 with +8 at diagnosis. Both patients were treated with the AML10 protocol and reached a complete haemotologic remis- sion; one after one course and one after two courses of chemotherapy. PBSCT was performed in both cases after a further course of ther- apy and tested by FISH with an α-satellite probe for chromosome 8. In pt. 1, three signals were observed in 1,6% of BM cells and 3,3% of PBSCT; in pt. 2, three aphereses resulted in 2,2, 1,8 and 1,2% of trisomic cells. In normal controls, the percentage of cells showing three spots with the val- ues of trisomy 8 cells exceeded the mean±2 SD of the controls and all sam- ples were considered to be positive for MRD. Autotransplant was done fol- lowing the protocol with a conditioning regimen of BuCy; both patients relapsed, one 4 months, the other 2 months, after autotransplant. Although more data are needed, these results suggest that the persistence of MRD, as detected by FISH, in stem cell collections is associated with a poor outcome in AML patients with trisomy 8 undergoing autologous trans- plant and indicate the need for alternative therapeutic regimens in this subgroup of patients.

PO-0269 Amifostine in a high-dose alkylating therapy for mobilisation or conditioning autograft procedure

Santini G, De Souza C, Marino G, Congiu S, Nalb S, Damasio E, Department of Hematology, S. Martino Hospital, Genoa, Italy

Clinical trials suggest that Amifostine may protect normal tissue without compromising anti-tumour response. Administered prior to chemotherapy or radiotherapy, it may provide a broad spectrum of cytoprotection including from drugs such as alkylating agents, cis-platinum and anthracyclines. We report a phase II study using Amifostine as drug protection against high-dose cyclophosphamide (HDCY)(7 g/m2) used to reduce tumour burden and to mobilise peripheral progenitor cells, and against high-dose melphalan (from 140 to 200 mg/m2) used alone or as part of a combined condition- ing autotransplant regimen (HDT). We enrolled 26 pts, in the study, who received 36 high-dose treatments (HDCY=17 pts.; HDT=19 pts.). We compared these patients with a previous group of patients who had received simi- lar treatment without Amifostine protection. Amifostine was used at the dosage of 740 or 910 g/m2. The most common side effects were nausea and vomiting (47%), hypotension (44%), hypocalcaemia (20%) and flu-like syndrome (5%). Only a few cases required therapy and side effects were easily controlled. The median time of haemotological recovery assessed in time (days) to achieve 500 PMN/ml and 20 000 PLT/ml and fever or infections were similar in both groups of patients. The most important results in favour of Amifostine were the reduction of the frequency and severity of HDT-related cardiac, pulmonary, renal and hepatic damage (5% vs 9% grade I vs grade II-IV toxicity). The use of Amifostine was also associated with a reduction of mucositis (37% vs 100%), cardiac and pul- monary toxicity (0% vs 8%) both HDT related. We conclude that Amifostine protects normal tissue from HDCY- and HDT-related non-haematological toxicity. Amifostine does not reduce time of haemotological recovery, fever or infections following high-dose chemotherapy.

PO-0270 A single-centre experience of autologous bone marrow transplantation for acute lymphoblastic leukaemia in an adult transplant unit

Douglas K, Liakopoulou E, Laing JE, Campbell M, Tansey P, Parker AM, Barnett AK, Franklin IM, Bone Marrow Transplant Unit, Glasgow Royal Infirmary Hospital, Glas-gow, UK

Objective. To determine the long-term outcome in all patients treated with autologous bone marrow transplant for acute lymphoblastic leukaemia (ALL) between May 1984 and January 1994. Design and Methods. Retros-pective analysis was carried out in all patients identified as received an autologous transplant for ALL. Results. A total of 21 patients received autologous bone marrow transplant for ALL at our institution in this period. The sex distribution was 20 male, 1 female, with a median age of 21 (range 14-48). The subtype was T-cell ALL in 9 patients, common ALL in 6, early pre-B in 2 and unspecified ALL in 4 patients. The conditioning regimes used varied over time. Fourteen patients received melphalan and total body irradiation (TBI), three received etoposide & TBI; one received cyclophosphamide & TBI: and three patients who had received prior radiotherapy were conditioned with busulphan and cyclophosphamide. Overall 5-year survival was 57% (12 of 21 patients), with disease-free 5-year sur- vival of 47% (10 of 21 patients). Nine patients (43%) suffered relapse post-autograft: of these, seven have died and two remain in prolonged second remission following further treatment. Of the nine patients who died, seven- en died of relapsed disease, one of peri-transplant systemic sepsis, and one of hepatic insufficiency associated with hepatic C. Some significant late complications of the procedure were observed. Six patients developed iron overload with abnormal liver function of these, three improved with venesection. Two patients acquired hepatitis C as a result of the transplant procedure, one of whom has died. One patient developed cataracts requir- ing surgery. However, all twelve long-term survivors appeared to have nor- mal or near-normal functional status. Conclusions. Autologous bone marrow transplant appears to be an effective therapy for ALL, as seen in other studies, with an overall 5-year survival of 57% in this series and an acceptable quality of life in the long-term survivors.

PO-0271 Matching for HLA and other polymorphic genetic systems in unrelated bone marrow transplantation


The most successful bone marrow transplants in the treatment of haema- tological disorders have to date been seen between HLA-identical siblings, however, only 30% of patients have these donors available to them. It is
therefore possible to perform a bone marrow transplant between unrelat-
ed individuals, as long as there is some level of HLA identity. It was origi-
nally thought that matching for class II alleles was more important than for class I, but recent publications show that class I matching is just as impor-
tant. With HLA identity at the classically matched loci, however, complica-
tions are still seen post transplant, such as Graft versus Host Disease (GVHD) and graft failure. It is possible that these complications arise as a result of mismatches between HLA and other polymorphic alleles which are not routinely matched. We are collecting DNA samples from the pre-transplant blood of patients and their unrelated donors provided by the Anthro-
...
severe portal hypertension and intractable ascites. This was performed on Day +181 without any bleeding or thromboembolic complications. The hepatic venous pressure gradient fell from 27 mmHg to 9 mmHg post TIPS. The patient’s body weight started to drop 9 days after the procedure and fell progressively from 128 kg to 100 kg, over a period of 5 weeks. The patient required no further ascitic tap post TIPS and diuretics were stopped 16 days after the procedure. Serum bilirubin stayed the same. The patient discharged home and thus seen frequently as outpatient. The patient died 52 days after the procedure from a short illness precipitated by Candida pneumonia and ended with hepatic encephalopathy and multiorgan failure. In adults, TIPS might be a suitable treatment procedure to relieve intractable ascites, awaiting liver transplantation for end-stage liver failure from VOD. (Histological and radiological illustrations together with literature review will be exhibited on the Poster).

PO-0276 Long-term follow-up of pulmonary function after autologous transplantation for multiple myeloma

Lannemeyr L, Johansson A, Mielkevitz U-H
Departments of Haematology and Clinical Physiology, Sahlgrenska University Hospital, Gothenburg, Sweden

Autologous stem cell transplantation (ASCT) is a generally accepted treat-
ment for multiple myeloma (MM). Forty-nine patients underwent a region-
al programme using 9 total body irradiation (0 Gy in 4 fractions), BCNU (300 mg/m²), regimens with known pulmonary toxicity; and melphalan (140 mg/m²) as conditioning treatment. Three patients died in an ARDS-like syndrome 12 and 14 months after transplantation. In the ASCT-related mortality in this cohort 6%. Our aim was to investigate the pul-
monary function in patients with non-progressive disease. Twenty-one patients were included and were invited to the study and of these, 16 patients (7 male), median ages 56 (range 39-69) were interviewed and examined a median 34 months (10-72) after ASCT. Vital capacity and forced expiratory flow were examined by single-breath technique and diffusing cap-
acity (DLCO) was measured with the carbon monoxyde single breath tech-
nique. The results were adjusted for haemoglobin and corrected to pretransplant tests results. A significantly impaired (p<0.0001) DLCO, 74.6% (range 54.5-89.4) of predicted, was seen in the pretransplant tests. After ASCT slight restrictive pulmonary changes were found but no signifi-
cant change in diffusing capacity. Four of the patients suffered from dys-
phonia during early activities of daily life. No correlation was found between respiratory symptoms and measured pulmonary function. These results imply that the measurable long-term pulmonary effects of ASCT with the used regimen in MM are minor. Reduced diffusing capacity prior to trans-
plantation is a common finding in these patients and we have therefore launched a follow up study to evaluate the pulmonary function at diagno-
sis and during subsequent treatment.

PO-0277 Extramedullary relapse after allogenic stem cell transplantation

Garcia Malo MD, Gómez-Espino J, Moraleda JM, Vallejo C, de Arriba F, Sánchez I, Corral J, Vicent V
Oncology Haematology Department, Hospital General Universitario, Murcia, Spain

Introduction. Extramedullary relapse after allogenic stem cell transplan-
tation (alloSCT) is a rare event and is usually followed by systemic relapse. Serum LDH was studied by systemic relapse only. Serum LDH was studied. It has been suggested that the presence of a complete chimera in bone marrow at the time of relapse is a favourable prognostic factor. Patients and Results. Case #1: A 38 year-old woman diagnosed as having AML-M0 received an alloSCT in first complete remission (CR) after cyclophos-
phamide and TBI in March 1996. She developed an acute and chronic GVHD that were treated with steroids. In July 1997 she presented with a painful node in her right breast. A fine needle aspira-
tion showed infiltration with blast cells of lymphoid lineage. The extension of relapse was found in bone marrow, using D1S80 and SE33 microsatel-
lites amplified by PCR. Conclusions. The best treatment option for extramedullary relapse after alloSCT needs to be defined, specially if there is no medullary relapse. Our two cases achieved CR with no subsequent systemic relapse after treatment with local radiotherapy.

PO-0278 Autologous hematopoietic stem cell transplant in women with metastatic or high risk non-metastatic breast cancer

Sánchez I, Ayala F, Moraleda JM, Heras I, de Arriba F, García-Malo MD, Perez Ceballos E, Vicente V
Department of Oncology, Hospital General Universitario, Murcia, Spain

Aims. To analyse retrospectively our experience in women with metastatic and high risk non-metastatic breast cancer treated with high-dose chemotherapy and hematopoietic progenitor cell support. Methods. Between 1993 and 1997, 58 patients were treated. Their median age was 38 (29-61). Sixteen patients had metastases: 9 had only one and 7 two or more locations: lymph node (9), lung (3), local (2), bone (2) and liver (2). Two patients were disease-free after surgery for a second breast relapse. Fifteen of 16 had received adjuvant chemotherapy (with anthracy-
clines in 5 cases). At autotransplant 15 were in CR and 4 in PR. In 42 patients transplant was performed in adjuvant setting (S=IV+III). TNM: T1TL3, T1-4, T1-4, T4-d0, Tm2; N1(N=0), N1=3, N2=2, N3=1. Posi-
tive axillary lymph nodes: T4a=1, T4b=1, T4c=1, T4d=0, Tm2=5, N1=2, N2=0, N3=0. A total of 13 patients received 2, 2 and 14 months after transplantation, making the ABSCT- like condition, 2, 2 and 14 months after transplantation, making the ABSCT-
related mortality in this cohort 6%. Our aim was to investigate the pul-
monary function in patients with non-progressive disease. Twenty-one patients were included and were invited to the study and of these, 16 patients (7 male), median ages 56 (range 39-69) were interviewed and examined a median 34 months (10-72) after ASCT. Vital capacity and forced expiratory flow were examined by single-breath technique and diffusing cap-
acity (DLCO) was measured with the carbon monoxyde single breath tech-
nique. The results were adjusted for haemoglobin and corrected to pretransplant tests results. A significantly impaired (p<0.0001) DLCO, 74.6% (range 54.5-89.4) of predicted, was seen in the pretransplant tests. After ASCT slight restrictive pulmonary changes were found but no signifi-
cant change in diffusing capacity. Four of the patients suffered from dys-
phonia during early activities of daily life. No correlation was found between respiratory symptoms and measured pulmonary function. These results imply that the measurable long-term pulmonary effects of ASCT with the used regimen in MM are minor. Reduced diffusing capacity prior to trans-
plantation is a common finding in these patients and we have therefore launched a follow up study to evaluate the pulmonary function at diagno-
sis and during subsequent treatment.

PO-0279 Filgrastim stimulated whole blood transplantation in 47 patients with relapsed non-Hodgkin’s lymphoma

Jonkhoff AB, Huijgens PC, Schuurhuis GJ, O. sensenkopp G
Department of Haematology University Hospital Vrije Universiteit, Amst-
terdam, The Netherlands

Background. High dose chemotherapy with stem cell rescue results in a 50% cure rate in relapsed non-Hodgkin’s lymphoma (NHL) patients. A drawback of autologous stem cell transplantation is a combination of dif-
ficulties including facilities for leukapheresis, logistics and costs. Some years ago we started to use 1 liter of filgrastim (10 µg/kg, 5 days) stimu-
lated whole blood (WB) as stem cell rescue after semi-ablative high dose melphalan (HDM) in multiple myeloma. Compared with a historical con-
trol group (20 pla) WB rescue after HDM in 51 patients resulted in a reduc-
tion of mortality from 20% to 6% and of median hospital stay from 43 to 17 days. Design and Methods. Subsequently WB was used in patients with relapsed or refractory NHL using a myeloablative regimen consisting of BCNU 300 mg/m² (d1), ARA-C 6 gr/m² (d2) and melphalan 140 mg/m² (d3) (BAM), and re-infusion of WB (stored unprocessed at 4°C) after 72 hr. Four mobilisation schedules using different dosages of filgrastim with or without IVMP chemotherapy were tested. Steady state mobilisation with filgrastim 12 µg/kg b.i.d. was used in the last 15 patients. A back-up leu-
kapheresis was available in all cases. Six out of 47 patients got their back-up stem cells re-infused because of insufficient platelet recov-
ery at day 32. The WB contained: CD34+ 0.36±10⁹/kg (range: 0.3-3.61) and CFU-GM: 6.41±10⁹/kg (range: 0.3-135) (median values). A threshold of 0.3±10⁹/kg CD34+ was defined to ensure save haematological recov-
ery. Hematological recovery was as follows: WBC>1.0×10⁹/L 14 days (10-27); platelets>100×10⁹/L 23 days (11-87) and hosi-
larly recovery 18 days (range: 13-31). Platelet recovery was improved i.e. 18.5 and 17 days with CD34+ thresholds at 0.5 (n=14) and 1.0×10⁹/kg (n=7) respectively. No patient needed a back-up re-infusion when transplanted with 9.3±10⁹/kg CD34+. Colony-forming units (CFU) >17 G/Ml were always achieved at both chimera with a median of 9 months. Back-up WB was reinfused in 6 patients with >1.0×10⁹/L 12 days (range: 11-16); platelets>20×10⁹/L 17 days (range: 11-23) and hospital stay: 18 days (range: 13-25). Conclusions. WB trans-

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Chimaerism studies in allogeneic peripheral blood stem cell transplantation with CD34 selection

Senet et al., Gonzalez Y, Sanz GF, de la Rubia J, Jimenez C, Perez-Sirvent M, Gomis F, Sempere A, Sanz MA

Hematology Department, Hospital Universitario La Fe, Valencia, Spain

T cell depletion is associated with an increased incidence of mixed chimaerism, graft failure and leukemia relapse after allogeneic haematopoietic stem cell transplantation. Donor T cells may play a major role in eliminating residual host immunocompetent lymphocytes that survive conditioning. This report describes haematopoietic chimaerism in 11 patients who underwent allogeneic peripheral blood stem cell transplantation (allo-PBSCT) with CD34 selection performed by means of immunobiovirum columns (Ceptrak). Median age was 35 yr (range 20-51 yr). Four patients had AML, 2 ALL, 2 MM, 1 NHL, 1 RAEB-1 and 1 CMML. In 3 cases there was a 1 vs 3 HLA-antigen mismatch. Conditioning was BuCy-2 in S, BuCy and thiopeta in 4 and busulphan and melphalan in 2. Analysis of haematopoietic chimaerism was done by amplification (PCR) of 5 VNTR loci (APOB, 33 AY-22, DSSR and D10S142). PCR products were analysed on 10% PAGE in 2% agarose and visualised by ethidium bromide staining. Haematopoietic chimaerism in the early post-transplant period (<1 mo) was analysed in 7 patients. Six had complete and one mixed chimaerism. Serial haematopoietic chimaerism studies (from 1 to 24 mo after transplant) were performed in all 11 cases. Seven always showed a complete chimaerism, two had a stable mixed chimaerism and 2 evolved from complete to mixed chimaerism. Thus, 4 out of 11 patients (36%) had mixed chimaerism at the time of analysis. Lymphoid chimaerism was analysed in two cases with mixed chimaerism. In one T-cells were exclusively of recipient origin and granulocytes remained of donor origin, whereas in the other T-cells showed a mixed chimaerism. Only one patient developed grade II acute GVHD and two had chronic GVHD. One patient, who showed a complete chimaerism 6 weeks earlier, relapsed 6 months after transplantation. Although preliminary, our results indicate a high frequency of mixed chimaerism after allo-PBSCT with CD34 selection. Partial T-cell depletion by CD34 selection could result in a higher immunologic tolerance of donor cells to host immunocompetent T cells. The clinical significance of this finding, in terms of graft versus leukemia effect, remains unclear.

Autologous stem cell transplantation as salvage chemotherapy for patients with relapsed or resistant malignant lymphoma


Department of Medicine, University Hospital “Merkur”, and “Croatian Institute for Transfusion Medicine, Zagreb, Croatia

Chemotherapy and/or irradiation provide a cure for many patients with malignant lymphoma. However, a number of patients either do not respond well to first-line treatment or relapse ultimately after initial response to treatment. Managing these, often heavily pretreated, patients still remains a major therapeutic challenge. Objective. In a group of heavily pretreated patients with malignant lymphoma we are investigating feasibility of stem cell transplantation as well as efficacy of this treatment and its toxicity. The cost effectiveness of this treatment is also assessed. Design and Methods. From 1993 until 1999 a total of 55 patients (32 male, 23 female; median age 33, range 18-63; 31 NHL and 24 HD) have received stem cell rescue after BEAM myeloablative treatment. Patients received heavy prior treatment with a median of 2 different lines of chemotherapy (range 1-6) and a median of 8 chemotherapeutic cycles (range 2-31). An average of 4.52 (range 0.79-32.8, SD 4.76) >10^9/kg CD34+ cells was re-transfused and G-CSF (5 mg/kg) was administered during the leukopenic period to all but one patient (mean 10.67 days, range 5-22). Median CD34+ cells infused were 5.8±10^6/kg (range 5.8-203.1), 4.4±10^6/kg (range 6.81-141.8), and 6.55±10^6/kg (range 2-29.41), respectively. CSA and Mtx were given as GVHD prophylaxis. All but 1 patient engrafted and the overall med. time to ANC >0.5±10^9/L was 12 (range: 6-51 days) and ANC >10±10^9/L was 14 (range 6-69 days). The median recovery times of PLT >20±10^9/L and PLT >50±10^9/L were 12 (range: 3-51) and 14 (range: 11-35), respectively. We observed correlations between CD4+ and CD8+ cells and time to PLT>20 (r=0.325 and 0.045) and CD42+ cells and time to PLT>20 (r=-0.335 and p=0.035). In our series of patients assessment of harvest with CD41 and CD42 positivity, had a positive impact on the platelet engraftment in the allo-PBSCT setting.

Patients who have received heavy prior treatment. It is cost effective because it induces high response rates, durable remissions and is associated with acceptably low transplantation related mortality.

Platelet engraftment after allo-PBSCT: the role of CD41 and CD42: stem cells


Ankara University Medical School, ibni Sina Hospital, Department of Hematology and Apheresis Unit, Ankara, Turkey

Between 7/97 and 10/98, 29 patients (med. age 32 yrs, range 5-47) with AML (n=14), CML (n=11), MDS (n=1), MM (n=2), and severe AA (n=1), were transplanted using peripheral blood stem cell stem (PBSCT) from their HLA identical siblings. All patients except that with severe AA, who had Cyloane, received BuCy as a preparation regimen. After treatment of donors with 10 µg/kg d c.s. for 4 days PBSBCs were collected on the 5th day of HS-G-SF (Neupogen, Amgen-Roche) and on the following day if needed. Apheresis procedures were performed with three different continuous flow cell separators. The median no. of TNC and MNC collected were 10.58±10^9/kg and 7.55±10^9/kg, respectively. The median number of CD41+, CD42+ and CD43+ cells infused were 5.4±10^6/kg, 5.8±10^6/kg and 5.8±10^6/kg (range 6.81-141.8), and 6.55±10^6/kg (range 2-29.41), respectively.

Experience of ibni Sina hospital in allogeneic peripheral blood stem cell transplantation for standard risk leukaemia patients


Ankara University, Faculty of Medicine, ibni Sina Hospital Department of Hematology and BMT Unit, Ankara, Turkey

Fifty-three patients with standard risk leukemia who underwent allogeneic peripheral blood stem cell transplantation (allo-PBSCT) from HLA-identical siblings were analysed particularly for their engraftment, incidence, severity of GVHD, and relapse rate. Standard risk leukaemia was defined as having ANLL in first complete remission or CML in first chronic phase within the first year after diagnosis. Recipients had a median age of 34.5 years (range: 13-47), and stem cells were mobilised using 10 mg/kg of G-CSF over 5 days of G-CSF (Neupogen, Amgen-Roche), collecting a median of 5.5 (2.1-18.9) x10^9/kg CD34+ cells. Median number of apheresis procedures was 2 (1-5). Cyclosporine-A (CSA) plus short course of Mtx was used for GVHD prophylaxis. Engraftment times to a granulocyte count >0.5 x10^9/L and platelets >20 x10^9/L were achieved at a median of day +13 (range: 8-32) and day +13 (range: 8-51) respectively. Median follow-up is 16 (4-38) months. Acute GVHD occurred in 18 of 49 (36.7%) evaluable patients and only 6 (12.2%) of them had severe disease (grade III-IV). Chronic GVHD occurred in 28 of 42 (66.6%) evaluable patients. Relapse rate at 2 years was 4.3%. Overall survival and leukaemia free survival were 12 (0.5-38) and 12 (0-38) months respectively. In conclusion, allo-PBSCT in standard risk leukaemia is an alternative to allogeneic bone marrow transplantation, and seems to show a low relapse rate and no increased risk of acute or chronic GVHD.

PO-0284 Interferon α2b versus no treatment after intensive therapy and autologous stem cell transplantation for relapsing lymphoma. Preliminary results of an international randomised study on 174 patients


for the GELA and European, Australian and New Zealand Groups; Yvelin, Belgium

High dose therapy (HDT) followed by autologous stem cell transplantation (ASCT) prolongs survival in relapsing lymphoma patients in comparison with standard chemotherapy. However, second relapse after HDT occurs frequently. Immunetherapy with interferon was chosen in this prospective multicenter (Europe and Australia-New Zealand) randomised trial because interferon α2b 3 × 10^6 IU TIW for 18 months (85

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pts or no further treatment (89 pts) after HD+(ASCT) for patients in second remission. Forty-four pts had low grade lymphoma (HL-LG): 62 diffuse small lymphocytic, 29 follicular, 8 mantle cell, 89 had high grade lymphoma (HL-HG): 69 diffuse large B cell, 13 peripheral T leukaemic lymphoma and 41 had Hodgkin’s disease (HD). Median duration from diagnosis to randomisation was 23 months (6–188). Seventy-two pts of the 85 randomised pts effectively received IFN. At this time, only 4 pts completed the 18 months of treatment. Ninety percent of the planned dose could be given. Relapses after HDT were classified as very early (<3 months), early (>3 <6 months) and late (>6 months) and occurred respectively in NHL-LG in 1, 1, and 5 cases, in NHL-HG in 11, 13, and 9 cases and in HD in 0, 2, and 2 cases. With a median follow-up of 10 months, the 2 yr-EFS was 42% (95 CI: 36–48). In the time of analysis, 34/44 in NHL-LG, 49/84 in HD or age, diagnosis, and marrow invole-

| PO-0285 | Long-term haematopoietic reconstitution after autologous transplantation using peripheral blood or marrow grafts in lymphoma patients | Domenet J, Berloubier I, Raingard F, Renault O, Delaim M, Truglio D, Linares G, Desbois I, Colonobat P, Binet C, Laboratory of Haematology and Department of Medical Oncology, Bre-\ntonneau Hospital, Tours, France |

Despite the wide use of peripheral blood (PB) grafts, their capacity to reconstitute haematopoietic functions in the long-term is not clearly established as for bone marrow (BM) grafts. In this study, we compared marrow progenitor cell recoveries for 2 years in 94 lymphoma patients (61 non-Hodgkin’s lymphomas and 33 Hodgkin’s diseases) who received unprepared autologous transplants from PB (APBT, n=58) or BM (ABMT, n=61). All APBT patients had GM-CSF for graft collection and post-transplant (APBT-GM+ group) while, among the ABMT patients, 13 had GM-CSF post-transplant (ABMT-GM+ group) and 43 did not receive any growth factor (ABMT-GM– group). Recovery of marrow progenitor cells (CFU-GM, CFU-E, BFU-E and GM-CSF+ group) was evaluated from d10 to d730 post-transplant and compared to control values. Statistics were performed by univariate analysis (Mann-Whitney’s test) and by multivariate analysis (stepwise regression) including the following variables: sex, age, diagnosis, and marrow involve-

| PO-0286 | Autologous stem cell transplantation for high-risk Hodgkin’s disease at a single institution: improvement over time and impact of conditioning regimen | Subira M, Sureda A, Martín R, García J,* Domingo-Albós A, Brunet S, Sierra J, Clinical Hematology Division, Hospital de la Santa Creu i Sant Pau, *Cyrometry and Cellular Therapy Department Cancer Research Insti-

tute, Barcelona, Spain |

Fifty-six consecutive patients (34 males and 22 females with a median age of 31 years) with poor prognostic Hodgkin’s disease (HD) have been autografted at our institution. At transplantation, 24 patients (43%) were in complete remission (CR1) (7 in first CR, 11 in second CR and 6 in third CR) and 32 (57%) were autografted with active disease (17 in relapse, 6 resistant relapse, 4 in untreated relapse and 5 with primary refractory disease). Twenty-nine patients were autografted before January 1993 and 27 after (January 1993). Bone marrow (BM) was used as the source of stem cells for 50 patients (92%). In patients transplanted afterwards (14% vs 4%) and in patients conditioned with TBI (18% vs 7%), although these differences did not reach statistical significance in univariable analy-

| PO-0287 | Hepatitis C associated thrombocytopenia in pediatric bone marrow transplant (BMT) recipients | Franco S, Sklar C, Small TN, Kemen NA, O’Reilly RJ, Bouland F, Memorial Sloan-Kettering Cancer Center, New York USA |

Design and Methods. After hepatitis C testing became available in 1992, we undertook a retrospective analysis of patients who underwent autolo-

gous or allogeneic marrow transplantation in our pediatric BMT service, from 1992 to 1993, and who were long-term survivors. Thirteen patients were identified who tested positive for hepatitis C by RIBA (Radio Recombinant Immunoblot Assay), or PCR (Polymerase Chain Reaction). Median age at time of BMT was 10.6 years (1–27). Median time of follow-

up post-BMT was 9.2 years (4–17). We retrospectively analyzed liver funct-

tion tests, ferritin, liver biopsy results, treatment for hepatitis C or haemosiderosis and blood counts. Results. Patients were divided in 4 groups:

| PO-0288 | A prospective comparison of reconstitution in haplo-identical stem cell versus conventional marrow recipients | Evrich M, Lang P, Schumm M, Lal S, Wessels JF, Handgretinger R, Klimges C, Niethammer D, Schielig PG, Children’s Hospital, University of Tübingen, Germany |

Immune reconstitution is one of the critical parameters in T-cell depleted stem cell transplantation. In order to compare the patterns of immune reconstitution after haploidentical stem cell transplantation (HSCT) and conventional BMT in children we carried out a prospective pilot study, in which we analysed lymphocyte subsets and T-cell proliferation at monthly intervals. To date, 18 patients after T-cell depleted HSCT have been com-

pared to 6 patients after nonmanipulated BMT. Stem cells for HSCT were harvested from the peripheral blood of a G-CSF mobilized platelet donor with subsequent positive selection on a MACS device using CD34 as a marker. The median stem cell dose given for HSCT was 24.6±0.10^6 CD34+ cells/kg BW with a T-cell content as low as 10.6±0.10^5 CD34+ cells/kg BW. BM recipients were transplanted with a median number of 2.1±10^6 nucle-
ated cells/kg BW. Engraftment defined by an ANC >0.5 and a platelet count >25.000/mm^3 was achieved by 16 (10 to 57) days and 16 (9 to 76) days, respectively. Hematological recovery was signific-
antly faster in patients transplanted from PB progenitor cells. Early trans-
plant related mortality (TRM) was 9%, TRM was higher in patients trans-
planted before January 1993 than in patients transplanted afterwards (14% vs 4%) and in patients conditioned with TBI (18% vs 7%), although these differences did not reach statistical significance in univariable analy-

sis. Overall TRM was 14%, TBI containing regimens significantly increased overall TRM (36% vs 9%, p = 0.03). Actuarial 3.5-year overall survival (OS), event-free survival (EFS) and progression free survival (PPS) were 57%, 58% and 65%, respectively. On multivariable analysis, TBI contain-

ing regimen and transplant before 1993 were the variables that signifi-
cantly reduced OS and EFS. Our results confirm that high-dose therapy fol-

lowed by autologous stem cell transplantation is associated with sustained PPS in a remarkable proportion of patients with HD unlikely to be cured by standard chemotherapy.

<table>
<thead>
<tr>
<th>Liver function</th>
<th>Normal</th>
<th>Abnormal</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

Six of 13 patients were found to have thrombocytopenia. Thrombocytope-

nia was mild (platelet count 50,000-150,000/mm^3) in 3 patients and se-
vere (platelet count >20,000/mm^3) in 2 patients. Platelet counts were low for 1 to 10 years (median, 5 years) following the diagnosis of throm-

bocytopenia. Four of the 6 patients were also found to have haemapsidero-
sis. Contributing factors for thrombocytopenia were CS/GHD (n=1), TIP (n=2), implicating a possible immunological causative etiology for the thrombo-

cytopenia. Three of the 6 patients had normal platelet count recovery coinci-
ding with improvement in liver function following interferon (n=1), phle-

bectomy (n=1) or spontaneously (n=1). In summary, thrombocytope-

nia can be found in patients who develop hepatitis C in the context of BMT. Its etiology is multifactorial, and probably immune-mediated. It may resolve with improvement of the hepatic process.
pared to BM recipients with a median time of 67 days (range 27-162) vs. 27 (range 17-44) to reach a CD3+ >100/µL and a median time of 100 days (range 53-183) vs. 31 days (range 1-7) for BM recipients. In order to ascertain whether PBSC transplantation is associated with a different degree of shortening we isolated neutrophils and T-cells from BM and PBSC recipients of allogeneic bone marrow transplantation, Wynn et al. Lancet 1998; 351:178. In conclusion, transplantation of megadoses of highly purified HLA-identical stem cells resulted in successful engraftment and a consecutive restoration of the T-cell compartment. However, T-cell reconstitution in HSCT recipients was delayed when compared to BM recipients.

**PO-0289**

telomere shortening following allogeneic bone marrow transplantation and allogeneic peripheral stem cell transplantation

Robertson JD,* Russel N,* Wynn RF,* Robinson SA,* Stainer C,* Robertson JD,* Russel N,* Wynn RF,* Robinson SA,* Stainer C,* T-cell reconstitution in HSCT recipients was delayed when compared to BM recipients.

**PO-0290**

defibrotide is an effective agent for the treatment of hepatic veno-occlusive disease in patients undergoing bone marrow transplantation

*Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Manchester; °City Hospital, Nottingham; #Royal Manchester Children’s Hospital, Manchester; 8Royal Victoria Hospital, Newcastle-upon-Tyne, UK

Allogeneic peripheral stem cells (PBSCS) are increasingly being used as an alternative to bone marrow for transplant procedures, their advantages include a higher progenitor cell yield and earlier engraftment. Mean telomere length (MTL) shortening has been described in peripheral blood leucocytes following bone marrow transplantation (Wynn et al. Lancet 1998; 351:178). In order to ascertain whether PBSC transplantation is associated with a different degree of shortening we isolated neutrophils and T-cells from BM and PBSC recipients of allogeneic bone marrow transplantation, Wynn et al. Lancet 1998; 351:178. In conclusion, transplantation of megadoses of highly purified HLA-identical stem cells resulted in successful engraftment and a consecutive restoration of the T-cell compartment. However, T-cell reconstitution in HSCT recipients was delayed when compared to BM recipients.

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appearance of dyshaemopoietic changes, assessed according to a pre-established score. We also studied 25 bone marrow samples obtained at the time of diagnosis, prior to treatment, but we did not find morphologi-
cal hypoplasia. Results. Myelodysplasia was present in bone marrow before transplantation in most patients and continued to be in evidence for a long time afterwards. This suggests that chemotherapy and radio-
therapy used prior to transplantation are responsible for dyshaemopoietic changes. Peripheral blood cytopenias were observed in 51% and 44% of patients 6 and 12 months after transplantation and dysplastic features in 73.6% and 75% respectively. Moreover, coexistent presence of cytopenia and myelodysplasia was observed in 37.7% of patients at six months after transplantation and 25% at 12 months, and therefore they could be diagnosed as having MDS. This data contrasts with the incidence of secondary MDS reported in previous publications. According to our findings, the value of FAB criteria for the diagnosis of MDS following autologous transplantation is questionable.

PO-0293 Risk factors influencing survival after allogeneic bone marrow transplantation for 73 patients with severe aplastic anaemia - a single center study
Lee JW, Han CW, Jin Y, Min WS, Kim HK, Kim WL, Kim CC
Catholic Haemopoietic Stem Cell Transplantation Center, St. Mary’s Hos-
pital, The Catholic University of Korea, Seoul, Korea

Objective. We investigated the survival rate and risk factors affecting sur-
vival in patients with severe aplastic anaemia (SAA) who underwent bone marrow transplantation (BMT) from HLA-identical siblings. Methods. Sev-
enty-three patients with SAA who received allogeneic BMT between Sep-

tember 1983 and July 1995 were analyzed. Their median age was 22

years (2-37). Most patients (82%) were high-risk for rejection which was
defined as the presence of a history of extensive exposure to random
packed red cells 10 or platelet concentrations 40 or family blood trans-
fusions before BMT. Patients were conditioned by cyclophosphamide (Cy)
alone (n=4) or in combination with TNI (n=14), procarbazine (PCZ) (n=10),
TNI+PCZ (n=2), ATG+PCZ (n=40), and TNI+ATG+PCZ (n=1). GVHD pro-
phylaxis consisted of CSA alone (n=19) or in combination with CSA+MTX
(n=54) or CY. The median number of BM nucleated cells infused was 2.4±10^6/kg of the recipient’s body weight (1.36-6.4). Results. The Kaplan-Meier estimate of 5-year survival rate was 71%. There was a trend of improving survival over time (1987-89; 58.8%, 1990-92; 70%, 1993-
95; 84%). The incidence of graft failure was 17.8% (4 primary, 9 delayed). The
incidence of grade II-III acute GVHD was 15.1% (no grade IV), and that of chronic GVHD was 16.4% (10 limited, 2 extensive). Fifteen died of TRM;
graft failure (n=10), major GVHD (n=2), chronic GVHD (n=2), TIP-like syn-
drome (n=1). Graft failure (p=0.0001), major ABO incompatibility (p=0.016), and sex mismatch (p=0.034) were significant risk factors for survival in univariate analysis. In multivariate analysis, graft failure was also the most important risk factor affecting survival (RR 48.71, p=0.0001). Other marginal risk factors were major ABO incompatibility (RR 2.94, p=0.089), sex mismatch (RR 2.71, p=0.084), presence of more than grade II acute GVHD (RR 1.78, p=0.049), and recipient’s age (RR 1.99, p=0.085). Conclusions. Although the majority of patients were high risk in this study, allogeneic BMT from HLA-identical sibling in SAA patients showed not only a promising survival rate but also a acceptable rate of com-
plications. Because of the high mortality rate in cases of graft failure, a sec-
ond transplantation should be actively considered for a better clinical out-
come.

PO-0294 Long-term outcome of autologous bone marrow transplanta-
tion for acute myeloid leukaemia - a single centre experience
Liakopoulou E, Douglas KW, Laing JE, Campbell M, Tansey P, Parker AN,
Bumet AK, Franklin IM
Bone Marrow Transplant Unit, Glasgow Royal Infirmary, Glasgow, UK

Objective. To determine the long term outcome in patients treated with autologous bone marrow transplant for acute myeloid leukaemia (AML) in Glasgow Royal Infirmary between October 1981 and December 1993.

Design and Methods. Retrospective analysis of survival data was carried out in all patients identified a having received autologous transplant for AML. Patients who received autologous transplant in remission or who were excluded from the study. Results. Eighty patients diagnosed with AML received autologous bone marrow transplant at our centre between Octo-
ber 1981 and December 1993. Seventy-four patients in first complete remission achieved with UKAML8 and UKAML10 protocols were studied, comprising 32 males and 42 females with a median age 34 years (15-56 years). The overall survival was 42%. The five year survival was 43% and the five year disease free survival 38%. In a population of 43 patients who were transplanted between October 1981 and December 1988, ten year survival was 44% and the corresponding disease free survival as 34%.

Long term complications related to transplant procedure were observed, in many of the long-term survivors. Iron overload combined with abnormal liv-
er function was documented in 18 patients, but most normalised after venesection. Cataracts were documented in 4 cases, hypothyroidism in 4, cardiomyopathy in 3, pulmonary fibrosis in 3, ovarian failure in 3 patients. One patient developed secondary malignancy and acquired hepatitis C was documented in five patients. Conclusions. The data from our centre show that autologous bone marrow transplant for AML has a long term five and equal ten year survival of 43% with normal or near normal functional status for most of the survivors, and is shown to be a viable option for treat-
ment of the disease. However, given the frequent occurrence of potential-
treatable long-term complications such as iron overload, the importance of careful long-term follow-up is apparent.

PO-0295 Recruitment to autologous peripheral blood progenitor cell transplantation in patients with acute leukaemia and multiple myeloma
Nguyen K, Robertson J, Summers Y, Dougall M, Chang J, Testa NG,
Scarfe JH
Department of Haematology and Medical Oncology, Christie Hospital
Hospital NHS Trust, Manchester, UK

Autologous peripheral blood progenitor cell transplantation has become the support mechanism of choice to rescue patients receiving high dose treat-
ments for acute myeloid leukaemia (AML), acute myelodysplastic leukaemia (ALL) and multiple myeloma (MM). Two hundred and sixty-eight patients
up to the age of 65 years presenting with a diagnosis of AML (104), ALL
(51), or myeloma (121) were potential candidates for our institu-
tion since 1991. On an intention to treat analysis the proportion of patients with AML who proceeded to a harvest, achieved an adequate col-
lection (CFU-GM: 5×10^6/kg, CD34+ve: >2×10^6/kg) and underwent sub-
sequent transplantation was significantly lower compared to patients with ALL or MM as summarised in the table.

Patients

<table>
<thead>
<tr>
<th></th>
<th>AML</th>
<th>ALL</th>
<th>MM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presented</td>
<td>104</td>
<td>52</td>
<td>112</td>
<td>268</td>
</tr>
<tr>
<td>Harvested</td>
<td>49</td>
<td>43</td>
<td>88</td>
<td>180</td>
</tr>
<tr>
<td>Adequate</td>
<td>29</td>
<td>43</td>
<td>84</td>
<td>155</td>
</tr>
<tr>
<td>Transplanted</td>
<td>29 (27%)</td>
<td>29 (56%)</td>
<td>76 (68%)</td>
<td>130 (50%)</td>
</tr>
</tbody>
</table>

Patients over the age of 50 yrs with AML were less likely to have an ade-
quate harvest compared to patients with ALL or MM. Cumulative progeni-
tor cell yields and estimated LTC-IC numbers for two harvest cycles were
significantly lower for the AML cohort. The overall survival of patients with
ALL or MM was not affected by age (<50, 50+). Patients over the age of 50
years with AML had a significantly worse 5-year survival than younger
patients (p<0.003). Our experience suggests that, on an intention to treat
analysis, not all patients eligible for autologous PBPC transplant undergo trans-
plantation. This is particularly true in patients with ALL or MM, because the
majority of patients older than 50 years are unable to receive conventional
support mechanism of choice to rescue patients receiving high dose treat-
mements. Of the patients screened, 13 (all of whom were CMV
negative) were potential candidates for PBPC transplantation (BMT) has led to the adoption of pre-emptive therapy
approaches Conventional detection methods (eg CMV antigenemia) may
be unreliable in this group. We have undertaken a prospective study to eval-
uate Quantitative Real Time PCR, CMV DNA was extracted from whole blood and CMV viral load was measured using the Taqman® real time PCR
machine. This approach allowed us to study the significance of viral load in neutropenic patients who receive chemotherapy or bone mar-
row transplantation. Of the patients screened, 13 (all of whom were CMV
negative at presentation) had detectable CMV copy numbers by PCR. Four of these patients (1 pt alloBMT; 3 pts autoPBSCT) were PCR positive on at least 2 occasions, had an incremental rise in the viral load ranging from 305 to 6,700 copy numbers/ ml of blood and developed CMV pneu-
omonitis. Ganciclovir was commenced but all 4 patients died. We therefore
terminated pre-emptive therapy in subsequent patients who were
PCR positive on more than one occasion, irrespective of the viral load.
Subsequently, 9 patients have been PCR positive; 4 (3 conventional

PO-0296 Quantitative real time PCR for the management of CMV infection in patients undergoing bone marrow transplantation
Chopra R, Guiver M, Barnes AJ, O penheine BA, Mutton K, Chang J,
Morganstein GA, Scarfe JH
Christie Hospital NHS Trust, Withington, Manchester; *Manchester
PHL, Withington Hospital, Manchester, UK

The mortality associated with CMV disease occurring post bone mar-
row transplantation (BMT) has led to the adoption of pre-emptive therapy
approaches Conventional detection methods (eg CMV antigenemia) may
be unreliable in this group. We have undertaken a prospective study to eval-
uate Quantitative Real Time PCR, CMV DNA was extracted from whole blood and CMV viral load was measured using the Taqman® real time PCR
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Subsequently, 9 patients have been PCR positive; 4 (3 conventional

Clinical transplantation I

Haematologica vol. 84 (EHA-4 Abstract Book); June 1999
PO-0297 Factors that influence long term haematopoietic function following autologous stem cell transplantation
Amigo ML, del Cañizo MC, Caballero MD, Corral M, Fernández ME, Bruñau JA, San Miguel JF
Dept. Haematology, Salamanca University Hospital, Spain

Several groups have studied factors which influence early haematopoietic reconstitution following autologous transplantation. However, the factors that determine haematopoietic function after transplant long-term, have hardly been explored. Objective. The aim of the present study was to assess the variables that condition late quality of haematopoietic engraftment in autologous transplantation. Design and Methods. We have analysed a series of 79 patients who had undergone autologous transplantation in our hospital. After transplantation, patients were followed up for 4 years. We have analysed the number of CFU-GM and CD34+ cells infused, the quality of cells infused and age as parameters that could influence the long-term haematopoietic function. Results. Significant lower dose of CFU-GM and CD34+ cells than patients without cytopenias (p<0.012 and p=0.007 respectively). The same correlation, with even higher statistical significance, was observed 12 months after transplant (p=0.04 and p=0.005). Alkylating agents and radiotherapy administered prior to transplantation and age did not seem to influence the presence of cytopenias. The incidence of cytopenias did not vary significantly according to the stem cell source (bone marrow or peripheral blood). Conclusions. The number of CFU-GM and CD14+ cells infused were the most important factors in the maintenance of adequate haematopoiesis.

PO-0298 Tandem transplantation, autografting followed by mini-allo-grafting, for resistant haematological neoplasia and metastatic breast cancer
Carella AM, Lerma E, Corsetti MT, Basta P, Deiana A
N.O.A. Hematology/ABMT, DEMA, Osp. S. Martino, Genoa, Italy

We have recently demonstrated that immunosuppressive therapy alone is able to achieve the engraftment of donor BPC and that autografting (ASCT) followed by mini-allografting (m-ALLO) can be combined to achieve tumour debulking after ASCT and the control of MDR after m-ALLO. Design and Methods. Our data refer to 18 patients with advanced disease: Hodgkin's disease (8), non-Hodgkin's lymphoma (2), chronic myelogenous leukaemia (2), and metastatic breast cancer (4). Two pts with RAEB received only m-Allo. In preparation for autografting, the patients underwent high-dose therapy on protocols appropriate for the underlying disease. At a median of 40 days after engraftment of autologous HPC, all pts were conditioned for allografting with immunosuppressive agents alone (fludarabine 30 mg/m2/d × 3 days with cyclophosphamide 160 mg/m2/d × 5 days) (Flu-Cy protocol). Then, HLA matched donor HPC mobilised with G-CSF were infused to the patients. GVHD prophylaxis consisted of CSP and MTX.

Results. After ASCT, lymphomas: CR 3, PR 6 and PD1; CML: 2nd CP-CML in both pts; breast cancer: all pts had just reduction of pain. After m-Allo, complete chimaerism was achieved in 8/18 pts (44%) and mixed chimaerism in the other 6 pts (33%). Two pts are under evaluation and only two pts (BP-CUL and 1 RAEB) achieved CR after mini-allograft (HD; 3; RAEB; 1; AP-CML; 1; NHL; 1). Eight of these 9 pts had achieved complete (4) or mixed (5) chimaerism while one pt is under evaluation. Subsequently, the RAEB patient relapsed and 1 pt with HD died in CR of aspergillus infection in the brain. Another 3 pts achieved sustained PR after m-Allo and DLI (breast; 2; HD; 1). Grade III-a GVHD was the single major complication, in only two pts did ANC decrease to ≤1×109/L. No patients required a sterile room. Conclusions. Immunosuppressive therapy with the Flu-Cy protocol allowed engraftment of HLA-matched sibling donor HPC without procedure-related deaths; moreover, we demonstrated that the combination ASCT/m-Allo can be pursued in safety in a severely ill population and that some of these pts achieve a remission.

Clinical transplantation I

Chair and Division of Hematology, Dept. of Medical and Morphological Research and Dpt. of Bone Marrow Transplantation, University Hospital Udine, Italy

Hepatitis B virus (HBV) reactivation may result in hepatitis, hepatic failure and death. This has been documented following both chemotherapy (CHT) withdrawal and high-dose therapy; in several cases the detection of HBsAg appears to precede the clinical manifestations. Lately, it has been known that hepatitis C virus (HCV) carriers undergoing standard and high-dose CHT. We report our experience with the outcome of 11 patients (pts) (6 carriers of HCV-RNA and 5 of HBsAg) who were autotransplanted at our Institution between March '92 and June '98. M/F ratio was 7/4, median age 41 years (26-56). Nine pts (4 HBsAg and 5 HCV-RNA) were affected by non-Hodgkin's lymphoma (NHL), 1 (HCV) by chronic myelogenous leukaemia (CML) and 1 (HBsAg) by breast cancer (BC). The patient with CML was treated with IFN and conditioned with busulphan/melphalan. The patient with BC was treated with 6 FEC and then conditioned with thiopeta/cyclophosphamide. All the NHL pts were treated with F-MACHOP and conditioned with BEAM. In the immediate post-transplant period only in 1 patient (HBsAg carrier and affected by BC) a hepatitis was documented (about 1 month from ASCT) with an elevation of transaminase levels (≤20x-40x n.v.) not associated with the detection of HBV-DNA. According to the definition of Lau et al. this pattern can be defined as hepatitis (probably drug-induced) but not as hepatiti- B virus reactivation. No other complications, nor toxic deaths were observed. During post-transplant follow-up (median 29 months, range 7-81) no hepatic abnormalities were observed. Overall survival of the whole population is 54 months (18-124). Currently 10/11 pts are alive. In complete remission, while 1 patient affected by follicular centre lymphoma is alive with disease 50 months from ASCT. This study shows that ASCT can be performed safely in HBsAg and HCV-RNA carriers.
Autologous stem cell products are at risk of bacterial and fungal contamination during the collection procedure. Additional manipulations may increase the incidence of contamination. Because patients may be neutropenic on the day of reinfusion and are often immunosuppressed, the infusion of a contaminated stem cell product may add to the morbidity and mortality of the transplant procedure. Potential sources of contamination for autologous stem cell products are the collection procedure, ex-vivo manipulations, bag breakage at the time of thawing and infusion and even contamination of the catheter at the time of harvesting due to a permanent catheter (or a port for drug application). In this study 525 autologous stem cell products drawn by leukapheresis from 94 patients were tested for microbiological contamination after processing performed in a laminar flow cabinet. Seven (1.3%) autologous stem cell products had not been reinforced because of a bacterial contamination. In these cases coagulase negative staphylococci (CNS) were found. CNS were also found in the blood cultures of the two correspondent patients, due to a permanent catheter. Although the patients had shown signs of bacteraemia, such as fever, headache, they had not been treated with antibiotics and leuka- pheresis had been performed. Without doing cultures of the stem cell preparations through the course of collection and processing, it is difficult to determine the source of contamination. Stem cell processing following collection performed in a laminar flow cabinet (airborne particulate classification, grade A, EU Guide to GMP, Annex 1) placed in a laboratory of a grade B seems to be the safest way of stem cell preparation.

Duran i Reynals Hospital, Barcelona, Spain

Poster discussions  Progenitor cell processing, mobilisation, expansion I

Karakassopoulos A German Red Cross Blood Service, Institute Muenster, Germany

ICAM-1, and LFA-3 were evaluated among steady-state bone marrow (BM) CD34+ cells. CD34+ cells from PBSC have a significantly lower mean fluorescence than BM counterparts. Grade B seems to be the safest way of stem cell preparation.

A technique of differentiation and cell sorting (ICAM-1, CD95, b2 integrin, N-CAM, ICAM-1, and LFA-3) were evaluated among steady-state bone marrow (BM) and G-CSF mobilised CD34+ progenitor cells (PBSCs), of samples collected from healthy donors. Comparison was made following CD34+ cell enrichment (Mini MACS Miltenyi Biotec) before cytometric analysis. Coexpression of lineage antigens was used to determine the nature of mobilised CD34+ cells. CD34+ cells from PBSC have a significantly lower mean fluorescence intensity (MFI) than BM for L-selectin expression (16±1 vs 37±3; p<0.005). C-KIT was also co-expressed with a lower antigen density compared to BM cells (4.6±2 vs 26.7±6; p<0.005) and on a lower percentage of CD34+PBPC (37±16% vs 59±1%; p<0.05). No differences among co-expression of ICAM-1, LFA-3 and J2 intergins were detected and NCAm was not expressed on cells from any of the sources. CD34+ cells from PBPC contained fewer B lymphoid progenitors than BM and heterogeneous expression of CD34 was also observed. CD34+GR-1 low and CD34+CD38 low were maintained in both sources and were mainly L-selectin+. Our data suggest that the release of CD34+ cells into the circulation may be associated with a down-modulation of L-selectin. Also, a down-modulation of ligands and receptors may be a prerequisite for the mobilisation of HPCs from BM into circulation.


Groupe de recherche en Hématologie, Pédiatrie Clermont-Ferrand, France

Conclusions. The STELLer CD34+ assay is useful in CD34 enumeration in cord blood, leukaapheresis and peripheral blood samples and provides comparable results to other methods. Nevertheless, peripheral blood samples with low CD34 absolute counts (below 10 cells/µL) should preferably be analysed by alternative flow cytometry protocols. Even though the same operator performed the study in a single laboratory, the mild high-method CV suggests that differences in sample preparation and gating strategy are limiting factors in order to decrease the variability. Protocols with fewer intermediate steps or that are fully automated such as the STELLer CD34+ assay are expected to reduce intra and interlaboratory variability.

Cabezudo E, Querol S, Cancélas JL, Garcia J

Department of Cryobiology and Cell Therapy, Cancer Research Institute, Duran i Reynals Hospital, Barcelona, Spain

An accurate determination of CD34+ stem cells is mandatory in cord blood banks and stem cell collection units. We have assessed the feasibility of a new-volumetric cytometry system (IMAGN/2000 microvolume cytomoter) for the enumeration of CD34+ cells (STELLer CD34+ assay) in apheresis products, peripheral blood and cord blood samples in our routine laboratory work. We compared this system with the following flow cytometry protocols: Milan, ISHAGE, ISHAGE with 7-ADD and Flow-Count fluorospheres. Methods. We performed correlation, linearity and reproducibility studies of the various methods. Clonogenic progenitor cultures were performed as an external control, to assess the correlation between the number of CD34+ cells/µL determined by each method and the number of CFU/µL Results. The linear regression analysis demonstrated that the five methods were comparable (R2 ranged from 0.86 to 0.96 and slopes were close to 1). The STELLer CD34+ and the Flow-Count methods showed poor linearity for CD34 cell counts below 10 cells/µL (R2=0.46 and 0.47). The reproducibility assay (n=10 replications) for a CD34 count of 10 cells/µL showed a CV of 12% and 25% for Milan and STELLer CD34+ methods respective. The mean CV among all five methods for the 46 evaluated samples was 20%. There was a strong correlation between the number of CD34+ cells/µL and CFU/µL either in cord blood and apheresis samples (r=0.71-0.81).

Bonhomme J, Travade P, Deméocq F

Groupe de recherche en Hématologie, Pédiatrie Clermont-Ferrand, France

Conclusions. The STELLer CD34+ assay is useful in CD34 enumeration in cord blood, leukaapheresis and peripheral blood samples and provides comparable results to other methods. Nevertheless, peripheral blood samples with low CD34 absolute counts (below 10 cells/µL) should preferably be analysed by alternative flow cytometry protocols. Even though the same operator performed the study in a single laboratory, the mild high-method CV suggests that differences in sample preparation and gating strategy are limiting factors in order to decrease the variability. Protocols with fewer intermediate steps or that are fully automated such as the STELLer CD34+ assay are expected to reduce intra and interlaboratory variability.

PO-0304 Collection of progenitor cells with paclitaxel mobilising schemes in patients with haematological malignancies

Gómez-Espuch J, Moraleda JM, Ortuño F, de Arriba F, Heras I, Vallejo C, Cano H, Vicent V

Oncohaematology Department, Hospital General Universitario, Murcia, Spain

Objective. To study the role of Paclitaxel (PT) priming schedules in the collection of haemopoietic progenitor cells in patients with malignant haemopathies. Design and Methods. From June 1997 to October 1998, 8 patients (4 males/4 females) were mobilised for an autologous stem cell transplant. Four patients were primed with PT 170 mg/m2 (24 hr iv infusion) day 1 and G-CSF 8 mg/m2 sc. from day 2 until the last apheresis day (PT-G group). Four patients were treated with a similar schedule but with the addition of cyclophosphamide 4g/m2 iv in day 2, and beginning G-CSF on day 3 (PT-Cy-G group). The first apheresis procedure was performed when the number of CD34+ cells in peripheral blood was ≥30 µL or when the total number of WBC was ≥30000/µL which ever occurred first. There were 4 patients with multiple myeloma (2 in each group), 3 with non-Hodgkin’s lymphoma (1 in TG and 2 in Cy-TG group) and 1 patient with Hodgkin’s disease (TG). Results. The first day of apheresis (mean±SD) was 11.1±2.1, the number of apheresis procedures performed was 1.5±1 and the number of aphereses required to obtain at least 2×10^9 CD34 cells/kg BW was 1.37±1. The mean number of CD34+ cells obtained was 626.9±479.7×10^9 (8.27±5.6×10^9) CD34+ cells/kg BW. The total yield of CD34+ CD38- CD45dim subpopulations was 31.28±23 and 31.0±278 respectively. Five out of eight patients developed a severe neutropenia (ANC<500/µL) and thrombocytopenia (Plt <50,000) a mean of 3.2±2.8 and 3.4±2.6 days respectively. There were no episodes of fever or bleeding, nor any complications that required hospitalization. We did not find any differences in the yield of CD34+ cells or CD34+ subpopulations between the PT-G and the PF-Cy-G groups although the first day of apheresis was earlier in the PT-G group (9.25±0.5 vs 13±0.8, p<0.001). Moreover, the number of days with neutropenia was fewer in the PT-G patients (1.5±1.3 vs 6.2±1, p<0.004) whereas the duration of thrombocytopenia was similar. Conclusions. Our data suggest that Paclitaxel-containing schedules are safe and useful for mobilisation of HPC in patients with haematologic malignancies even though considered bad mobilizers such as patients with multiple myeloma.


Groupe de recherche en Hématologie, Pédia trie Clermont-Ferrand, France

Introduction. We have previously demonstrated feasibility of cryopreservation of human peripheral blood stem cells (PBSCs) at -80°C without rate-controlled freezing using a cryopreservation solution containing: hydroxyethyl starch (HES), dimethylsuloxide (DMSO) and human serum albumin.

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(HSA). Objective. To evaluate the feasibility of PBSC cryopreservation without any human derived products. Design and Methods. We compared WBC, CD34+ cells, CFU-GM, and BFU-E recovery rates from the same PBSC sample cryopreserved at -190°C in (1) standard cryopreservation solution with addition of HSA (final concentration 1%) and (2) cryopreservation solution without any HSA addition (PBSC n=10). Results:

<table>
<thead>
<tr>
<th>Volume (mL)</th>
<th>Total cells (10^9)</th>
<th>MN cells (10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=25)</td>
<td>22.9±2.34</td>
<td>4.12±1.57</td>
</tr>
<tr>
<td>B (n=25)</td>
<td>34.88±2.42</td>
<td>5.99±1.6</td>
</tr>
<tr>
<td>C (n=25)</td>
<td>72.3±20.12</td>
<td>8.5±2</td>
</tr>
</tbody>
</table>

Comparison among these three groups using one way ANOVA revealed significant differences in total nucleated and mononuclear cells between groups A and C, and groups B and C (p<0.01). Conclusions. According to the EUROCORD report the minimum total cellularity is 4 x 10^9. Our data show that the increase of collected volumes determines a greater number of total nucleated and mononuclear cells, but that UCB units with volumes less than 30 mL possess a mean total cellularity of 4.12 ± 10^9, which would probably be a sufficient number of total transplantable cells for low weight recipients.

PO-0306 Influence of double or single immunomagnetic purging on haematologic recovery after PBSC

Hospital Universitario de Canarias, Tenerife, Spain

Objective. Ex vivo procedures may affect stem cells the ability of to engraft. We analyzed haematologic recovery during the first year post-transplantation in a group of patients who underwent PBSC with immunomagnetic selected products. Methods. Twelve patients (7 multiple myeloma, 4 non-Hodgkin lymphoma and 1 breast cancer) (mean age 55±1.40 years) were harvested and immunomagnetic selection was performed with the Isolspin device (Baxter, USA), 9 with double selection (positive and negative) and 3 with only positive selection. After collection, all patients received an autologous PBSC with the selected products as the source of cells. Results. The average number of CD34+ cells >10^5 kg infused was 3.31±0.31. Median number of days to neutrophils > 0.5 x 10^9/L and platelets > 20 x 10^9/L were 11.33±14 and 7.10±12, respectively. Conclusions. We conclude that autologous albumin present in PBSC products seems to be sufficient to assure a good quality of PBSC cryopreservation. These simplified cryopreservation solution minimizes the risk of transmission of diseases via human blood derived products and reduces the cost of cryopreservation.

PO-0308 Immunophenotype and ex vivo expansion of foetal cord blood

Engel H, Kaye E, Kolhagen H, Mallmann P
*Medical High School Hannover, Department of Haematology, Hannover; **University of Cologne, Department of Gynecology, Cologne, Germany

Objective. Neonatal cord blood (CB) has been identified as an alternative source of haematopoietic stem cells. The main topic of the study was to analyse the character and ex vivo expansion potential of foetal CB compared to neonatal CB. Design and Methods. Forty-three CB samples (19-30 weeks of gestation) were analysed for their immunophenotype by three color flow cytometry and cultured with r-hSF and r-HL3-ligand for up to 21 days. Cell counts and immunophenotyping were performed on days 0, 2, 4, 7, 14 and 21. For comparison we also studied 50 neonatal CB samples. Results. Foetal CB contained a statistically significant higher percentage of CD34+ cells than neonatal CB (1.24±0.82% versus 0.3±0.18%; p=0.0001). This correlated inversely with the age of gestation. Ex vivo expansion with r-hSF and r-HL3-ligand showed an increase of CD34+ cells (up to 20-fold) with a peak at day 4 (49.8±6.72%; 1.63 ±10^5) and day 7 (54.01±5.13%; 1.53 ±10^5). Mean CD34+ cell recovery was 67% (38-99) with a GM-CFC recovery of 25% (9-40%).

PO-0309 CD34+ cell selection can give rise to a falsely low measurement of clonogenic graft adequacy

Ings SJ, Watts M, Ardelesha KM, Linch DC
University College London Medical School, London, UK

It is usual practice to assess the adequacy of peripheral blood stem cell harvests with measurements of CD34+ cell numbers and clonogenic assays. We have noted a significant discrepancy between the expected and actual colony numbers from purified CD34+ cells plated in commercial methylcellulose media (Terry Fox media with added growth factors, SCF 10 ng/mL, IL-3 30 ng/mL, GM-CSF 25 ng/mL, G-CSF 25 ng/mL and IL 3 U/mL). Enrichment is reflected in a recent audit of 216 clinical scale PBSC CD34 selection procedures in our unit in which median CD34+ cell yields of 52% were obtained compared to only 36% for GM-CFC. We have also noted apparently low GM-CFC yields with the Isolspin 200® (n=12) and the ClineMACS® 8 (n=11), where we found median CD34+ cell recoveries of 60% and 77% compared to GM-CFC yields of only 28% and 37% respectively. These data are in accord with most published studies and could be due to poor cell function caused by cell damage during the selection process, the preferential selection of a poorly clonogenic subfraction of CD34+ cells or the lack of accessory cells in the methylcellulose assay medium in which the purified cells are cultured. In this study the effect of accessory cells on the colony growth of CD34+ purified cells from eight ClineMACS procedures was investigated. Colony assays were measured on either purified CD34+ cells alone or the same number of these cells were added to accessory cells (CD34 depleted fraction). The colony numbers were considerably increased in these "add-back" assays. The median CD34+ cell recovery was 67% (38-99) with a GM-CFC recovery of 25% (9-100).
In the presence of the accessory cells the GM-CFC yields increased cell number compared to the AC133+/CD34+ expanded cells with FLT3, IL-3 and SCF. Expanded cells lydimsrned compared to the expanded AC133+/CD34+ population with FLT3-ligand, thrombopoietin (TPO) and interleukin-6 (IL-6). Expanded cells proliferative activity is especially important if WB is used as a stem cell source for autologous transplantation. We investigated three methods of CFU enumeration in WB. Methods. 1. Mononuclear cells (MNC) were isolated by Ficoll separa-
tion from a WB sample. CD34+ cells were determined both in the MNC and in the original WB sample using the ProCount™ assay. Sub-
sequently, MNC were plated into CFU culture medium. In this way, the number of CFU-GM/mL of WB can be calculated independently of the cell count after Ficoll separation. 2. WB was lysed with NH4Cl and washed twice. The lysate was plated into CFU medium. 3. WB was plated directly into CFU medium. In all cases, the cells were diluted with fresh medium before plating into CFU medium (MethoCult GFH4434, Stem Cell Techn.) to a final concentration of 300 CD34+ cells/mL at maximum to prevent overplating.

In conclusion, these data demonstrate that enrichment into CFU medium (MethoCult GFH4434, Stem Cell Techn.) to a final concentration of 300 CD34+ cells/mL at maximum to prevent overplating.

Conclusions

1. CD34+AC133+ cells ranged from 4.3% to 33.9% of total CD34+ cells. A wider range of variation was seen in BM than PBSC (4.35-
22.77% vs 18.18-33.9%). In the patient from whom two samples were taken, there was a marked difference between the proportions of CD34+ 
and CD34+ cells in the BM and PBSC. Conclusions. These preliminary results indicate considerable variation in the proportion of CD34+ cells co-express-
ing the AC133 antigen. Furthermore, mobilised PB may be considerably enriched in AC133-expressing cells. This indicates that the precise method of mobilizing, and processing stem cells may differentially affect yields of CD34+ positive and negative CD34+ cells. This issue should be closely examined before clinical use of cells harvested using AC133-based tech-
nology and warrants further investigation.

Enrichment and ex vivo expansion of mobilised peripheral blood AC133 hematopoietic stem cells


University Children’s Hospital, Tübingen, Germany

A novel cell surface antigen present on a CD34 bright subset of human hematopoietic stem and progenitor cells (HSCs) has recently been described. Discovery of the monoclonal antibody against the AC133 pan-
taspan molecule enabled the enrichment of this new cell subpopulation.

In this study, we focused on phenotypic and functional characterisation of peripheral blood (PB) AC133 HSCs from donors mobilised with granulo-
cyte colony-stimulating factor (G-CSF). Using MACS (Magnetic Activated Cell Sorting) we could enrich the AC133 subpopulation to a purity of 98% with a recovery of 60-80%. Almost all of the AC133 enriched cells coex-
pressed CD34 antigen (AC133+/CD34+), whereas only a small proportion of them were AC133+/CD34+ (0.5-2%). In contrast to the bone marrow AC133+/CD34+ population which contains a minor population of BFU-E, in vitro clonogenicity assays have demonstrated that the mobilised peripheral blood double positive AC133+/CD34+ population contains the major-
ity of BFU-E, CFU-GM and a significant number of CFU-Mix. Interestingly, AC133+/CD34+ also contained a considerable number of progenitors for dendritic cell colonies (DCs). In addition to clonogenic assays, repro-
duction and self-renewal potential of the enriched AC133+ population was assessed by their capacity to generate cobblestone area forming cells (CAFC) and by assessing the telomerase activity. Freshly isolated AC133+ CD34+ cells showed an equal telomerase activity and a similar frequ-
cy of CFUCs with the freshly isolated CD34+ cells. Ex vivo culture of the highly purified AC133+/CD34+ population with FL3-ligand, inter-
leukin-3 (IL-3) and stem cell factor (SCF) resulted in a 20 to 40-fold expan-
sion of myeloid progenitors, with a decreased capacity to generate CFU-
Mix and week 6 CAFCs. Telomerase activity in these cells was significant-
ly diminished compared to the expanded AC133+/CD34+ population with FL3-ligand, thrombopoietin (TPO) and interleukin-6 (IL-6).

Expanded cells with such a cytokine combination, also generated a significantly higher number of GM-CFC in secondary clonogenic assays and week 6 CAFCs, compared to the AC133+/CD34+ expanded cells with FL3, IL-3 and SCF cytokine mixture. In conclusion, these data demonstrate that enrichment and ex vivo expansion of the peripheral blood AC133+/CD34+ cell population might be a reasonable transplantation alternative for the 30-40% of CD34+ leukemias which do not express AC133 antigen.

Reproducibility of CFU-GM enumeration in G-CSF stimulated whole blood using three different methods
de Kuik AM, Hendriks ECM, Zeewenberg A, van Oosterveen JW, Jonkhoff AR, Huijgens PC
University Hospital Vrije Universiteit, Dept. Of Haematology, Amsterdam, The Netherlands

The reproducibility of CFU enumeration in G-CSF stimulated whole blood (WB) collections can vary largely depending on the method used. This vari-
ability is especially important if WB is used as a stem cell source for autologous transplantation. We investigated three methods of CFU enumeration in WB.

Methods. 1. Mononuclear cells (MNC) were isolated by Ficoll separa-
tion from a WB sample. CD34+ cells were determined both in the MNC and in the original WB sample using the ProCount™ assay. Sub-
sequently, MNC were plated into CFU culture medium. In this way, the number of CFU-GM/mL of WB can be calculated independently of the cell count after Ficoll separation. 2. WB was lysed with NH4Cl and washed twice. The lysate was plated into CFU medium. 3. WB was plated directly into CFU medium. In all cases, the cells were diluted with fresh medium before plating into CFU medium (MethoCult GFH4434, Stem Cell Techn.) to a final concentration of 300 CD34+ cells/mL at maximum to prevent overplating.

In conclusion, the direct plating method gives the same number of CFU-
GM per mL of WB as both the Ficoll and the Lysis method, but has a better reproducibility. Besides, this method is much easier to perform.

Kinetics of immature reticulocytes (IR) during peripheral blood stem cell (PBSC) mobilisation in the treatment of aggressive lymphoma

Tsujiyama H, Kumagai K, Sakai C, Takay T

Hematology-Oncology Div, Chiba Cancer Center Hospital, Chiba, Japan

Background. Monitoring and searching for the maximum number of PBSC in the circulation are essential for successful PBSC collection. CD34+ cells or CFU-GMs are useful indicators, but laborious to measure and sometimes inaccurate. IR has been suggested to be another useful parameter (Remacha et al. 1996 Bone Marrow Transplant, 17, 163-168). We studied the kinetics of IR during PBSC mobilization. Design and Methods. We treated aggressive lymphoma with CHOP-ABVP therapy. PBSC collection was scheduled during the days of neutrophil recovery in the 3rd course of therapy. In 14 patients, IR was measured simultaneously with WB, CD34+ cells and CFU-GMs by the flow cytometer GEN-S (Coulter, Miami, Florida, USA) and was expressed as the high light scatter reticulocyte (HLR). To test the correlation with PBLC, CD34+ cells and CFU-GMs, absolute HLR and IR fraction rate (IR/HLR) of total reticulocyte) were used. Results. The kinetics of each parameter is described as follows:

<table>
<thead>
<tr>
<th>Exp</th>
<th>Ficoll</th>
<th>Lysis</th>
<th>Direct</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>9,793</td>
<td>9,475</td>
<td></td>
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<tr>
<td>2</td>
<td>4,377</td>
<td>4,442</td>
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<td>3</td>
<td>21,418</td>
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<td>25,200</td>
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In conclusion, per mL of WB as both the Ficoll and the Lysis method, but has a better reproducibility. Besides, this method is much easier to perform.

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</table>

In conclusion, per mL of WB as both the Ficoll and the Lysis method, but has a better reproducibility. Besides, this method is much easier to perform.
PO-0314 St Louis Hospital’s transfusion service experience in peripheral blood progenitor cell (PBPC) mobilisation and collection: an over view of 1171 patients in the CD34 technology era


From 1/1/94 to 31/12/98, 1171 patients were referred to our apheresis unit (Unité de Cytaphérèse et d’Immunothérapie - Hôpital St Louis, Paris, France) for autologous PBPC collection. Patients’ characteristics, age = 45 years (median) 18 (range: 5-80), 800 (88%) males and 371 (20%) females were assessed on day 7 and 21 by cell counting and CFU-GM, BFU-E, NPY, N-PSE 106 CD34 cell/kg in less than 5 daily LK sessions to support a myeloablative treatment afterwards with unprocessed autologous PBPC. Four types of blood cell separator were used: Cobe Spectra version 4 (Cobe 4) or version 6 (Cobe 6), Fresenius AS 104 (Fres) or Baxter CS 3000 (CS).

RESULTS.
- Product volume (mL) 682.8±175.0 (689.5) 376.4±92.10 (383) <.001
- Total CD34+ cells (x108) 10.53±13.78 (5.59) 3.87±3.23 (3.29) .003
- PBSC mobilization.
- Total CD34+ cells (x106) 16.8±19.8 (11.26) 5.82±4.26 (4.60) .002
- Post procedure platelets
  - 87.9±26.7 (72.8) 178.1±43.0 (192) <.001
- CD34+/kg receptor
  - 10.3±3.78 (5.59) 3.87±3.23 (3.29) .003
- CD34+/l of processed volume(x106)
  - 0.87±1.09 (0.35) 0.35±0.26 (0.27) .02

CONCLUSIONS.
- Decision to begin leukapheresis (LK) depended on PB CD34 cell count in patients at risk of PBPC mobilisation failure: the requirement was a minimum of 10 CD34 cells/µl. The standard of the PBPC collection was to collect at least 2.5 to 3x10^6 CD34 cell/kg in less than 5 daily LK sessions to support a myeloablative treatment afterwards with unprocessed autologous PBPC.
- more detailed informations from our data base concerning especially risk factors for inadequate PBPC mobilisation or collection (including impact of blood cell separator type on the PBPC collection efficiency) will be presented at the meeting.

PO-0315 Peripheral blood progenitor cell (PBPC) collections using two different generations of separators in normal donors

Marques JFC, Vigorito AC, Aranha FJP, Eld KAB, Azvedo AM, Roveri EG, Res ARC, Miranda ECM, De Souza CA, State University of Campinas, Sao Paulo, Brazil

Objective. We analysed the PBPC collections performed in allogeneic donors, and compared 2 different generations of equipment (Dideco Vivace A and Fresenius AS 104). Methods. Eighteen procedures (6M; 12F), from Jan/94 to Nov/96, were performed on Dideco Vivace, using the kit 40FL-40S, and analysed on automatic counters. Our main aim was to collect more than 2 x 10^6 mononuclear cells per kg receptor. At that time, we had no flow cytometer analyser available. The samples were frozen and stored until we CD34+ quantification was performed. Twenty-two procedures (10M;12F), from Aug/96 to Jun/98, were performed as recommended by manufacturers, on Fresenius AS 104. Our goal was to collect more than 5 x 10^6 CD34+ cells per kg receptor. This analysis was performed prospectively. Results. The results are shown on the table at the end of the text. Conclusions. We concluded that Dideco Vivace was able to collect more CD34+ cells, in spite of a higher post-procedure thrombocytopenia incidence. A more accurate study of the selectivity, CFU-GM quantification, CD33+, CD4+, CD8+ cells contents in the products collected in both equipment.

PO-0316 Influence of the purification procedure on the expansion of human umbilical cord blood CD34+ cells

Rodríguez-Calvillo M, Rifon J, Pinazo C, Cuesta B, Rodríguez-Wilhelmi P, Rocha E, Hematology Department, University Clinic of Navarre, School of Medicine, University of Navarra, Spain

UCB stem cells have become an alternative source of progenitors for allogeneic transplantation. Nevertheless, their extensive use has been restricted by the low number of cells available from most samples. In recent years, ex-vivo expansion of UCB cells has been widely investigated. We compared the expansion of CD34+ enriched UCB samples, collected from 18 full-term deliveries, obtained from two different selection systems. Nine samples were isolated using the CeprateL selection system and 9 collections were processed with the MiniMACS system. The mean recovery of the CD34+ fraction after MiniMACS separation was 90% compared to 52.7% using the Ceprate column, and the purity of the positive subset was 48.4% versus 28.31% respectively. The CD34+ enriched cells were cultured in suspension in 20% FCS-IMDM in the presence of SCF+IL-3+G-CSF at 100 ng/mL of each factor. Cells were seeded at 1 x 10^6 cells/mL and cultured in suspension for 7 and 21 days, monitored by flow cytometry and CFU-GM. Preliminary results are presented.

Table: Cell yields and CFU-GM activity

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Dideco Vivace</th>
<th>Fresenius AS 104</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.5±10.69 (30)</td>
<td>29.8±10.22 (30)</td>
<td>NS</td>
</tr>
<tr>
<td>Weight receptor (kg)</td>
<td>62±13.5 (59)</td>
<td>67.2±24.5 (64.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Procedure time (min)</td>
<td>376±31.35 (385)</td>
<td>258±62.35 (253)</td>
<td>NS</td>
</tr>
<tr>
<td>Volume processed (mL)</td>
<td>12.6±23.89 (12.000)</td>
<td>11.17±22.74 (12.421)</td>
<td>NS</td>
</tr>
<tr>
<td>PMN before (x 10^6/mm)</td>
<td>5.9±1.5 (6.0)</td>
<td>5.0±2.0 (5.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PMN after (x 10^6/mm)</td>
<td>3.29±0.87 (3.2)</td>
<td>2.15±0.97 (2.07)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pre-procedure platelets</td>
<td>3.23±0.27 (3.25)</td>
<td>239.9±24.8 (237.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Post-procedure platelets</td>
<td>87.4±6.26 (72.5)</td>
<td>178.1±43.0 (192)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Donor platelet decrease (%)</td>
<td>64.8±11.9 (63)</td>
<td>25.8±3.3 (27.2)</td>
<td>.01</td>
</tr>
<tr>
<td>CD34+/kg receptor (x10^6)</td>
<td>16.8±19.8 (11.26)</td>
<td>5.82±4.26 (4.60)</td>
<td>.002</td>
</tr>
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<td>Total CD34+ cells (x10^6)</td>
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<td>CD34+/l of processed volume (x10^6)</td>
<td>0.87±1.09 (0.35)</td>
<td>0.35±0.26 (0.27)</td>
<td>.02</td>
</tr>
</tbody>
</table>

* Mann-Whitney test.

We observed better nucleated cell expansion of CD34+ progenitors after MACS separation than after Ceprate isolation. Median fold expansion of CFU-GM at day 7 was also higher with the MiniMACS system (67.72-fold increase vs. 9.37-fold). We found that: 1) UCB CD34+ cells can be highly expanded after MiniMACS isolation; 2) The lower expansion of the Ceprate-selected progenitors is consistent with the lower purity yield obtained with this system, raising the question as to whether mononuclear cells present in the positive fraction may play a role in the inhibition of the expansion of CD34+ progenitors.

PO-0317 Mobilisation of previously autografted patients by a combination of cisplatin, etoposide and G-CSF


It is possible to obtain responses in patients with solid tumors in progression after previous high-dose therapy by using sequential intensive chemotherapy (SIC) with cellular support. The access to these programs depends on the feasibility of obtaining satisfactory mobilizations in such a heavily
pre-treated population. Design and Methods. Twenty-four patients with metastatic solid tumors who relapsed after high-dose chemotherapy with autologous stem cell support were admitted to a programme of SIC with cellular support after every course. Diagnoses were: 14 breast carcinoma, 4 ovarian, 3 germ-cell, 3 others malignancies. Patients were mobilised during the recovery from the first course. Chemotherapy (CT): cisplatin, 120 mg/m² in 2 days plus etoposide 600 mg/m² in 2 days. G-CSF: 5 to 12 mcg/kg/day was administered subcutaneously from the 9th day post CT. Peripheral blood (PB) CD34+ cells was performed from day +13. Descriptive statistics are expressed in median with extremes. Results: Med-ian number of CD34+ cells in PB the day of the first aphereses was 55 cells/mm³ (3-234). CD34+ cells obtained in the first aphereses were 3.6 x 10^9/kg (0.12-29.4). Seventy-five percent of the aphereses yielded more than 2 x 10^9/kg CD34+ cells. The post-CT day when the first aphereses could be performed was +19 (14-27), with an absolute leucocyte count of 25.5 x 10^9/L (14-47) and platelet count of 53 x 10^9/L (25-134). Time interval since the previous autograft did not influence the mobilization. In 54% of the courses patients required hospital admission because of fever or documented infection. There was one episode of non-haematological non-infectious grade III-IV toxicity (tiphlytis). There was no toxic mortality. Conclusions. This CT can induce potent mobilisation of CD34+ cells in previously autografted patients.

PO-0318 Evidence for the presence of primitive progenitors with lymphoid (NK or B) potential in ex vivo expanded bone marrow cells

Giaratana MC, Vergè V, Barret C, Kobari L, Droux L
INSERM U417, Hôpital Saint-Antoine, Paris; Service d’Hématologie Biologique, Hôpital Trousseau, Paris, France

Background. We have previously reported that CD34+ bone marrow cells can be successfully expanded ex vivo upon the myeloid pathway, in gas-permeable bags, in stroma and serum free conditions in the presence of Flt3-1 for 4 to 8 weeks. For NK assays, cultured on a preformed adherent monolayer in 24 wells plates. For B-cell culture, from June 1997 to October 1998, 26 patients (4 males/22 females) were mobilised for an eventual autologous stem cell transplant. Eleven patients were primed with PT 170 mg/m² in a 24 hr infusion day 1, cyclophosphamide 4 g/m² in day 2, and G-CSF 8 mg/m² sc. from day 3 until the last apheresis day (PT-Cy-G group). Fifteen patients were treated with PT 170 mg/m² in a 24 hr infusion day 1 and G-CSF 8 mg/m² sc. from day 2 (PT-G group). The first apheresis procedure was performed when the number of CD34+ cells in peripheral blood was ≥ 30 µL or when the total number of WBC was ≥3000/µL which occurred first. There were 18 patients with breast cancer (7 T-G/11 T-G), 4 patients with multi-pIle myeloma (2 in each group), 3 with non-Hodgkin’s lymphoma (2 T-CY-G/11 T-G) and 1 patient with Hodgkin’s disease (T-G). Results. The first day of apheresis (mean±SD) was 10±2.7. The mean number of aphereses procedures performed was 1.46±0.85. The mean number of CD34+ cells collected was 803.8±541.4 x 10^9/kg (11.6±5.7 x 10^9/kg). The mean number of aphereses required to obtain at least 2 x 10^9/kg CD34+ cells was 1.26±0.82. There were no differences in the number of CD34+ cells nor in the CD34+/CD38- or CD34+/CD56+ subpopulations obtained between the PT-Cy-G and the PT-G groups. The first apheresis day was significantly earlier in the T-G group (8.66±0.89 vs 13.36±0.92 p<0.01). There were fewer days of neutropenia (ANC<1000/µL) in the PT-G than in PT-Cy-G group (0.66±0.97 vs 6.1±2.3, p<0.01), as well as fewer days of thrombocytopenia (2.4 days of platelets<100,000/µL vs 8±3.6 respec-tively). There were three episodes of neutropenic fever in the PT-G group and none in the PT-G group. There were no deaths. Conclusions. Our data shows that Paclitaxel plus G-CSF is a useful scheme for mobilisation of HPC. The inclusion of cyclophosphamide was not associated with better collect-yields.

PO-0320 Comparison of two different doses of G-CSF for stem cell mobilisation and collection in normal donors


Objective. To know the optimal G-CSF dose and schedule for stem cell mobilisation in normal donors. Design and Methods. Retrospective analysis of the number of CD34+ cells collected after the administration of G-CSF to 459 normal donors included in the Spanish National Donor Registry at two different doses: 10 µg/kg day for 3 until the last apheresis day (PT-Cy-G group). There were no differences in terms of age and sex between the two groups. The median dose of G-CSF administered in the higher dose group was 12 (range, 10.8-20) µg/kg day. Median duration of G-CSF administration was five days in both groups (range, 4-5). Results. The median number of CD34+ cells collected from donors receiving >10 and 10 µg/kg day was 7.4±5 x 10^9/kg and 6.6±3 x 10^9/kg, respectively. This difference was not statistically significant. When only donors receiving >10 µg/kg day were considered, the CD34+ cells collected from people treated for five days was higher (7.8±6.1 x 10^9/kg) than those collected after four days of G-CSF (6.9±3 x 10^9/kg), although differences were not significant. Finally, side effects were more frequently reported in donors receiving >10 µg/kg/day of G-CSF than in those with the lower dose (82.8% versus 61.8%; p=0.004), though severity was similar in both groups. Conclusions. These findings suggest that G-CSF dose of 10 µg/kg day for four to five days appears to be the best regimen for mobilisation and collection of CD34+ cells in the majority of normal donors, and that it should be the standard mobilisation regimen in this population.
We have previously documented autologous cytolytic activity against AML blasts in patients after autologous bone marrow transplantation (ABMT). Here we present a patient with poor-risk AML M2 who relapsed from first CR despite 2 courses of consolidation therapy with cytarabine (1.33 g/m²/day; days 1-3); daunorubicin (45 mg/m²/day; days 1-3) and etoposide (400 mg/m²/day; days 8-10). She achieved second CR following Fludarabine (30 mg/m²/day; days 1-5); Ara-C (2 g/m²/day; days 1-5); Idarubicin (5 mg/m²/day; days 1-5) and G-CSF (600 µg/day; days 1-5). Two weeks after completion of this course the patient's bone marrow was in morphological CR and she then received a repeat course of FLAG/IDA for consolidation. The patient was unwilling to undergo stem cell transplantation. In second chemotherapy-induced CR the patient had no evidence of anti-leukaemia cytolytic activity in an in vitro assay and she commenced IFN-α (Roferon). She subsequently developed high levels of leukaemia-specific cytotoxicity (Figure 1) and has remained in second CR for two years. These findings support the use of IFN-α in patients with poor-risk AML and suggest that one mechanism of action may be immunological. The storage of leukaemic cells at presentation will allow subsequent testing for LSC and help our understanding of the control of leukaemia.

Po-0321 Generation of autologous immunity to acute myeloid leukaemia and maintenance of complete remission following interferon treatment
Lowdell MW, Crabton R, Prentice HG
Department of Haematology, Royal Free & University College Medical School, RF Campus, London, UK

We have previously documented autologous cytolytic activity against AML blasts in patients after autologous bone marrow transplantation (ABMT). Here we present a patient with poor-risk AML M2 who relapsed from first CR despite 2 courses of consolidation therapy with cytarabine (1.33 g/m²/day; days 1-3); daunorubicin (45 mg/m²/day; days 1-3) and etoposide (400 mg/m²/day; days 8-10). She achieved second CR following Fludarabine (30 mg/m²/day; days 1-5); Ara-C (2 g/m²/day; days 1-5); Idarubicin (5 mg/m²/day; days 1-5) and G-CSF (600 µg/day; days 1-5). Two weeks after completion of this course the patient's bone marrow was in morphological CR and she then received a repeat course of FLAG/IDA for consolidation. The patient was unwilling to undergo stem cell transplantation. In second chemotherapy-induced CR the patient had no evidence of anti-leukaemia cytolytic activity in an in vitro assay and she commenced IFN-α (Roferon). She subsequently developed high levels of leukaemia-specific cytotoxicity (Figure 1) and has remained in second CR for two years. These findings support the use of IFN-α in patients with poor-risk AML and suggest that one mechanism of action may be immunological. The storage of leukaemic cells at presentation will allow subsequent testing for LSC and help our understanding of the control of leukaemia.

Po-0322 The influence of interleukin-2, -7 and -15 on the development of natural killer cells
Crabton R, Prentice HG, Lowdell MW
Department of Haematology, Royal Free & University College Medical School, London, UK

We have demonstrated autologous leukaemia-specific cytolytic activity mediated by CD56+ve cells which had been cultured in vitro. Following immunomagnetic selection of these cells, we investigated the roles of IL-2, IL-7 and IL-15 in their activation and proliferation. The majority of CD56+ve cells were CD8-ve (~70%), and this subset was studied to see if it was possible induce CD8 activation and proliferation. The majority of CD56+ve cells were CD8-ve (~70%) and this subset was studied to see if it was possible induce CD8 activation and proliferation.

Po-0323 Autologous lystate-pulsed dendritic cells stimulate T-cells in patients with B-cell chronic lymphocytic leukaemia
Goddard RY, Lewis S, Prentice AG, Kaminski E
Plymouth Postgraduate Medical School, Derriford Hospital, Plymouth, UK

Dendritic cells have emerged as potent stimulators of anti-tumour responses. Our main aim was to investigate whether the pulsing of dendritic cells with B-cell lysates from patients with B-cell chronic lymphocytic leukaemia would stimulate autologous T-cells. Patients who had not received treatment in the last 6 months were selected. Removal of 99.9% of the peripheral blood mononuclear cells with CD19 (Pan B) Dynabeads facilitated the isolation of adherent monocytes. Dendritic cells were cultured adherent monocytes for 6 days in the presence of IL-4 and GM-CSF. The dendritic cells were HLA-DR positive and CD83 negative indicating that they were at the optimum stage of differentiation for peptide loading. The dendritic cells were pre-treated with IL-12 for 16 hours then pulsed with a B-cell lysate from the patient for 4 hours. Autologous T-cells were cultured alone or with lymphocyte-pulsed and unpulsed dendritic cells. Interferon gamma secretion was assessed using ELISA. All the cultures were fed IL-2 (5U/mL) on day 3 and 7 of culture. Lysate-pulsed dendritic cells cultured with T-cells were restimulated with soluble protein on day 7 of culture. After 14 days in culture cytotoxicity to the patients cells was assessed using flow cytometry. After 72 hours in culture T-cells cultured with lymphocyte-pulsed dendritic cells showed a significant increase in IFN-γ secretion. After a total of 14 days in culture T-cells which had been stimulated with lysate-pulsed dendritic cells and re-stimulated with soluble protein showed specific cytotoxicity of 10±5% at the highest effector:target ratio of 40:1. T-cells cultured with dendritic cells not pulsed with B-cell lysate showed specific cytotoxicity of 2.5±8%. We conclude that autologous dendritic cells can stimulate autologous helper T-cells and cytolytic T-cells to respond to a B-cell lysate from patients with B-cell chronic lymphocytic leukaemia.

Po-0324 Rituximab and complement induced lysis of neoplastic B cells is regulated by CD55
Golajy J, Zaffaroni L, Borleri G-M, Tedesco F, Dastoli G, Barbui T
Istituto Mario Negri, Milano, *Division of Hematology, Ospedali Riuniti, Bergamo; °Roche Italia, Milan; #University of Trieste, Italy

Rituximab (humanised anti-CD20 MAb) is proving a useful therapeutic agent in the treatment of NHL. Its mechanism of action includes antibody-depen- dent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) leading to depletion of normal and neoplastic B cells from the circulation. In follicular lymphomas (FL), the response rate is about 50% of the patients. Rituximab is also being considered for the treatment of oth- er CD20+ B cell neoplasias such as chronic lymphocytic leukaemia (CLL) and high grade lymphomas. We have therefore initiated an in vitro study to investigate the basis for the heterogeneity of the response in FL patients and to determine the response of fresh CLL cells to Rituximab. We inves- tigated the effect of Rituximab on several cell lines. Effects on prolifer- ation in the absence of complement and induction of ADCC were comparab- le for all lines. On the other hand CDC, measured by acridine orange staining and FACS analysis, was very heterogeneous. Study of the surface complement inhibitors CD46, CD55 and CD59 indicates that CD55 inhibits CDC in several lines. The relative levels of CD20 expression on the cell sur- face may also affect efficiency of killing. Expression of CD20 and CD20 may thus be of prognostic value to predict the response of neoplastic cells to CDC. More recent data obtained on CLL cells will be presented.

Po-0325 Maintenance therapy with histamine-dihydrochloride (masamine) and interleukin-2 (IL-2) in acute myelogenous leukaemia (AML)
Depts of Hematology, Göteborg, Umeå, Sundsvall, SM EDEN

Objective. T-cells and NK-cells become anergic to IL-2 after exposure to monocyte (MO) derived reactive oxygen metabolises (ROM). Histamine, an inhibitor of ROM formation in MO, was administered in addition to low-dose chemotherapy (CT) and compared with CT alone in acute myelogenous leukaemia (AML). Methods. A total of 13 AML patients, including 12 CR1 patients and one CR2 patient, were studied. The study period was 14 days in culture cytotoxicity to the patients cells was assessed using flow cytometry. After 72 hours in culture T-cells cultured with lymphocyte-pulsed dendritic cells showed a significant increase in IFN-γ secretion.
subsets deserve special attention in evaluating antiretroviral therapy. Expansion of selected CD8 T-cell subsets and NK cells. These lymphocyte immune function in HIV disease. Cellular immune function correlates with total CD8 T-cells (P=0.001), primed CD8 T-cells (P=0.006) and NK cells. However, a significant correlation was seen between mitogen reactivity and increased cells, or cytotoxic, activated and stimulated CD8 T-cell subsets. However, in a 43 year old man with IgGk stage III myeloma refractory to VAD and a first relapse 2 months after chemotherapy. Seven patients had used topical DNCB for 2-7 years, and nine patients had taken for 7 days. Phenotype was assessed by 2-color FACS. The responses did not correlate with HIV PI therapy, viral load, CD4 T-cells, B-cells, or cytotoxic activated and stimulated CD8 T-cell subsets. However, a significant correlation was seen between mitogen reactivity and increased total CD8 T-cells (P=0.001), primed CD8 T-cells (P=0.006) and NK cells (P=0.008). There was no correlation between mitogen response and naive CD4 T-cells, but a significant correlation was found with increased naive CD8 T-cells (P=0.001) and memory CD8 T-cells (P=0.014). Conclusions. Topical DNCB therapy is associated with improved cellular immune function in HIV disease. Cellular immune function correlates with expansion of selected CD8 T-cell subsets and NK cells. These lymphocyte subsets deserve special attention in evaluating antiretroviral therapy.

**PO-0326 Improved cellular immune function associated with topical dinitrochlorobenzene (DNCB) therapy in HIV disease**

Stricker RB, Goldberg B, Winger EE, Epstein WL

*California Pacific Medical Center, International DNBC Study Group, University of California School of Medicine, San Francisco, CA, USA*

Background. Human immunodeficiency virus (HIV) infection is associated with progressive deterioration of cellular immune function. Topical dinitrochlorobenzene (DNCB) treatment of HIV-infected patients increases quantitative markers of cellular immunity, including CD8 T-cells and natural killer (NK) cells (Stricker et al, Immunol Lett 1997;59:145-150; Oracion et al, J Invest Dermatol 1996;106:476). Objective. To examine the effect of topical DNCB on peripheral blood mononuclear cells of HIV-infected patients. Design. Fourteen HIV-infected patients were included in the study. Nine patients had used topical DNCB for 2-7 years, and seven patients had taken for 1-3 months. Six patients were using a combination of DNCB and HIV PI therapy, and two patients were untreated. Cellular immune function was assessed by lymphocyte proliferation to three mitogens: phytohemagglutinin (PHA), concanavalin A (Con-A) and pokeweed mitogen (PWM). Lymphocyte subsets were measured by flow cytometry, and viral load was measured using the HIV RNA polymerase chain reaction. Results. Eight of the 14 patients had a normal proliferative response to at least one mitogen. Mitogen responsiveness showed a significant correlation with topical DNCB therapy (P=0.038). The responses did not correlate with HIV PI therapy, viral load, CD4 T-cells, B-cells, or cytotoxic activated and stimulated CD8 T-cell subsets. However, a significant correlation was seen between mitogen reactivity and increased total CD8 T-cells (P=0.001), primed CD8 T-cells (P=0.006) and NK cells (P=0.008). There was no correlation between mitogen response and naive CD4 T-cells, but a significant correlation was found with increased naive CD8 T-cells (P=0.001) and memory CD8 T-cells (P=0.014). Conclusions. Topical DNCB therapy is associated with improved cellular immune function in HIV disease. Cellular immune function correlates with expansion of selected CD8 T-cell subsets and NK cells. These lymphocyte subsets deserve special attention in evaluating antiretroviral therapy.

**PO-0327 Graft versus myeloma (GVM) effect with allogeneic lymphocyte transfusion after autologous peripheral blood stem cell transplantation in refractory myeloma**


Hôtel-Dieu, Paris, Hôpital Foch, Suresnes, France

Donor lymphocyte infusion has been proposed as treatment of relapsed haemopoietic after allogeneic bone marrow transplantation (allo-BMT). Response suggests an immunological effect against the disease. Adverse effects are possible graft versus host disease (GVHD) and aplastic anaemia. High dose chemo-radiotherapy with haemopoietic stem cell transplantation (HSCT) increases overall survival in patients with myeloma. GVM effect is reported after donor lymphocyte infusion in patient with myeloma relapsing after alloBMT. However, the transplant related toxicity after alloBMT is high and chance of cure is not higher than after autologous HSCT. Thus autologous HSCT is preferred to alloBMT. We report a case of a 49 year old man with IgGk stage III myeloma refractory to VAD and a first relapse 2 months after chemotherapy. Seven patients had used topical DNCB for 2-7 years, and nine patients had taken for 1-3 months. Six patients were using a combination of DNCB and HIV PI therapy, and two patients were untreated. Cellular immune function was assessed by lymphocyte proliferation to three mitogens: phytohemagglutinin (PHA), concanavalin A (Con-A) and pokeweed mitogen (PWM). Lymphocyte subsets were measured by flow cytometry, and viral load was measured using the HIV RNA polymerase chain reaction. Results. Eight of the 14 patients had a normal proliferative response to at least one mitogen. Mitogen responsiveness showed a significant correlation with topical DNCB therapy (P=0.038). The responses did not correlate with HIV PI therapy, viral load, CD4 T-cells, B-cells, or cytotoxic activated and stimulated CD8 T-cell subsets. However, a significant correlation was seen between mitogen reactivity and increased total CD8 T-cells (P=0.001), primed CD8 T-cells (P=0.006) and NK cells (P=0.008). There was no correlation between mitogen response and naive CD4 T-cells, but a significant correlation was found with increased naive CD8 T-cells (P=0.001) and memory CD8 T-cells (P=0.014). Conclusions. Topical DNCB therapy is associated with improved cellular immune function in HIV disease. Cellular immune function correlates with expansion of selected CD8 T-cell subsets and NK cells. These lymphocyte subsets deserve special attention in evaluating antiretroviral therapy.

**PO-0328 Culture of dendritic cells from cancer patients for immunotherapy**

Mayordomo IJ, Laeruella P, Palomera L, Isla D, Cajar R, Uybero A, Larraz L, García MD, Trea A

Division of Medical Oncology, Immunology and Haematology, Hospital Clinico Universitario, Zaragoza, Spain

Background. Dendritic cells are the most potent antigen-presenting cells known. The development of methods to culture large numbers of mouse dendritic cells from haematoepoietic progenitors grown in granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 has made testing of immunisation with T-cell recognised tumour peptide-pulsed dendritic cells possible. This strategy has induced regression of established tumors in mouse models (Mayordomo et al, Nature Medicine 1995, 1:1297). Aims. To verify whether large numbers of autologous dendritic cells can be generated from peripheral blood mononuclear cells in cancer patients. Design and Methods. Peripheral blood mononuclear cells from 30 oncologic patients undergoing chemotherapy after stem cell mobilisation with G-CSF chemotherapy were cultured. Mononuclear cells were separated and cultured in GM-CSF (1000 UI/mL) and interleukin-4 (1000 UI/mL) for 7 days. Phenotype was assessed by 2-color FACs. Results. Tumour types were: breast cancer (20 pts), non-Hodgkin’s lymphoma (5 pts), germ cell tumour (2 pts), ovarian cancer (2 pts) and rhabdomoyosarcoma (1 pt). The phenotype of cultured cells was consistent with dendritic cells: intense positivity for HLA-DR and CD 86, with negativity for markers of other lineages, including CD3, CD4, CD8 and CD14. The identity of the cultured cells as dendritic cells was confirmed by their typical round morphology with multiple cytoplasmic projections on light microscopy and by their immunocytochemical positivity for HLA-DR. Although the number of dendritic cells cultured differed from case to case, more than 10^7 dendritic cells can be generated from a single leukapheresis in more than 50% of patients. Conclusions. Sufficient numbers of dendritic cells for ongoing cancer immunotherapy trials can be reproducibly generated from cancer patients by culture of peripheral blood mononuclear cells in GM-CSF plus interleukin-4.

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**PO-0329 Rituximab: phase II (PII) treatment study in 28 patients (PTS) with relapsed follicular and low-grade (F/LG) NHL**


*Hosp. Vall D’Hebron, Barcelona; 12 Octubre, Madrid; Sant Pau, Barcelona; G. Marañon, Madrid; V. Rocío, Sevilla; Insular, Las Palmas; V. Victoria, Malaga; A. Amixaca, Murcia, Spain*

A PII study was conducted to determine the safety and efficacy of treatment with anti-CD20 Mab rituximab in relapsed pts with F/LG NHL. No more than
three prior relapses following standard therapy were allowed. The dose regi-
imen was 375 mg/m2 of Rituximab once weekly for four weeks. Pts char-
teristics included: 46% male, median age 54, median 2.3 years from diagnosis (0.8-13.4). Disease characteristics: 85% stage III/IV, 24.8 cm2 (1-160) average overall size of their measurable lesions (sum of all the measured two main diameters). Twenty-eight pts were included. When the statistical analysis was performed, 24 had completed the treatment. 2 had withdrawn from the study before completing the treatment and in 2 pts the treatment was ongoing. The pts did not receive any concomitant anti-
cancer treatment (including corticosteroids) before a response was docu-
mented. Sixteen of 28 pts were evaluable for efficacy (3 months of follow-
up) and the overall response rate (ORR) was 56% with 1 (6%) CR and 8
(50%) PRs; the median time to maximum response was 49 days (21-84).

To determine the capacity of Fc receptors (FcR) to function as cytotoxic trigger molecules on different effector cells using monoclonal (MoAb) or bispecific antibodies (BsAb) directed against EGF-R, EGF-R was significantly enhanced, whereas G-CSF therapy induced target cell killing by an FcR or EGF-R BsAb. As effectors source, whole blood and isolated effector cells from healthy volunteers, tumor cells were killed by humanised MoAb 425, FcRRII or FcCRII to function as cytotoxic trigger molecules on different effector cells using monoclonal (MoAb) or bispecific antibodies (BsAb) directed against renal cell carcinoma. Design and Methods. Several renal carcinoma cell lines were tested as targets in antibody-dependent cellular cytotoxicity (ADCC) assays. The epidermal growth factor receptor (EGFR)- which is overexpressed on a majority of renal cell carcinomas – was used as target antigen for different EGFR-directed antibody constructs (murine- or humanised MoAb 425 from E. Merck, Germany, or corresponding [FcRII or EGF-
R], [FcRI x EGF-R] or [FcRII x EGF-R] BsAb). As effectors source, whole
blood from patients receiving G-CSF, GM-CSF, or a combination of IL-
2 and IFN-[g], [FcRII x EGF-R] BsAb in combination with blood from healthy volunteers, target cells were killed by humanised MoAb 425, FcRRII or [FcRII x EGF-R] BsAb in combination with the CD1a antigen from the CD1a expression from an effector source, and then finally adding the absolute number of CD1a+ cells from each effector. Patients mobilised with r-metHuG-CSF (cohort A) possessed a median total of 106 x 10^6 CD1a+ cells (range = 8-278 x 10^6, n = 12) and a mean total of 117.0±23.5 x 10^6 CD1a+ cells. By contrast, patients mobilised with r-metHuG-CSF + HuSCF (cohort B) possessed a median total of 212 x 10^6 CD1a+ cells (range = 29-499 x 10^6, n = 12) and a mean total of 208.4±64.0 x 10^6 CD1a+ cells. CD1a+ cells in cohort C also showed increased total numbers of CD1 a+ cells [median = 228 x 10^6, range = 83-414 x 10^6, n = 12; mean = 238.0±32.5]. These data indicate that large numbers of phenotypically identifiable dendritic cells are mobilised by metHuG-CSF and, that this mobilisation is signifi-
cantly enhanced by concurrent treatment or pretreatment with HuSCF. Functional analyse of the cells mobilised by metHuG-CSF is as yet incomplete, but further analysis of the phenotype expression of DC markers linked to the ability to present antigen will further elucidate the role that growth factor (G-CSF and SCF) mobilised DC can play in immunotherapy.

**PO-0330** Recruitment of cytotoxic cells via different Fc receptors: new approaches for immunotherapy of renal cell cancer

Stadick H, Elsässer D,** Valerius T, Stockmeyer B, Glennie M,* van de Winkel JG*, Kalden JR, Schrott KM,* Gramatzki M**
Departments of Medicine I and *Urology, University Erlangen-Nürnberg, Germany; **Tenovus Research Laboratory, Southampton, UK; +Medarex Europe BV and Department of Immunology, Utrecht, The Netherland

Objective. To determine the capacity of Fc receptors (FcRI, FcRII or FcCRII) to function as cytotoxic trigger molecules on different effector cells using monoclonal (MoAb) or bispecific antibodies (BsAb) directed against renal cell carcinoma. Design. Methods. Several renal carcinoma cell lines were tested as targets in antibody-dependent cellular cytotoxicity (ADCC) assays. The epidermal growth factor receptor (EGFR)- which is overexpressed on a majority of renal cell carcinomas – was used as target antigen for different EGFR-directed antibody constructs (murine- or humanised MoAb 425 from E. Merck, Germany, or corresponding [FcRII or EGF-R], [FcRI x EGF-R] or [FcRII x EGF-R] BsAb). As effectors source, whole blood from patients receiving G-CSF, GM-CSF, or a combination of IL-
2 and IFN-[g], [FcRII x EGF-R] BsAb in combination with blood from healthy volunteers, target cells were killed by humanised MoAb 425, FcRRII or [FcRII x EGF-R] BsAb in combination with the CD1a antigen from the CD1a expression from an effector source, and then finally adding the absolute number of CD1a+ cells from each effector. Patients mobilised with r-metHuG-CSF (cohort A) possessed a median total of 106 x 10^6 CD1a+ cells (range = 8-278 x 10^6, n = 12) and a mean total of 117.0±23.5 x 10^6 CD1a+ cells. By contrast, patients mobilised with r-metHuG-CSF + HuSCF (cohort B) possessed a median total of 212 x 10^6 CD1a+ cells (range = 29-499 x 10^6, n = 12) and a mean total of 208.4±64.0 x 10^6 CD1a+ cells. CD1a+ cells in cohort C also showed increased total numbers of CD1 a+ cells [median = 228 x 10^6, range = 83-414 x 10^6, n = 12; mean = 238.0±32.5]. These data indicate that large numbers of phenotypically identifiable dendritic cells are mobilised by metHuG-CSF and, that this mobilisation is signifi-
cantly enhanced by concurrent treatment or pretreatment with HuSCF. Functional analyse of the cells mobilised by metHuG-CSF is as yet incomplete, but further analysis of the phenotype expression of DC markers linked to the ability to present antigen will further elucidate the role that growth factor (G-CSF and SCF) mobilised DC can play in immunotherapy.

**PO-0332** Measurement of autologous immunity to leukaemia can predict maintenance of complete remission at two years

Departments of Haematology, Royal Free & University College Medical School - RF Campus, London; Colchester General Hospital, Essex, UK.

In this study we have tested eleven patients at various stages through anti-
leukaemia therapy for evidence of leukaemia-specific cytotoxic activity (LSC). Five patients received conventional chemotherapy alone while a fur-
ther six underwent autologous BMT(aBMT) after high dose chemotherapy. All patients were undergoing treatment for acute leukaemia (AML-12, ALL-1). Ten of these patients had detectable leukaemia-specific immune reactivity at one or more time point during treatment. Analysis of the lev-
els of LSC at six months post CR or post aBMT was informative. Five patients remain in CR at 24 months post treatment, six relapsed within 18 months. The mean LOWEST LSC for the 11 “survivors” was 17.3% [sd 5.65]. The mean HIGHEST LSC of the group who relapsed was 8.56% [sd 5.25] (Figure 1). None of the patients who relapsed achieved LSC >17.3% in any sample tested. This gives a sensitivity of prediction of relapse of 100%. Ten of the five “survivors” had individual LSC >17.3% (but both patients had higher LSC at other sample times). This gives an assay specificity of 81.8%. The storage of leukaemic cells at presentation will allow subsequent test-
ing for immune responsiveness and help our understanding of the control of leukaemia in man.

**Figure 1**
Po-0333 Induction of anti-tumour immune response by activation of autologous B lymphocytes

Banat GA, Cocholivus B, Christ O, Zöller M, Praille HB
Innere Medizin IV, Justus-Liebig Universität Giessen, Giessen, Germany

Immunosuppressive features of tumour cells are a major obstacle for immunotherapy of cancer. We recently noted that some RENCA (renal cell carcinoma) tumour line of BALB/c mice (H-2d) cell-derived clones effectively interfere with the activation of RENCA-specific T cells. To unravel the underlying mechanism, we evaluated the influence of RENCA cells of mounting an allogeneic mixed lymphocyte reaction (MLR). We therefor performed an MLR, a lymphocyte/tumour cell culture as well as a double chamber culture and measured the proliferation rate of the effector lymphocytes by 3H-thymidine incorporation. Further analyses were done by the flow cytometry.

We observed that RENCA cells are not directly immunosuppressive. Instead, we noted the initiation of production of immunosuppressive factors, e.g. by lymphocytes, which likely triggers NK 1.1+ T cells to kill CD4+ and CD8+ lymphocytes. Interestingly, this immunosuppressive pathway could be circumvented when tumour cells were presented by conventional antigen-presenting cells or expressed an appropriate costimulatory molecule (B7.1). Thus activation induced cell death (AICD) can be a major obstacle in immunotherapy of cancer, independent of whether the tumour itself expresses the Fas ligand. Our data demonstrate that the first contact between elements of the immune system and the tumour cell may be of great importance for directing immunoregulatory circuits.

Po-0334 MDS dendritic cells are phenotypically and functionally different from those obtained in healthy subjects

Ripoll GD,* Sneddon C,* Castoldi G,* Mufli G*
Department of Haematological Medicine, King's College of Medicine, London, UK; Department of Biomedical Sciences, Haematology Section, University of Ferrara, Italy

Encouraging the immune system of patients to eradicate malignant cells by using immunotherapeutic induction may represent a novel treatment strategy for patients with haematological malignancies. The development of a specific and potent cell-pulse requires optimal presentation of antigens by a MHC class II molecule, and delivery of one or more costimulatory signals provided by professional APC such as dendritic cells (DC) to naive T cells. Regarding the use of DC for in vivo adoptive immunotherapy, vaccination, however, it is important to define the optimal cell type and the most feasible source. Using a combination of GM-CSF and IL-4 we have been able to generate virtually in vitro DC from the peripheral blood mononuclear cells of patients affected by MDS and we have investigated their phenotypic and functional similarity to DC generated in a similar way from healthy donors. DCs were studied by flow cytometry using a combination of monoclonal antibodies (CD1a, CD4, CD14, CD45, CD54, CD80, CD83, CD86, and HLA-DR) while their ability to induce allogeneic cell proliferation was evaluated in the mixed leucocyte reaction (MLR).

After 10 days of culture, comparable yields of cells were obtained in normal and MDS subjects. Flow cytometry analysis revealed a predominant population of large cells exhibiting increased forward and light scatter, which were identified as DC by the coexpression of CD 1a and CD45 and were stimulated under FCS-free conditions with either Id-transduced or Id-pulsed DC. Induction of Id-specific cytotoxicity was assessed with Id-transfected autologous lymphoblastoid cells (LLC) or K562 as target cells. Results. Id-pulsed DC were shown by confocal microscopy. Efficient transduction of DC by Id-Fc vectors was shown by Western blot analysis. Fab-pulsed DC efficiently induced specific lytic activity against heavy chain-expressing LLC with half-life degradation of 4-5 days. In contrast, these stimulation conditions resulted in moderate NK activity. Conclusions. A-PCR and bacterial expression permitted rapid, convenient and reliable production of lymphoma-derived Id-Id from non-Ig-secreting BCL for vaccination therapy. In a strictly autologous human lymphoma system, Id-pulsed DC appeared to induce Id-specific cytotoxicity without NK activity more efficiently than Id-transduced DC. These in vitro data have important implications for rational design of Id-directed immunotherapy of human lymphoma patients.

Po-0336 Progenipetin, a chimeric growth factor which mobilizes hematopoietic progenitors and dendritic cells

Doshi PD, Klein BK, Streeter PR, Kahn LE, MacVittie TJ, Farase AM, Fleming WH, Mc Keam JP, Wouffe SW, G. D. Searle, Monsanto Company, University of M aryland, Oregon Health Sciences University, St. Louis, USA

Objective. We have examined biological activities of Progenipetin (ProGP), a novel chimeric molecule, that activates both the human flt-3 and G-CSF receptors in mouse and rhesus monkey models. Design and methods: ProGP was administered subcutaneously (50-500 µg/kg/day, QD) in mice for 10 days and cells from peripheral blood (PB), spleen and lymph nodes were analysed by flow cytometry. Mobilisation and engraftment of transplantable hematopoietic stem cells was analysed in lethally irradiated mice. In primate models, stem cell mobilisation was investigated in normal rhesus and hematopoietic reconstitution in rhesus following SC administration of ProGP (50-400 µg/kg/day, QD for 10 days). Results. Administration of ProGP in mice increased the numbers of Gr-1+CD11b+ neutrophils, CD11c+ class II+ dendritic cells (DC) and B220+ lymphocytes in PB by up to 400, 120, and 5-fold, respectively. ProGP treatment also increased the number of CD 11c+/Class II+ DC in both spleen and lymph nodes. Purified CD 11c+/Class II+ DC isolated from ProGP treated mice dose-dependently stimulated T cell proliferation in a mixed leucocyte reaction (stimulation index 2x), as well as lysis of peptide-pulsed target cells by cytotoxic T lymphocytes. In a murine serial transplantation model, 5 µL of unfractionated PB showed donor-derived multiline hematopoietic reconstitution whereas 100 µL of PBS/BSA mobilised PB failed to protect lethally irradiated recipients. Administration of ProGP to rhesus monkeys increased the numbers of mononuclear cells, CD3+ cells, and total colony-forming cells in peripheral blood by up to 3-, 3-, and 377-fold, respectively. Furthermore, ProGP treatment of myelosuppressed rhesus monkeys stimulated effective recovery of neutrophils and platelets.

Conclusions. Data from both murine and non-human primate models suggest that ProGP may be useful for multi-lineage hematopoietic restoration following cytotoxic cancer therapies. Furthermore, if comparable numbers of functional DC are mobilised into human peripheral blood by ProGP, this chimeric cytokine is likely to enhance immunotherapeutic approaches to cancer treatment.

Po-0337 Induction of an efficient anti-idiotypic immune response by vaccination with a DNA vaccine encoding the VH domain of the lymphoma immunoglobulin

Benvenuti F, Burrone O, Eremin D.G
ICGEB Trieste, Italy, and Dept. of Hematology, Faculty of Medicine, Skopje, Macedonia

The variable regions of the immunoglobulin (Ig) expressed in malignant B cells contain tumour specific idiotypic (Id) determinants which can be used as targets for active immunotherapy. Vaccination with immunogenic formulations of tumour derived Ig can elicit strong anti-Id Ab responses which have proved beneficial in murine B-cell tumour challenge experiments and

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in recent human clinical trials. Anti-id immune responses can also be elicited with naked plasmid DNA vaccines encoding the id determinants as scFv fragments. In this study we have produced a scFv gene construct linked to the CMV enhancer region from the human CD34+ cell line H9 and tested its efficacy in inducing protective immunity against the mouse BCL1 lymphoma. Two other constructs in which the BCL1 V\(_i\) gene was deleted (scFvAN\(_i\)) or substituted with a human V\(_i\) gene (scFvAN\(_i\)) were also generated to investigate whether the V\(_i\) region domain contains sufficient anti-id Abs were detected by ELISA using BCL1 IgM protein and by flow cytometry using BCL1 tumour cells in animals from all groups, with the highest titres in those immunized with the complete scFv vaccine. The three groups of immunized animals and a control unimmunised group were challenged with 10\(^5\) BCL1 tumour cells. The unimmunised animals died by day 100 with a mean survival of 86 days. All animals immunised with the complete scFv construct mounted protective immune responses and were still alive after 220 days of follow-up (p<0.001). The survival of mice immunised with the scFvAN\(_i\) construct (mean 99 days) did not differ significantly from the survival of the unimmunised mice (p=0.1), but mice immunised with the scFvAN\(_i\) construct showed significantly longer (mean 135 days, as a result). In conclusion, a scFv DNA vaccine in which the V\(_i\) and V\(_\lambda\) genes of the BCL1 Ig are linked to the human IgG1 CH3 exon can induce complete protection in the BCL1 lymphoma model. An anti-idiotypic immune response leading to prolonged survival can also be induced with a DNA vaccine encoding only the VH domain of the lymphoma Ig. This approach, although not as effective as vaccination with a complete scFv construct, may be advantageous in terms of simpler production of patient specific DNA vaccines.

**PO-0338 Serum-free culture conditions for the generation of dendritic cells from cord blood CD34+ hematopoietic progenitors: phenotypic and functional analysis. Efficiency of gene transfer methods**

Serravinyals M, Flores R, González-Barca E, Querol S, García J, Rueda F. Dpt. of Cyrobiology and Cell Therapy, Institut de Recerca Oncològica, Hospital Duran i Reynals, Barcelona, Spain

Dendritic Cells (DC) are potent hematopoietic Progenitor (HP)-derived Antigen Presenting Cells (APC), which could be used in specific immunotherapies. Cord Blood HP (CBHP) transplantation is becoming an alternative to other forms of HP transplantation in the treatment of hematopoietic malignancies. A lower incidence of GvHD has been described in this transplantation modality, but possibly it is accompanied by a lower GvL effect. The aim of this work is to study whether a limited number of CBHP are able to generate sufficient quantities of active mature DC for clinical treatments under serum-free conditions. Different combinations of cytokines were tested to optimise the culture of purified CD34+ cells from CB to generate DCs. 1.0*10\(^5\) CD34+ cells were cultured in SF-3 serum-free medium or RPMI with 10% FBS as a control. The cytokine combination that rendered the best survival of CBHP, to obtain sufficient quantities of DC for treatment.

**Poster discussions Gene transfer, gene therapy**

**PO-0339 Efficient transduction of human NOD-SCID repopulating cells using a pseudotyped retroviral vector**

Barquerojo J,* Segovia JC,* Ramirez M,* Guenechea G,* Limón A, Puig T, García J, Buena JA.* Department of Cell Therapy, Institut de Recerca Oncològica, Hospital, Barcelona, Spain; and *Department of Molecular and Cell Biology, CIBERAT, Madrid, Spain

With the aim of stably transducing human repopulating cells, retroviral vectors packaged with the env protein of the gibbon ape leukaemia virus (GALV) were generated. PG\(_i\)3 cells were infected with supernatants of the amphotrophic PA317/EGFP1 cell line (Limon et al. Blood 1997; 90: 3316-21). More than 600 clones were isolated, expanded and screened on the basis of green fluorescence intensity and titer on HeLa cells. The clone PG\(_i\)13/EGFP with a titer of 10\(^5\) was finally selected for further studies of gene transfer into human hematopoietic cells. Purified CD34+ cells from cord blood (n=22, median purity 92%) were prestimulated for 48 hours with recombinant human stem cell factor (rhSCF) (100 ng/ml), MGDF (10 ng/ml) and Flt-3 ligand (50 ng/ml). Thereafter, cultures were fed daily with infective supernatants for 48 hours in reconstituant C29-26 fibroblasts, with or without doxycycline. The cytokine combination that produced the highest transduction efficiency was found to be recombinant human stem cell factor (rhSCF) (100 ng/ml), MGDF (10 ng/ml) and Flt-3 ligand (50 ng/ml). Culture supernatants from PG\(_i\)13/EGFP were used to transduce NOD-SCID mice and the transduction efficiency was assessed by flow cytometry. Transduction efficiency was also analyzed in the CD34+ subpopulation and in the more immature CD34+ CD38- sub-set by triple staining (CD34-PE, CD38-APC and EGFP) using a dual laser cytometer. To evaluate the efficacy of this procedure to transduce human repopulating cells, infected samples were transplanted into imidod NOD-SCID mice. In some experiments GFP positive cells were sorted out and transplanted into further groups of NOD-SCID mice. Preliminary results obtained in 5 transplanted mice indicate that the transduction levels of the NOD/SCID repopulating cells is also high. A mean of 33.8% (range 17-53%) of human CFUs obtained from the bone marrow 30 days after transplantation were EGFP+. We conclude that the described infection procedure is highly efficient for transducing, very primitive human hematopoietic precursors capable of engraftment in vivo. This work was supported by a grant from CICYT (SAF96-0130) and "Fundación Ramón Areces".

**PO-0340 Improvement of mouse b-thalassaemia upon erythropoietin delivery by encapsulated myoblasts**

Daille B,* Payen E,* Regulier E,* Deglon N,* Rouyer-Fessard P,* Beuzard Y,* Aeberscher P.* Laboratory of Experimental Gene Therapy, Saint-Louis Hospital, Paris, France; *Division of surgical research and gene therapy center, Lusanne, Switzerland

Despite a remarkable understanding of molecular and cellular defects responsible for b-thalassaemia, there is no safe and specific therapy. Erythropoietin (EPO), the regulator of erythrocytosis in mammals is secreted primarily by kidney peritubular cells in response to hypoxia. This glycoprotein hormone stimulates proliferation and terminal differentiation of erythrocyte progenitors. 

mEPO injections in b-thalassaemic mice improve the b-thalassaemic phenotype. High dose injections in b-thalassaemic patients have also been shown to improve the syndrome. However, because mEPO must be injected at very high doses, frequently and permanently, an in vivo EPO delivery by gene transfer has been developed in a mouse model of b-thalassaemia. C2C12 myoblasts were stably transfected with a plasmid containing the mouse Epo cDNA and loaded in polyethersulfone microporous hollow fibers which were subsequently implanted subcutaneously in b-thalassaemic mice. We observed an increase in hematocrit associated with an elevated EPO level and an improved red blood cell phenotype. Implanted mice had a reduced amount of free a-haemoglobin chain, the hallmark of globin chain imbalance in b-thalassaemia. Furthermore, we observed an increase in the number of red blood cells. These results hold promise for the treatment of b-thalassaemia associated chronic anaemia.
In gene transfer experiments including gene therapy studies, expression of the integrated transgenes in host cell often declines with time. The molecular basis of this phenomenon is not clearly understood. We used the Green Fluorescent Protein (GFP) gene as both a selectable marker and a reporter to study long-term gene expression in transfected K562 cells. We found that transfected, fluorescent cells, selected by flow cytometry, eliminated the integrated GFP transgene over 60-80 cell generations and ultimately became non-fluorescent. Hence, a great majority of the transgenic integration sites in the host genome that permitted transcription of the GFP transgene were incompatible with long-term, stable integration of the transgene. Additional in vivo drug selection based on a co-integrated drug resistance gene enriched for a minor subset of the transfected cells containing the transgenes integrated into host sites that permitted not only transcription but also stable integration of the transgenes. Our findings suggest that for long-term stable expression of the GFP gene, and other transgenes which do not confer a selective growth advantage on host cells, it may be necessary to co-integrate such genes with a drug resistance gene and select with drugs for cells containing transcriptionally active and stably-integrated transgenes.

Regulation of erythropoietin delivery from engineered myoblasts in mice


Laboratory of Experimental Gene Therapy, Saint-Louis Hospital, Paris, France; Bioengineering Laboratory, Saint-Antoine Hospital, Paris, France

Recombinant human erythropoietin (rEPO) is a glycoprotein hormone currently used in the treatment of acquired chronic anemia due to kidney failure. Preliminary results strongly suggest that it could also be beneficial in hemoglobinopathies such as β-thalassemia. However, because rEPO has to be injected at very high doses, frequently and permanently, an in vivo EPO delivery by gene transfer has been developed. As in other diseases, this type of delivery could be proposed if the protein level can be regulated. For this purpose, we combined two types of modulation/ regulation. The first one is related to the method of transgenic protein production which is made of encapsulated and genetically modified cells producing EPO. Cells are immunoprotected by hydrogel of AN-69. One can theoretically fix the secretion level by adjusting the cell number. The second level of modulation is transcriptional control of the transgene, regulated by the tetracycline controlled transactivator (TcR). We stably transfected C2C12 mouse myoblasts and selected cells producing high levels of erythropoietin (300 IU 106 cells 24h) from which secretion was efficiently repressed by tetracycline (more than 100 times). When differentiated into myotubes in vitro, the cells still produced large quantities of regulated erythropoietin. The former selected cells were encapsulated into hollow fibers and induced for differentiation. The devices were then subcutaneously introduced into normal mice. We observed that whereas hematocrit remained stable in tetracycline treated mice, it increased up to 80% in untreated mice. These data suggest that several points of interest in term of usefulness and safety might be combined. A therapeutic protein can be stably secreted, easily regulated and produced by a biological device that can be easily removed. These results hold promise for the long term treatment of several chronic diseases with transgenic proteins, for example anemia (erythropoietin), haemophilia (factor IX), vascular ischaemic disease (angio genic factor) or cancer (angiostatic factor).
PO-0345 Systemic mast cell disease and prognostic role of c-kit mutations
* Dept. of Biology and Genetics, Medical Faculty, University of Milan, Italy; ° Division of Hematology, Neumann, Milan, Italy; ° Dept. of Dermatology, University of Padua, Italy; ° Dept. of Internal Medicine, S. Gerardo Hospital, Monza, Italy.

Mastocytosis is a heterogeneous clonal disease ranging from indolent to the aggressive form through stages with variable extent of haematological involvement. Point mutations in the c-kit gene (Asp816Val, Asp166Tyr, Asp820Gly) have been identified in patients with severe mastocytosis pointing out a role for this gene in mast cell growth and differentiation. We have recruited 12 mastocytosis patients, 8 of whom with the cutaneous form (including 2 familial cases) and 4 with different degrees of bone marrow involvement. Screening for c-kit mutations was performed by PCR- DGGE/CGGE and direct sequencing on DNA from skin lesions, peripheral blood, bone marrow and whenever possible the mast cell enriched fraction. Tissues were examined in parallel by using morphological, histochemical and immunocytochemical techniques. No c-kit mutation was detected in any of the patients. Further biochemical and genetic analysis of genetic c-kit polymorphisms ruled out c-kit involvement in the 2 families in which the cutaneous form was transmitted. D816V mutation, not detectable in the unaffected bone marrow, was found in the mast-cell enriched fraction from a patient with initial haematological presentation providing a means of assessing prognosis and developing intervention strategies. D816Y mutation was identified in the bone marrow blasts from an AML M2 patient with mast cell involvement and rapid progression of disease. In culture of leukemic blasts supported the in vivo findings on the origin of mast cells from the leukaemic clone and transformation to mast cell leukaemia.

PO-0346 Myeloblastin is a major c-myc target gene controlling normal growth and transformation of hematopoietic cells
*UniNorte-HAIP, Uppsala, Sweden; ° Institute of Immunology, The Weizmann Institute of Science, Rehovot, Israel; ° Cancer Institute, New York Medical College, Hawthorne, USA.

Haematopoiesis is maintained by a limited subset of quiescent hematopoietic stem/progenitor cells (HSPC). The c-myc proto-oncogene is critical to the control of subsequent proliferation of hematopoietic progenitors. Myeloblastin (MBN) has been described as being associated with proliferation of human leukemia cells. Our results show that inhibition of MBN expression leads to suppression of HSPC proliferation via maintaining stem cells in a quiescent state, resulting in an increased number of LTC-I and prolonged expansion of SCID-repopulating cells and their progeny. We demonstrate that MBN expression is up-regulated by G-CSF and that its constitutive expression is sufficient to transform L3-2/G-CSF-dependent murine bone marrow-derived Ba/F3 cells to factor-independent growth. Our results show that, in myeloblastic PLB-985 cells, MBN expression is necessary for continued proliferation and is up-regulated by ectopic expression of the myeloid c-myc oncogene. Our study confirms the human and murine MBN promoters which both harbor binding sites for myeloid specific transcription factors (PU.1, c/EBP and c-myc) that are all critical for constitutive MBN expression. We demonstrate that MBN expression is dependent upon a histone acetyltransferase/histone deacetylase (HAT/HDAC) balance and that inhibition of MBN expression is mediated through c-myc down-regulation in leukemia cells growth-arrested by retinoic acid (RA)-treatment or serum starvation. Altogether our data indicate that MBN is a major target of c-myc and a key protease for controlling normal growth and transformation of hematopoietic cells, and suggest a mechanism which bypasses an unbalanced HAT/HDAC oncogenic role.

PO-0347 De novo methylation of tumour suppressor gene p16INK4a is a frequent finding in multiple myeloma patients at diagnosis
Dept. Hematology, Salamanca University Hospital, Spain.

The p16INK4a gene is mapped to the 9p21.2 region, frequently altered in several types of tumours. The p16 protein, a cyclin-dependent kinase inhibitor, acts as a suppressor protein. Inactivation of p16 gene can be caused by different mechanisms, homozygous deletions being the most frequent, followed by translocations, and more rarely by point mutations. Recently, de novo methylation of a 5’ CpG island within exon 1 of p16 gene has been observed, associated with transcriptional silencing of the p16 gene. Objective. To analyse the methylation pattern of exon E1 of the p16 gene in multiple myeloma (MM) and primary plasma cell leukaemia (PCL). Design and Methods. 181 patients with untreated MM and five primary PCL were included in this study. AB studies were carried out in bone marrow samples containing a variable number of plasma cells (5 to 80%). A PCR assay relying on the inability of some restriction enzymes (EagI, SacI) to digest methylated sequences was used to analyse the methylation status of the first exon E1 of the p16 gene. By SB, genomic DNA was restricted with one methylation non sensitive enzyme (HindIII) and one methylation sensitive enzyme (EcoRI or SacI). The filters obtained were hybridised with a probe of exon E1 of the p16 gene. SB analysis was rude in 20 random samples that had experienced amplification of the exon E1 by PCR and 20 random samples with no amplification of the exon E1. Results. Since the methylation sensitive enzymes do not cut the exon E1 of the it is methylated, this exon E1 was amplified in 41/101 MM patients (40.5%) as well as in four of the five (80%) patients with primary PCL. By SB, of the 20 cases used as positive samples, the methylation status was confirmed in 18. The two discrepant cases could be explained by the low plasma cell infiltration (<10%) in one case, and by a suboptimal DNA quality in the second case. None of the 20 samples used as negative cases, displayed a 6Kb band observed in the methylated samples. Conclusions. Our study confirms, that even at diagnosis, hypomethylation of the p16 gene is already a very frequent event in MM. Large prospective studies are required in order to evaluate whether or not this methylation pattern is a marker of clinical and biological implications or whether it is an accessory finding marginally associated with disease outcome.

PO-0348 Analysis of bc12, p53 and MDM2 oncprotein expression in adult acute leukaemia cells
Bartkowiak P, ° Biskop JS, ° Najder M, ° Niewiadomska H, ° Robak T
*Dept of Oncology and °Dept of Hematology, Medical Univ. of Lodz, Lodz, Poland.

Heterogeneous expression of many oncproteins in several tumour cells seems to have clinical significance. These proteins are important regulators of apoptotic processes and are also involved in the control of cell cycle development. However, the real mechanisms of their function are still unclear and especially in adult acute leukemias their biological role is poorly understood. The aim of our present study was to establish the frequency and the intensity of BCL-2, p53 and MDM2 oncproteins expression in cells of human adult AML and ALL. For analysis were 13 acute leukaemia cases (9 and 4 of AML and ALL, respectively) studied. Mononuclear blood cells were isolated from peripheral blood and the process of their membrane permabilisation was performed with FicollPerm (Catalt, Austria). Next, the cells were incubated with the following FITC or PE-conjugated monoclonal antibodies (mAbs): CD3, CD5, CD10, CD13, CD14, CD19, CD20, CD23, CD33, CD34, MPO, K67, PCNA and antiBCL2, p53, MDM2. Fluorescence analysis were performed by two-color measurement of 10,000 cells on a Coulter Cytorubofor System, EPICS. Mann-Whitney U test and Spearman rank correlation order for data analysis was used. Results. BCL-2 oncprotein expression was found in 69% (9/13 cases), p53 23% (3/13 cases) and MDM2 38% (5/13 cases). There was a positive correlation between p53 and K67 and the PC resistance analysis was used. Chemotherapy response was only observed in several patients with p53-negative immunostaining. p53-positive patients were always chemoresistant. The above results initiated some preliminary studies of P53 and MDM2 molecular abnormalities.

PO-0349 Mutations of RB-1 gene in children with leukaemia and neuroblastoma
Markakis EA, ° Tsiponimochalou M, ° Dimitrakou E, ° Spanidiodos DA, ° Kalamard M
*Department Of Pediatric Hematology/Oncology, University Hospital, Heraklion, Crete; °Laboratory of Virology, Medical School, University of Crete, Heraklion, Greece.

RB-1 is a tumour suppressor gene located in the 13q14 chromosome region and comprises 27 exons. The RB-1 gene, coding for a 110 KD product, which is a nuclear phosphoprotein acting as a cell cycle regulator and which blocks the transition of normal cells from G0/G1 into the S phase of the cycle, is normally expressed in hematopoietic cells. It is inactivated by deletions but more often by mutations. Point mutations may affect most of the exons, but have a certain predominance in exons 20-24 and their splicing sites. In hematopoietic malignancies, deletions or rearrangements of the RB-1 gene have been reported in 5 to 10% of acute leukemias, in some of which they are associated with premature senescence. The RB-1 gene is not detectable in the unfractionated bone marrow, was found in the mast cell rich fraction from a patient with initial haematological presentation providing a means of assessing prognosis and developing intervention strategies. D816Y mutation was identified in the bone marrow blasts from an AML M2 patient with mast cell involvement and rapid progression of disease. In culture of leukemic blasts supported the in vivo findings on the origin of mast cells from the leukaemic clone and transformation to mast cell leukaemia.

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Poster discussions  Gene expression, gene regulators, receptors

PO-0350 Differential expression of signal transducers and activators of transcription-I isoforms in B-CLL and tonsillar B-cells

Department of Haematology, Royal Free & University College Medical School, London, UK

Signal transducers and activators of transcription (STATs) are central to cytokine signal transduction. STAT-1 (191Da) and STAT-1b (184Da) are generated via alternative splicing of the STAT-1 primary transcript. Homodimers formed by STAT-1a are transcriptionally more active than those which include STAT-1b. Therefore alternative splicing of the STAT-1 primary transcript may have a role in regulating the expression of genes involved in apoptosis and cell division. The expression of STAT-1a and STAT-1b was investigated in malignant cells from B-CLL patients and tonsillar B-cells. Protein and mRNA from 15 B-CLL and 5 tonsillar B-cell samples (protein = 15, mRNA = 12) were analysed for the expression of STAT-1a and STAT-1b. Western blot and reverse transcriptase polymerase chain reaction (RT-PCR) showed a linear and sequential confirmation of the STAT-1a and STAT-1b RT-PCR products. In all B-CLL cell samples STAT-1a exceeded STAT-1b expression at the protein and RT-PCR levels. The mean STAT-1a:STAT-1b ratio was 11.6 (range 1.5-6.9) at the protein level and 6.1 (range 0.1-2.1) at the RT-PCR level. In contrast STAT-1b exceeded STAT-1a protein expression in 10 out of 15 tonsillar B-cell samples with a mean a/b ratio of 0.73 (range 0.1-2.13). Using RT-PCR, STAT-1a:STAT-1b expression exceeded that of STAT-1a in 10 out of 12 tonsillar B-cells with a mean a/b ratio of 0.7 (range 0.1-2.11). The difference in the mean ratios for B-CLL compared to tonsillar B-cells was statistically significant at both the protein and RT-PCR levels (p < 0.05). The results obtained at the protein and RT-PCR levels were concordant in 15 B-CLL and 5 tonsillar B-cell samples. There was no correlation between the mean ratios and stage of disease in B-CLL. RT-PCR levels were concordant in 15 B-CLL and 5 tonsillar B-cell samples.

The induction of embryonic globin gene transcription was confirmed by quantitative RT-PCR. In untreated cells only β- and γ- but no βγ-globin mRNA was present. Hemin increased y mRNA two- to ten-fold (dose-dependent effect). These results suggest that KG-91 cells are pluripotent cells that retain the ability for differentiation along the erythroid lineage after induction by relevant agents. Furthermore, they provide an appropriate cell system for the study of biologic and molecular events in the early stages of erythropoiesis.

PO-0352 iDRE sequence in the rat βγ-globin promoter regulates transcription in REL cells

M Irovic T, Pavlovic S, Popovic Z
Institute of molecular genetics and genetic engineering, Belgrade, FR Yugoslavia

We have shown that the rat adult βγ-globin gene is transcriptionally active. Further studies of the role of the promoter in transcription regulation of this β-globin gene have been based on a REL (rat erythroleukemia) cell line, as a homologous model system. Stable transfection experiments with βγ-globin promoter-CAT reporter constructs have shown that the -98 bp promoter region (TATA-iDRE-CAT-CATCCG) is sufficient for high-level expression and inducibility during REL cell differentiation. Gel mobility shift assays and South-Western blot analysis have revealed increased binding of two erythroid-specific factors (150 and 60 kDa) to the iDRE in differentiating REL cells.

PO-0353 Lovastatin and ATRA as a potential therapy for leukaemia

Woodgate L, Walker EJ, Gilkes AF, Walsh V, Sweeney MC, Mills KJ, Burnett AK
LRF Differentiation Unit, Department of Haematology, University of Wales College of Medicine, Cardiff, Wales

Differentiation therapy with retinoic acid has an established benefit in the treatment of acute promyelocytic leukaemia. The mode of action involves the persuasion of leukaemic cells to complete the maturation process, including activation of apoptotic cell death. The molecular consequences of this may be applicable to other sub-groups of leukaemia, and we have investigated these by differential display to comparing the mRNA expression profiles of an ATRA sensitive and a ATRA resistant cell line (AS) to one that is less responsive to ATRA (AR). This approach identified HMGCoa reductase (HMGCor) as being highly expressed in the cell line that is less responsive or resistant to differentiation by ATRA. HMGCoAR is the rate-limiting enzyme in the mevalonate pathway and is essential in cholesterol biosynthesis and famesesylation of RAS protein. We now report the elevated expression of this mRNA in other ATRA resistant myeloid cell lines. Furthermore, high expression of HMGC-oAR was observed in primary material from all AML FAB M3 and M4 classes except the M3 FAB class which responds clinically to ATRA. The obvious role of HMGC-oAR in cell proliferation makes it a target for therapy, particularly as inhibition of HMGC-oAR is known to have an anti-proliferative effect. Protein inhibitors of HMGC-oAR include lipid lowering agents Lovastatin and Simvastatin, whilst ATRA inhibits mRNA transcription of HMGC-oAR. Therefore as a combined approach to the reduction of HMGC-oAR we treated myeloid cell lines with the combinations of Lovastatin (at a dose range of 5 nM to 2 µM) and ATRA (at 100 nM or 500 nM). ATRA resistant cells treated with the combinations showed an enhanced differentiation response as opposed to ATRA alone. These data provide a rationale for the combined use of HMGC-oAR inhibitors and retinoids as a novel therapy for AML.

PO-0354 Characterisation of the A-mb promoter in normal and neoplastic B cells

Facchinetti V, Lopa R, Spreafico F, Golya J, Intra M
Istituto Ricerche Farmacologiche Mario Negri, Milan, Italy

A-mb is a transcription factor with a restricted pattern of expression. In particular amongst hematopoietic cells, its expression is restricted to a very narrow window B cell differentiation taking place in the dark zone of ger...
nal centres. In order to investigate the B cell specific and cell cycle regu-
lated expression of A-myb, we cloned and characterised the human A-myb
promoter. A 12.5 kb DNA fragment was isolated by screening a genomic library
with the 5‘ fragment of the A-myb cDNA. A partial cDNA map and sequence of this
genomic region was obtained. The major transcription start sites were mapped by RNase protection, extending about by 270 bp
from the previously published cDNA sequence. Furthermore the minimal pro-
moter region as well as positive and negative regulatory regions char-
terised. The study of the expression of the A-myb gene in human B lym-
phoma pathology revealed that it is highly expressed in the majority of
Burkitt’s lymphomas and in 40% of CLL cases. In order to identify the
molecular basis for this heterogeneity and potential alteration of the A-myb
regulatory region in these cells, the A-myb promoter region is being used in
Southern blots of Burkitt’s lymphomas and CLL cases and the data will be
presented.

PO-0355 In situ detection by RT-PCR of SCF mRNA on
paraffin-embedded sections of mastocytosis sign lesions

*Dept. of Biology and Genetics, Medical Faculty, University of Milan; *Dott. di Biologia, University of Padua; *Dept. of Internal Medicine, S. Gerardo Hospital, Monza, Italy

Interaction between stem cell factor (SCF) and c-kit receptor plays a criti-
cal role in survival and terminal differentiation of mast cells in normal skin.
C-kit mutations have been identified in a subset of patients with severe
mastocytosis, mainly displaying haematological involvement. A search for c-kit
mutations in 5 patients with cutaneous mastocytosis did not evidence
any sequence alteration in the familial cases in which the gene was also
found to segregate independently of the disease. In order to establish
whether a local altered production of SCF might have a causal role in mas-
tocytosis we investigated the presence and cellular localisation of SCF
transcripts by using the highly sensitive technique of in situ RT-PCR. Fol-
lowing a morphological analysis and mast cell specific staining, two dif-
ferent primer pairs, suitable for detecting the soluble SCF isoform and both
soluble and transmembrane SCF isoforms respectively, were used on con-
tiguous tissue sections. So far the analysis has been performed on one
familial and one isolated patient; only the latter turned out to have DS616
a c-kit mutation in skin lesions and bone marrow. RT-PCR by both designed
primer pairs allowed to detect, in mast cells infiltrating skin lesions, high
levels of SCF mRNAs, identified by morphological criteria and specific stain-
ing. By contrast low expression levels were evidenced in skin fibroblasts,
endothelial cells and keratinocytes. The overall expression profile of SCF
was comparable in both RT-PCR experiments, although the presence/
absence of transmembrane SCF isoforms cannot be excluded. The observed
localisation of kit ligand mRNAs in the CD 117+ skin-mast cells may be
accounted for by the activation of an autocrine loop by altered metabolism of SCF.

PO-0356 Altered gene expression in 32d cells transfected with the
AML1 and AML1-eto genes

Robinson LG, Austin SJ, Owen-Jones CE, Kell J, Gilkes AF, Dann EJ,°
Haematology, University of Wales College of Medicine, Cardiff, UK and
*Haemato malignology, University of Wales College of Medicine, Cardiff, UK and
*Rambam Medical Centre, Haifa, Israel

Chromosomal translocations involving the AML1 transcription factor gene
on 21q22 is frequently observed in myeloid leukaemias. The (8;21)
translocation associated with AML M2 results in the fusion of the ETO gene
on 8q22 with AML1, leading to altered expression of genes involved in
haematopoietic differentiation. Objective, To study the differential expres-
sion of genes in 32D cells transfected with the AML1 and AML1-ETO gene sequences
showing altered expression. Results. Following cloning and sequencing, 2
groups of gene fragments were identified - one with altered expression in the
AML1 or AML1-ETO expressing cell lines and the other, over-expressed in the
32D-AML1-ETO cell line only. Those over-expressed in the former
include novel sequences and a steroid esterase, while the under-
expressed include novel sequences and an ATP synthase a subunit. Genes
overexpressed in the 32D-AML1-ETO cell line include Granzyme B and a
Na-H ion exchange protein. Granzyme B has been previously shown to be
highly expressed in an undefined myeloid multistep cell line and is
downregulated as cells are induced to differentiate. Conclusions. We have
identified some downstream genes targeted by the AML1 and AML1-ETO
genes which may play a role in determining the different phenotypes
observed in cell lines expressing them.

PO-0357 Lineage-restricted expression of protein kinase C (PKC)
isoforms in haematopoiesis

Dept. of Biomedical Sciences, University of Brescia, Inst. of Histology and
General Embryology, University of Bologna, Dept. of Morphology and
Embryology, University of Ferrara, Lab. of Clinical Biochemistry and
Cell Biology, Istituto Superiore di Sanità, and Inst. of Cytomorphology,
CNR, Bolonia, Italy

The pattern of expression of several protein kinase C (PKC) isoforms (α, β1,
β2, γ, η and ζ) during the course of hematopoietic development was investi-
gated using primary human CD34+ hematopoietic cells and stable cell
lines subcloned from the growth factor-dependent 32D murine hematopo-
etic cell line. Each 32D cell clone shows the phenotype and growth factor
dependence characteristics of the corresponding hematopoietic lineage. Clear-cut differences were noticed between erythroid and non-erythroid line-
eges: i) the functional inhibition of PKC-ε in primary human CD34+
 hemopoietic cells resulted in a two-fold increase in the number of ery-
throid colonies; ii) erythroid 32D EP01 cells showed a lower level of bulk
PKC catalytic activity, lacked the expression of α and β1 PKC isoforms and
showed a weak or absent upregulation of the remaining isoforms, except β1,
upon readdition of Epo to growth factor-stimulated cells; iii) 32D, 32D
GM1, 32D G1 cell lines with mast cell, granulomacrophagic, and granulo-
cytic phenotype, respectively, expressed all the PKC isoforms investigated,
but showed distinct responses to growth factor readdition: (i) 32D Epo 1.1,
a clone selected for IL-3-responsiveness from 32D EP1, expressed the ε
isoform only when cultured in IL-3. On the other hand, when cultured in Epo,
32D Epo 1.1 cells lacked the expression of both α and β1 PKC isoforms,
similarly to 32D EP01. In conclusion, the PKC isozyme expression during
haematopoiesis appears lineage-specific and, at least partially, related to
growth factor response.

PO-0358 Identification of genes differentially expressed upon CD30
activation of anaplastic large cell lymphoma

Hubinger G, Müller E, Emz M, Bergmann L,
Department of Internal Medicine III, University of Ulm, Germany

The expression of the cytokine receptor CD30 is one of the major charac-
teristics of anaplastic large cell lymphoma (ALCL). While CD30-mediated
signal transduction pathways leading to activating processes are well char-
acterized, little is known about CD30–induced antiproliferative effects. We have previously shown that activation of CD30 results in cell cycle arrest
of the ALCL-derived cell line Karpas 299 indicated by a p53-independent
upregulation of the cell cycle inhibitory protein p21 and a hypophospho-
ylated state of the retinoblastoma protein (Rb). We were therefore inter-
ested to examine further genes involved in CD30 signaling. We could char-
acterise CD30-induced alterations of gene expression in Karpas 299 cells
by differential display RT-PCR (DDRT-PCR) and Northern blot analysis.
So far we found a variety of genes differentially regulated by CD30. Stimulat-
ed cells showed a significant decrease of mRNA expression of Leu-13, an
interferon-inducible 17-kDa membrane protein implicated in cell growth
control, while expression of the ferritin heavy-chain gene product, known
to be involved in negative regulation of lymphocytic growth, is increased upon CD30-activation. Other differentially expressed genes such as
Mox2, first identified in the MDS-treated adenocarcinoma cell line A549, are not
further characterised or revealed no similarities to sequences present in
GenBank, suggesting previously unreported gene products possibly involved in
CD30-induced signal transduction pathways. Taken together, our data
suggest that CD30 can mediate a p21-dependent cell cycle arrest in ALCL
involving known and new signal molecules.
ribosylation. Two clones overexpressing Gs1 were further analyzed and compared to a mock-transfected clone and to non-transfected K562 cells. Transfected thymidine incorporation, cell morphology, cyclic AMP production, MAP kinase activation were evaluated. K562/Gs1 cells had a significantly increased rate of proliferation compared to mock-transfected and control K562 cells, as evident from day 3 - transfected thymidine incorporation. Intracellular cAMP levels were measured by RIA assay with and without PGE2 and PGE2 plus pertussis toxin incubation. K562/Gs1 cells stimulated with PGE2 did not enhance the levels of cAMP as control cells, but this ability was restored when the activity of Gs1 was inhibited by pertussis toxin. No variation in cell morphology could be detected in transfected clones, and maturation achieved in the presence of the differentiating molecules TPA and Na butyrate was not different from control cells. Total cell lysates were obtained from K562/Gs1 cells and SDS-PAGE 10% performed. Anti-MAP kinase and anti-Phyto blots indicated that transfected K562 cells (endowed with a higher proliferative potential than control non transfected cells) also had a significant spontaneous activation of MAP kinase p42p44. These data indicate that G-proteins may indeed play a significant role in proliferation signal transduction of acute myeloid leukemia cells and that there is a cross talk with downstream pathways such as Ras-Raf-MAP kinase, already involved in growth signaling.

**PO-0360 Activation of the δ globin gene by the β globin gene CACCC motif**

Battisti M, S. Casula S, S. Percu S, S. Piramut M, O. Cao A

**IRTAM CNR-Cagliari; #ICBEE,Università di Cagliari; °Ist. Genetica Molecolare, Alghero, Italy**

The CACCC motif is duplicated in the adult β globin gene promoter in human and others mammals. Previous studies have shown that introduction of the β globin CACCC or CAAT box is able to activate the δ globin gene promoter, but the effect of the distal CACCC element has not yet been tested. In the present study we introduced, by site-specific mutagenesis, in the wild type δ globin gene promoter the consensus sequence for the distal CACCC element alone or in combination with the CAAT and/or the δ globin gene promoter, in addition, we tested IFNα, IFNγ, TNF-α and IL-1β to investigate the influence on cell morphology mediated by GM-CSF which increased in frequency to up to 50% within six hours. This morphological variable protusions and cells of unregular, biconcave shape were present after stimulation with IL-3 or GM-CSF. Under both conditions, alterations of cell shape and formation of the pseudopodia were observed as described above. The action of both cytokines could be delayed by preincubation with the PI3-kinase inhibitor wortmannin and completely blocked by repeated treatment for the time of observation (up to 4 hours). Thus, IL-3 and GM-CSF can signal for morphology alterations of transfected Ba/F3 cells in a PI3 kinase and temperature dependent manner. As apparent from cells transfected with a single-chain chimeric GM-CSF receptor, this is true even in the absence of cytoplasmic sequences from human GM-CSF.

**1. Salgà et al., Clin Invest 1997; 100:46**

**PO-0362 Quantification of β-subunit isoforms of the human GM-CSF receptor mRNA by real-time TaqMan RT-PCR**


Dept. of Hematology and Oncology, Medical School Hannover, Germany

Signal transduction by the receptor for human granulocyte-macrophage colony-stimulating factor (GM-CSF) is initiated upon ligand binding to the β-subunit (δGMR), followed by activation of the signal transducing common β-chain (β-δGMR). Recently, a β-δGMR splice variant has been identified in which due to lack of a 104 nucleotide exon, the mRNA results in a truncated β-δGMR with only 46 intracellular amino acids (1). In order to investigate expression levels in normal mononuclear peripheral blood cells (PBMC) and in acute myeloid leukemias (AML) cells, we have established a real-time TaqMan PCR system for the isoform-specific quantification of both β-δGMR mRNA variants in the presence of each other. Using plasmids containing the wildtype (wt) and mutant (mut) β-δGMR isoform respectively, we are able to specifically detect less than 50 molecules of the corresponding template. Standard curves from plasmid dilutions routinely have correlation coefficients of 0.97 up to 0.99. The isoform specific fluorescent TaqMan-probes and pairs of PCR primers do not detect up to 105 molecules of the alternative splice variant. Moreover, specific quantification is possible even with a 100-fold excess of the alternative β-δGMR isoform. Analysis of mRNA expression in two cell lines by both conventional RT-PCR with subsequent gel analysis and real-time PCR confirms specific detection and validates the quantification by real-time RT-PCR. Analysing the expression levels of wt and mut β-δGMR in the PBMC of healthy volunteers, we noted expression of the mut isoform in all individuals (n=18). The ratio of mut:total β-δGMR as determined by real-time RT-PCR (n=6) was between 22.6% and 30.3% (mean: 27.2%). In contrast, in peripheral blood or bone marrow MNC from 24 AML patients the mut:total ratio varied considerably from <1% to up to 57.6% at the time of diagnosis. These data are currently related to the phenotype and genotype of the AML cases. Taken together, our approach allows sensitive and specific quantification of β-δGMR isoform expression in the context of normal and malignant haematopoesis.


**PO-0363 Expression of sorcin and µ-calpain is modulated in the T cells during normal aging and in lymphocytic leukemias**

Wildkowski IM, * Kazcmarek B, Swiercz J, Zmuda-Trzebiatowska E

*Department of Physio-pathology, Medical University of Gdansk, Poland

The control of lymphocyte proliferation is affected, both in opposite directions, both in physiological aging as well as in the development of lymphatic leukemias. We have demonstrated before that aging of murine T lymphocytes is accompanied by increased expression of a constitutively cell surface binding, regulatory protein sorcin and by increased activity of a strongly Ca2+-dependent thiol protease – µ-calpain, the latter being involved in the regulation of proliferative signal transduction and apoptosis. No data are currently available as to the expression of these two proteins in relation to aging and leukemic transformation of human lymphocytes. So, we attempted to quantify the expression of both proteins in peripheral blood T and B cells and in leukemic lymphocytes (CALL ALL) at the mRNA level using RT-PCR, and for the calpain also at the protein level using western blot analysis. Additionally, activity of the µ-calpain in the lysates of normal and lymphocytic leukemias was estimated by casein zymography. We found that sorcin expression was increased in the T cells of old people and in the lymphoblasts obtained from the leukemic patients’ blood. The activity of µ-calpain was significantly increased in the T cells of elderly people compared to the young individuals; the same activity was absent from most of the samples obtained from leukemic lymphoblasts. The activity of this protease paralleled to some extent the results of western blot and PCR analyses. Conclusing, the activity of µ-calpain, a pivotal signal transduction-related protease is unregulated in the T cells of elderly people and downregulated in leukemic lymphocytes; this might correspond with generally decreased proliferative capacity of aged T cells and its augmentation in leukemias. On the other hand, increased levels of sorcin expression in both normal and pathologic lymphocytes could affect the level of Ca2+ in these cells, thus influencing their proliferative capacity.
Epidemiology, viruses, genetic diseases

PO-0364 Suppression of telomerase reverse transcriptase mRNA expression in differentiated HL60 cells: regulatory mechanisms
Xu D,* Gruber A,* Björkholm M,* Peterson C,* Pisa P*
*Department of Medicine, Division of Hematology and *Department of Oncology, Radiumhemmet, Karolinska Hospital, Stockholm, Sweden; **Clinical Pharmacology, Faculty of Health Sciences, Linköping University, Linköping, Sweden

Telomerase activity, associated with cellular immortalisation and tumourigenesis, is suppressed during terminal differentiation of HL60 promyelocytic leukaemic cells. However, it is poorly understood how telomerase activity is regulated in differentiated HL60 cells. In the present study, we demonstrate that the downregulation of telomerase reverse transcriptase (hTERT) expression, the catalytic subunit, occurs prior to the suppression of telomerase activity in differentiated HL60 cells. In contrast, the expression of telomerase RNA template (hTR) and telomerase associated protein (TP1) is not reduced. This downregulation of hTERT expression is achieved through inhibition of gene transcription, in which process new protein synthesis is required. Moreover, the rapid downregulation of hTERT expression followed by the inhibition of telomerase activity is a specific component of the differentiation programme and not simply a consequence of cell cycle arrest. Serum-deprivation of HL60 cells causes cell cycle arrest without differentiation and this does not result in a significant reduction in hTERT mRNA levels within the first 24 hours. Our findings suggest that hTERT expression is stringently controlled at transcriptional level in HL60 cells. The downregulation of hTERT in the HL60 cell differentiation model may represent a general regulatory mechanism through which telomerase becomes repressed during development and differentiation of human somatic cells.

Poster discussions Epidemiology, viruses, genetic diseases

PO-0365 Enzyme replacement therapy in patients with Gaucher disease: the experience of Dr. Peset Hospital in Spain
Hematology Service, *Paediatric Service, Hospital Dr. Peset,Valencia, Spain

Gaucher disease (GD) is the most common sphingolipid storage disorder, caused by glucocerebrosidase deficiency, resulting in accumulation of glucocerebrosides within the macrophages of the reticuloendothelial system. The disease is characterised by great phenotypic heterogeneity, which can be explained only in part by the various mutations in the glucocerebrosidase gene and by the amount of storage material in affected organs and tissues. The aim of this study was to evaluate the efficacy of the treatment with alglucerase/imiglucerase in our patients diagnosed with GD type I in our centre. The study included four patients (age range: 5-35 years), belonging to two families, diagnosed in childhood except one in adulthood. The diagnosis in all cases was established by identification of Gauch er cells in bone marrow, enzyme and genotype studies. Three patients were receiving enzyme replacement therapy (ERT) with alglucerase (30 to 60 U/kg every two weeks) for 6 months to 3 years, after it was replaced by imiglucerase. The other case did not require treatment. Before ERT was available in our centre, one of the patients was splenectomised in childhood. Patients underwent clinical radiological, haematological and biochemical evaluation every three months. This report presents the clinical evaluation of patients on ERT. At diagnosis the genotype was N370S/I7 and levels of serum tetratose-resistant acid phosphatase (TRAP) were elevated in all patients. All of them had severe skeletal disease. The following changes were observed in different patients: a) increased splenomegaly and hepatomegaly; b) recovery of normal levels of haemoglobin and platelets; c) decreased TRAP activity and d) improvement of skeletal complications. ERT has been well tolerated in all cases and specific antibodies against the administered enzyme were not produced. In our experience, ERT induces the regression of haematologic, visceral and skeletal symptoms in some GD type 1 patients.

PO-0366 Somatic mutations of the proviral sequence results in a higher mutation frequency in HTLV-1 associated diseases than in asymptomatic carriers
Mortreux F,° Leclercq I,° Carevois M,° Geslain A,° Wain-Hobson S,° Wain J,°
In GTM U 124, Lille, Institut Pasteur, Paris, Service des Maladies du Sang, CHU, Lille, France

Clonal expansion of HTLV-1 bearing cells in vio is the rule in patients with or without malignancy. The Tax protein interferes with numerous processes which govern the cell cycle and gene transcription in a positive manner. It is therefore possible that somatic mutations (SM) within the HTLV-1 provirus may occur as a result of clonal expansion, increasing genetic variability in vio. An inverse PCR (IPCR) strategy was designed to distinguish SM from reverse transcriptase (RT) associated substitutions – one primer was HTLV specific while the other was defined by the clone flanking sequence. FBMC DNA samples from 2 asymptomatic carriers, 2 TSP/HAM patients and 2 patients with acute ATLL were studied. The last 399 bp of the 3' RUS sequences from 173 proviruses defined by 24 distinct cellular clones were analysed together with their integration sites. Sixteen of 24 celledular clones, harboured at least one mutated RUS sequence. With respect to an individuals consensus sequence, 25 clones encoded between 1-4 mutations, albeit usually one. A total of 45 additional mutations were detected within the RUS sequence: 26 transitions, 16 transversions and 3 single base deletions. This data indicate that the cellular clone specific mutations were somatically generated. The fraction of mutated clones (A) and the number of mutations of the RUS sequence 100b (B) in carriers, TSP/HAM and ATLL were respectively 37% and 4%; 82% and 7%; and 75% and 9%, respectively (for A, p=0.04; B, p=0.04, Mann Whitney U test). Only one integration site, from the malignant clone of an ATLL patient, was found to harbour a single mutation. Controls ruled out IPCR associated recombination and/or substitution. In conclusion, the intrapatient genetic variability of the HTLV-1 RUS sequence appears to result predominantly from somatic mutations. The fact that they are more frequent in HTLV-1 associated diseases than in asymptomatic carriers fits with a greater degree of infected cell proliferation. The impact of these substitutions remains to be elucidated. However, the fact that all but one are restricted to the proviral sequence (45 mutations in 69 kb vs. 1 in 20 kb, p<0.04) suggests that they may have been selected for.

PO-0367 Glutathione S-transferase M1 and T1 genotypes in haematologic malignancies
Hematology Unit, Department of Internal Medicine and *Genetics Unit, Ioannina University Hospital, Ioannina, Greece

Objective. Glutathione S-transferases (GSTs) are a group of enzymes involved in the detoxification process of carcinogens and other substances. The genes encoding isozymes M1 and T1 are polymorphic in humans. Reports have shown that the null genotypes of these genes are frequently associated with malignancies. Here we describe the frequency and the significance of the two genes in haematologic malignancies. Design and Methods. DNA was extracted from the peripheral blood of 106 patients with myelodysplastic syndrome, chronic lymphocytic leukaemia, chronic myeloid leukaemia, acute lymphoblastic leukaemia and acute myeloblastic leukaemia. Control DNAs were obtained from 147 cancer-free, age and sex matched individuals. A multiplex PCR method was used to analyse the genotypes and the κ2 test for comparisons between the two groups. Results. A significantly increased incidence of the GSTM1 null genotype was found in the group of patients compared to the controls (56.6% vs 38.1%, p<0.003). However the incidence of the GSTT1 null genotypes was comparable in patients and controls (15.1% vs 10.9%, p>0.003). The highest incidence of the GSTM1 null genotypes was observed in patients with occupational exposure to pesticides but not in patients exposed to tobacco smoke. Conclusions. Individuals with GSTM1 null genotype may be at increased risk of developing haematologic malignancies particularly when they are exposed to occupational and environmental carcinogens.

PO-0368 Natural history of hepatitis C virus in a cohort of individuals with clotting factor disorders
Griffioen A, Sabin CA,* Devereux H,*° Ye TT, Lee CA
Haemophilia Centre, *Department of Primary Care & Population Sciences and **Department of Rheumatology, Royal Free Hospital and School of Medicine, London, UK

Objective. To examine the natural history of HCV among individuals with inherited clotting factor deficiencies treated with unfractionated clotting factors (CFC) prior to 1986. Design and Methods. Data have been collected from the notes of 310 patients, 294 men and 16 women, registered at our
PO-0369 The influence of socio-economic factors on malignant lymphomas in children
Neamtu S, Miheu E, Cosnarovic R
Institute of Oncology "I. Chiricuta", Cluj-Napoca, Romania
Objectives. The aim of the study was to find a correlation between the indicators of socio-economic status and the incidence of malignant lymphomas in children. Design and Methods. The study included the families of 64 children with malignant lymphomas. Information was obtained through a standardised interview on socio-economic factors such as family size, parents' age, parents' level of education and occupation, family income and characteristics of housing, parental and childhood exposures to ionising radiation and chemicals. Results. The incidence of malignant lymphomas was higher in urban areas. No association was observed between parents' occupation and the type of malignant lymphoma in the children. Most families included in our study had a low socio-economic status with inadequate sanitation. There was a tendency for higher parental education level and small family size to be associated with the diagnosis of Hodgkin's lymphoma. Conclusions. These results suggest that socio-economic factors play an important role in the observed higher incidence of malignant lymphomas in children. Low socio-economic and nutritional status and delayed referral to paediatric centres may have contributed to the high stage of malignant lymphomas with predominance of histological subtypes with unfavourable prognosis.

PO-0370 Severe cytopenia with thiopeurine methyl-s-transferase (TPMT) deficiency, following azathioprine therapy
Reis MD, Einkerth PH, Potchinnay A, Dewar C, Tavaddia S, Sauder D
Sunnybrook and Women's College Health Sciences Centre, Toronto, ON, Canada
Background. Two patients treated with azathioprine developed pancytopenia 3 weeks and 4 months respectively after starting treatment. TPMT gene mutations lead to varying degrees of TPMT deficiency and predispose to severe myelosuppression. Case reports. Patient 1 received azathioprine for bullous pemphigoid. After 3 weeks therapy she became pancytopenic (Hb 89 g/L, WBC 0.7 × 10^9/L, platelets 6 × 10^9/L). Patient 2 with relapsing polychondritis was treated with azathioprine. After 4 months of therapy he had Hb 67 g/L, WBC 1.6 × 10^9/L, platelets 67 × 10^9/L. Both patients showed hypoplastic bone marrow. PCR-based genomic analysis for TPMT polymorphism on DNA obtained from patient 1 revealed the TPMT*3A/3B genotype, associated with low TPMT activity resulting from homozygosity for the G460A substitution, and heterozygosity for the A719G mutation. Patient 2 was found to have the TPMT*1/1B genotypes. Associated with intermedi- ate TPMT enzymatic deficiency, resulting from heterozygosity for the G460A mutation. Discussion. Azathioprine is extensively used in treatment of immunobiological diseases. A TPMT polymorphism with mutations leading to impaired metabolism and accumulation of azathioprine predisposes to sec- ondary hypoplastic haematopoiensis (about 10% of population has intermediate enzymatic activity and 0.3% has very low or absent enzymatic activity). As the main mutations of the TPMT gene associated with deficient enzymatic activity have been identified, it would be prudent to determine TPMT genetic status before commencing azathioprine treatment.

PO-0371 The role of N-acetyltransferase 2 genetic polymorphisms in chronic hepatitis B related hepatocellular carcinoma
Yee TT,* Yen CC,# Goldman E, Devereux H, Sabin C, Lee CA
*Department of Public Health, School of Medicine, National Taiwan University Hospital, Taipei; #Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan
The HCC risk associated with cumulative exposure to cigarette smoke was suggestive evidence of a synergistic interaction between cigarette smoking and NAT2 rapid acetylation genotypes in HCC. For smokers, genotypes for rapid acetylation were significantly associated with an increased risk of HCC (OR, 2.82; 95% CI, 1.13, 7.08; p=0.027). However, no association between NAT2 status and HCC was found among non-smokers. The HCC risk associated with cumulative exposure to cigarette smoke was also more striking among individuals who carried the rapid acetylation genotypes.

PO-0372 Low serum carotene level, low arsenic methylation capability, and arsenic-induced peripheral vascular disease
*Department of Public Health, School of Medicine, Taipei Medical College Taipei; †Department of Internal Medicine, National Taiwan University Hospital, Taipei; ‡Department of Nuclear Science, National Tsing-Hua University, Hsinchu; ′Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan
Long-term exposure to inorganic arsenic has been well documented to induce peripheral vascular disease (PVD), and there seem to be variations in individual susceptibility to arsenic-induced health hazards. The objective of this study was to explore the associations of serum micronutrients level and arsenic methylation capability with the development of the PVD. A total of 81 PVD patients and 216 healthy controls were recruited and examined in this study. Systolic blood pressure on bilateral ankle and brachial systolic blood pressure >0.90 on either side. Serum levels of α- and β-carotene, retinol and α-tocopherol were tested by high-performance liquid chromatography (HPLC). Urinary arsenic was examined by HPLC to speciate arsenite, arsenate, monomethylarsenic acid and dimethylarsenic acid, and then quantified by hydride generator linked to atomic absorption spectrometry. The cumulative arsenic exposure was derived from arsenic concentration in artemis well water and duration of consuming the well water. Multiple logistic regression analysis showed a significant dose-response relationship of PVD risk with the cumulative arsenic exposure, the percentage of inorganic arsenic in total amount of urinary inorganic arsenic metabolites as an indicator of poor arsenic methylation capability, and the serum level of α-carotene. The multivariate-adjusted odds ratios (and 95% confidence intervals) were 3.6 (1.1-11.6), 3.3 (1.2-7.9) and 0.3 (0.1-0.9), respectively, for the highest quartile group compared with the lowest quartile group of the cumulative arsenic exposure, the percentage of inorganic arsenic in total amount of urinary inorganic arsenic metabolites and the serum level of α-carotene. The risk of PVD was significantly associated with the cumulative arsenic exposure in a dose-dependent relationship. The higher the serum carotene levels, the lower the PVD risk; but the poorer the arsenic methylation capability, the higher the PVD risk.

PO-0373 Natural history of HIV infected haemophilic patients with biological offspring
Yee TT, Goldman E, Devereux H, Sabin C, Lee CA
Haemophilia Centre and Haemostasis Unit, Royal Free Hospital, Pond Street, London, UK
A proportion of haemophilic men were infected with HIV when they were relatively immatures or young men. They had stable relationships and had children. To determine the risk factors associated with heterosexual transmission of HIV, immunological, virological and clinical progression of
Epidemiology, viruses, genetic diseases

HIV infection in HIV-infected patients who have fathered children have been studied. HIV-1 viral load (AmplificHIV 1-monitor™ assay) was measured on archived serum samples and CD4 counts have been measured at six monthly follow up of patients since 1983. HIV-1 provirogen expressions were determined as the midpoint between the last negative and the first positive test by using a competitive enzyme immunoassay (Wellcozyme, Wellcome Diagnostics, Dartford, UK). The viral load at the time of conception was calculated as the mean of three measurements (results from two years prior conception and year of conception). One hundred and eleven haemophiliacs patients who were HIV-infected at a median age of 24 years (range 2-77) have attended the Royal Free Hospital regularly and of than 14 couples have raised 19 children. All the offspring are anti-HIV negative.

PO-0374 Haematologic syndromes associated with chronic hepatitis C

N exseresence Center for Haematology, *Moscow Medical Academy, Moscow, Russia

We studied bone marrow and serum indices of proinflammatory cytokines in 46 untreated patients (pts) (age 18-69) with different haematologic syn-
dromes associated with chronic viral hepatitis. The majority of pts had 1-3 lineage cytopaenia in peripheral blood, HCV RNA in serum (82% pts) and normal or mildly deranged serum levels of liver enzymes (87% pts). Liver biopsy obtained from 34 pts showed low histological activity index (Knodell score = 3-8) without cirrhosis. There were no signs of haemolysis, vitamin B12, folate or iron deficiency in our pts. Bone marrow aspiration biopsies showed ineffective hematopoiesis (82% pts) with prominent morphological abnormalities of erythroid cells and elevated numbers of monocyte/macrophages (53% pts), lymphocytes (48% pts), plasmocytes (33% pts) which correlated significantly with high serum levels of TNF-α (range 2-16); CD4 counts median 550 cells/µL (range 250-1300); viral load median 4600 copies/mL (range <400-38,800). All were p24 anti-

PO-0375 Geographical distribution and clinical characteristics of Gaucher’s disease in Spain

Giraldo P, Pocovi M, Pérez-Calvo J, Giralt M and the Spanish Group on Gaucher Fundación Española para Estudio y Terapéutica de la Enfermedad de Gaucher (FEETEG), Zaragoza, Spain

Aims. In may 1995 we activated the National Registry for Gaucher's disease (GD) in Spain. The purpose of this registry is to know the incidence and distribution of GD, in order to analyse the clinical, genetic and evolu-
tive features of these patients in our country. Design and Methods. A ques-
tionnaire including demographic, clinical, diagnostic, biological, radiol-
ogical and evolutive data was sent to hospitals. The cases without previous 

PO-0377 Incidence rates of chronic B-lymphoproliferative disorders (CBLPD) in a northern Spanish area

Perelía M, Giraldo P, Franco-García E, Gutiérrez M, Angóis JA, Puertas F
Polanco H, Barbastro H, Alcañiz H, S Jorge H, Aragón, Spain

Purpose. To determine incidence rates (IR) and age-adjusted incidence rates (AIR) of CBLPD in the Aragon population. The evaluated population showed a regressive demographic pattern and negative-vegetative growth (-1.84/103 inh/y) and it seems a good model to justify a prospective study, taking into account the strong relationship between ageing and CBLPD. To our knowledge, no studies have been performed previously on CBLPD incidence in the Aragon region. In conclusion, an increase in mortality rates in elderly haemopathies was observed, possibly related to high mean age observed in our population.

Comments. The distribution of clonal neoplastic PHD in our series is simi-
lar to that previously reported for other European countries. An increase in elderly haemopathies have been observed, possibly related to high mean age observed in our population.

References

Epidemiology, viruses, genetic diseases

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Giraldo P, Pocovi M, Pérez-Calvo J, Giralt M and the Spanish Group on Gaucher Fundación Española para Estudio y Terapéutica de la Enfermedad de Gaucher (FEETEG), Zaragoza, Spain

Aims. In may 1995 we activated the National Registry for Gaucher's disease (GD) in Spain. The purpose of this registry is to know the incidence and distribution of GD, in order to analyse the clinical, genetic and evolu-
tive features of these patients in our country. Design and Methods. A ques-
tionnaire including demographic, clinical, diagnostic, biological, radiol-
ogical and evolutive data was sent to hospitals. The cases without previous 

Perelía M, Giraldo P, Franco-García E, Gutiérrez M, Angóis JA, Puertas F
Polanco H, Barbastro H, Alcañiz H, S Jorge H, Aragón, Spain

Purpose. To determine incidence rates (IR) and age-adjusted incidence rates (AIR) of CBLPD in the Aragon population. The evaluated population showed a regressive demographic pattern and negative-vegetative growth (-1.84/103 inh/y) and it seems a good model to justify a prospective study, taking into account the strong relationship between ageing and CBLPD. To our knowledge, no studies have been performed previously on CBLPD incidence in the Aragon region. In conclusion, an increase in mortality rates in elderly haemopathies was observed, possibly related to high mean age observed in our population.

References

PO-0374 Haematologic syndromes associated with chronic hepatitis C

PO-0375 Geographical distribution and clinical characteristics of Gaucher's disease in Spain

PO-0377 Incidence rates of chronic B-lymphoproliferative disorders (CBLPD) in a northern Spanish area

Comments. The distribution of clonal neoplastic PHD in our series is simi-
lar to that previously reported for other European countries. An increase in elderly haemopathies have been observed, possibly related to high mean age observed in our population.
Gaucher’s disease (GD), the most prevalent of the genetic lysosomal storage disorders, is caused by a severe deficiency of glucocerebrosidase enzymatic activity. The accumulation of glucocerebroside in macrophages results in hepatosplenomegaly, cytopenia and bone lesions. Alglucerase (Ceredase), a macrophage-targeted human placental glucocerebrosidase, has proven to be elective in the treatment of type 1 GD, as well as immune-replacement therapy for Gaucher’s disease in cerebral spinal fluid infection: two cases mimicking malignant lymphoma

Varicella-zoster virus (VZV), particularly in the immunosuppressed host, may be associated with serious central nervous system (CNS) complications including encephalitis, myelitis, cerebral vasculitis and meningitis. The examination of cerebrospinal fluid (CSF) is non-specific, but may easily provide a rapid diagnosis orientation to viral infection. We report two cases with very unusual cytological features suggesting the possibility of primary CNS lymphoma (PCNSL). In spite of the intraparenchymal location of PCNSL, most authors report positive CSF and agree on the possibility of identifying diagnostic cells in 20-30% of cases of PCNSL. In our patients, smear examination showed hypercellular spinal fluid (cell count over 1000/mm³).

The large lymphoid cells had fine chromatin with one or many nucleoli, basophilic cytoplasm with uniform granulations and high mitotic activity. Patient #1: a 30-year-old man with AIDS was admitted for confusion, meningeal syndrome and a 48 hour history of throracic zoster rash. Patient #2: a 38-year-old woman with allogeneic bone marrow transplantation under treatment for a chronic myeloid leukaemia seven years ago. Clinical presentation was similar, but there was no antecedent zoster rash. The first case has been well documented; the available pathologic material was more limited for the second patient. Using immunocytochemistry, we showed the T origin of the large lymphoid cells (CD3+, CD8+) and the presence of a minor B contingent (CD22+). There was no evidence of TCR rearrangement but we found a minor B clone. The CSF was tested for many viral DNA (HSV, VZV, EBV, HHV6, CMV). The PCR assay gave dual amplification of both VZV and EBV DNA sequences suggesting either co-infection or dual CNS process: VZV infection associated with EBV+ B lymphoma. Finally, clinical evolution was favorable under acyclovir; this result may be in keeping with the fact that our patient was very unusual. The second patient showed amplification of VZV DNA alone. These data indicate that VZV central nervous system infection may have uncommon cytological features which could mimic lymphoma needing many investigations to provide convincing evidence of non malignant aetiology.

Enzyme replacement therapy for Gaucher’s disease in 19-65 years old, with type 1 GD in therapy: 8 patients with alglucerase injection, as well as imiglucerase injection, can be effective in the reversal of cytopenia and organomegaly in patients with Gaucher’s disease improving also quality of life in these patients. Results will be confirmed in more patients, while further studies are necessary to compare therapeutic responses on varying initial doses of enzyme among these patients.

The results obtained in the study indicate that VZV central nervous system infection may have uncommon cytological features which could mimic lymphoma needing many investigations to provide convincing evidence of non malignant aetiology.

Epidemiology, viruses, genetic diseases

Epidemiologic study of malignant haemopathies in our environment

Objective. To determine the accumulated incidence and distribution according to age and sex of the various malignant haemopathies in our region. Material and methods. Over 22 years (1975 to 1997) 718 cases of malignant haemopathies were observed in La Rioja. The ratio of accumulated incidence (per 1000 inhabitants) and distribution according to age and sex was determined through a statistical study. To carry out this study the population of La Rioja in 1986 (half way through the studied period) was taken as reference. The malignant haemopathies studied were: multiple myeloma (MM), non-Hodgkin’s lymphoma (NHL), myeloproliferative syndrome (MPS), lymphoproliferative syndrome (LPS), myelodysplastic syndrome (MDS), Hodgkin’s disease (HD), acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), others monoclonal gammopathies (MNG), malignant histiocytosis (MH). Results. The results obtained in the study are as follows: among these patients.

<table>
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<tr>
<th>Dx</th>
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<td>5.9</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Aged ≥60</td>
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<td>9.4</td>
<td>1.3</td>
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</table>

Comments. According to the age characteristics of the analyzed population, a high incidence of CBPLD, mainly MGUS, was observed, specially in males over sixty.

Varicella-zoster central nervous system infection: two cases mimicking malignant lymphoma in cerebral spinal fluid

Varicella zoster virus (VZV), particularly in the immunosuppressed host, may be associated with serious central nervous system (CNS) complications including encephalitis, myelitis, cerebral vasculitis and meningitis. The examination of cerebrospinal fluid (CSF) is non-specific, but may easily provide a rapid diagnosis orientation to viral infection. We report two cases with very unusual cytological features suggesting the possibility of primary CNS lymphoma (PCNSL). In spite of the intraparenchymal location of PCNSL, most authors report positive CSF and agree on the possibility of identifying diagnostic cells in 20-30% of cases of PCNSL. In our patients, smear examination showed hypercellular spinal fluid (cell count over 1000/mm³).

The large lymphoid cells had fine chromatin with one or many nucleoli, basophilic cytoplasm with uniform granulations and high mitotic activity. Patient #1: a 30-year-old man with AIDS was admitted for confusion, meningeal syndrome and a 48 hour history of throracic zoster rash. Patient #2: a 38-year-old woman with allogeneic bone marrow transplantation under treatment for a chronic myeloid leukaemia seven years ago. Clinical presentation was similar, but there was no antecedent zoster rash. The first case has been well documented; the available pathologic material was more limited for the second patient. Using immunocytochemistry, we showed the T origin of the large lymphoid cells (CD3+, CD8+) and the presence of a minor B contingent (CD22+). There was no evidence of TCR rearrangement but we found a minor B clone. The CSF was tested for many viral DNA (HSV, VZV, EBV, HHV6, CMV). The PCR assay gave dual amplification of both VZV and EBV DNA sequences suggesting either co-infection or dual CNS process: VZV infection associated with EBV+ B lymphoma. Finally, clinical evolution was favorable under acyclovir; this result may be in keeping with the fact that our patient was very unusual. The second patient showed amplification of VZV DNA alone. These data indicate that VZV central nervous system infection may have uncommon cytological features which could mimic lymphoma needing many investigations to provide convincing evidence of non malignant aetiology.

Enzyme replacement therapy for Gaucher’s disease in 19-65 years old, with type 1 GD in therapy: 8 patients with alglucerase injection, as well as imiglucerase injection, can be effective in the reversal of cytopenia and organomegaly in patients with Gaucher’s disease improving also quality of life in these patients. Results will be confirmed in more patients, while further studies are necessary to compare therapeutic responses on varying initial doses of enzyme among these patients.

Incidence rates of non-leukaemic chronic myeloproliferative disorders (CMPD) in a Northern Spanish area


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Epidemiology, viruses, genetic diseases

**PO-0382 Prevalence of hepatitis C virus infection in patients with B-cell lymphoproliferative diseases**

Domingo JM, Romero MS, Palomera L, Domingo JA, Calién L, Moreno JA, Azaceta G, Vano MJ, Rabasa P, Chueca P, Gutiérrez M, Reina Sofia Hospital, Tudela; *University Hospital, Zaragoza; "Ospino Polanco" Hospital, Teruel, Spain

Objective. To evaluate the prevalence of hepatitis C virus (HCV) infection in patients with B-cell malignancies in our geographic zone. Design and Methods. We included 112 patients affected by B-cell lymphoproliferative diseases (LPD) in our Hospital and diagnosed according to the R.E.A.L. classification. All the patients were tested for antibodies to HCV by ELISA and RIBA methods and by RT-PCR for HCV-RNA. Results. We detected antibodies to HCV in 19 (17%) patients; this prevalence was high in comparison to that in the healthy population in this area (1.3%). Viral RNA was detected in 14 (15.6%) patients. We found a very high prevalence of HCV infection in patients with immunocytoma (40%), multiple myeloma (18%) and follicle center lymphoma (22%). Conclusions. These results are in agreement with those reported from other countries such as Italy and USA (not confirmed by data from the United Kingdom) and support the pathogenetic role of HCV in the clonal proliferation of B-cells, probably due to lymphomatisis of this virus. However, the exact mechanism of this association and the viral genotypes implicated need to be investigated.

**PO-0383 Presence of human retroviruses in peripheral blood of patients with various forms of haematological malignancies**


Objective. To investigate the association of human retrovirus (HTLV-II, HRV-5) infection with various forms of haematological malignancies. Design and Methods. Blood samples were collected from 81 randomly selected patients with haematological malignancies. Serum antibodies to HTLV-II and HRV-5 were examined by using ELISA. DNA was extracted from frozen blood as well as from PBMC and examined by PCR for evidence of proviral DNA (HTLV-II, HRV-5) with the subsequent cloning and sequencing of PCR products. Three undet DNA samples isolated from whole blood of practically healthy blood donors were analysed in a similar fashion. Results. Blood serum of 81 patients with haematological malignancies (acute leukaemia - 9, chronic lymphocytic leukaemia - 30, non-Hodgkin's lymphoma [NHL] - 22, Hodgkin's disease - 5, other non-lymphoid malignancies 15) and 300 blood donors was analysed in ELISA for the HTLV-II antibodies. All sera were negative for IgG antibodies to these viruses. In their turn B DNA samples of the same patients (9.9%) and 19 DNA samples of blood donors (6.3%) were PCR positive for HTLV-I tax only. None of the blood donors was positive for HRV-5 DNA whereas 3 out of 22 patients (14%) with non-Hodgkin's lymphoma were positive for HRV-5 which is 3.7% of all examined patients with haematological malignancies. All three HRV-5 positive NHL patients had clinical features in common: 1) the lymphoma was of low-grade malignancy; 2) all were B-cell origin; 3) all had a proliferation of malignant cells in the spleen and abdominal lymph nodes, leading to splenomegaly and abdominal lymph node enlargement; 4) two of them had metastases of lymphoma in atypical sites: the pleural cavity and lumbar vertebrae. Conclusion. Our findings of a statistically significant difference in HRV-5 DNA positivity between healthy persons and NHL patients (p value 0.0003 judged by the Fisher exact test) suggest that there is an association between HRV-5 DNA positivity and low-grade NHL having the described features. In its turn difference in HTLV-I tax positivity is not statistically significant and the functional role of HTLV-I tax positivity alone is not yet clear.

**PO-0384 Enrythropathology study of the migrant African people of Maresme**

Las Heras G, *Juncà J, Gil M, Rovira M, Bosch A, Hernández JA, *Laboratory Services Sant Joan de Déu Hospital, Martorell and Consorci Sanitari de Mataró, Mataró, *Hematology Service University Germans Trias i Pujol Hospital, Badalona, "Institut Municipal d'Investigació Mèdica, Barcelona, Spain

Objective. A numerous community of African people lives in Maresme, a region of East Catalonia. These immigrants original from part of West Africa where sickle cell trait and glucose-6-phosphate dehydrogenase (G6PD) deficiency are common. The aim of our study was to establish the prevalence of these two disturbances in this population. Design and Methods. We studied 200 black migrant Africans. This group is representative of the migrant African people of Maresme. The G6PD deficiency was studied through a quantitative assay and the identification of abnormal haemoglobins by conventional electrophoresis. Results. We studied 137 males and 63 females. Their average age was 30.8 years. We found abnormal haemoglobins in 42 subjects (21%), 35 haemoglobin S carriers (17%), 6 haemoglobin C carriers (3%) and 1 haemoglobin C homozygous (0.5%). Moreover, we identified G6PD deficiency in 29 subjects (14.5%). Conclusions. Our results demonstrate a high prevalence of haemoglobinopathies and G6PD deficiency in this population. It would be necessary to take into consideration the design of screening programs for these two disturbances in this population.

**PO-0385 Cancer incidence in relatives of patients with haematological malignancies**

Brito-Babapulle F, Ekelidwei R, Woodward M, *Dept of Haematology, Royal Berkshire Hospital, Reading, and Dept of Applied Statistics, University of Reading, UK

Germ line mutations in genes such as BRCA1 and R1 are known to be involved in the causation of cancer. However, epidemiological studies in colorectal cancer and twins of patients with Hodgkin’s disease do not support the hypothesis of a genetic predisposition to cancer. Yet it is well known that there is an increased incidence of leukaemia in twins of patients with acute lymphoblastic leukaemia and a 1 in 4 risk of patients with CLL having a family member with cancer. We have compared the prevalence of cancer in relatives of patients diagnosed with haematological malignancies (Group A) to a control group of patients with non-malignant haematological disease (Group B) in a prospective study. Detailed 3-generation family histories were obtained from all patients attending the haematology clinic. One hundred and twenty-one patients gave detailed histories. These in Group A (n=89) included 39 with lymphoproliferative disease, 20 with chronic myeloid leukaemia, 11 with myeloproliferative disease (CML, 3 others), 18 with a plasma cell dyscrasia, 12 with myelodysplasia, 4 with acute leukaemia and 1 each of Hodgkin’s disease, systemic mastocytosis and Burkitt’s lymphoma. Those in Group B (n=36) included patients with auto-immune disorders, secondary polycythemia, hereditary disorders of white and red cells, rTP, nutritional anaemias, and coagulation disorders. The mean age of Group A was 68 (14-90) and of Group B was 53 (20-82). Sixty-six percent of patients in Group A had one relative affected by cancer compared with 35% in Group B. In Group A 27% of patients had two or more relatives affected by cancer compared with 1% in Group B. The odds ratio for a malignancy comparing those with at least one affected relative was 3 (1.27-7.46 p<0.01). The specific types of cancer identified in Group A were breast 19, colorectal 14, stomach 14, lung 10, leukaemia 7, prostate 5, and others 30, whereas in Group B there were 3 cases of breast cancer, 2 of stomach and others 11. In female relatives of Group A patients the prevalence of breast cancer was 19/58 (33%) vs 3/16 (19%) in Group B. There were 32 kindreds in Group A in whom multiple family members were affected by haematological malignancies or solid tumours and 1 such family in Group B. Siblings with cancer and/or haematological malignancies were identified in 20 families of which 4 had haematological disease alone. Our preliminary analysis indicates that there is an increased incidence of cancer among relatives of patients with haematological malignancies, which may indicate an inherited susceptibility to cancer. Further analyses of epidemiological and molecular genetic data need to be carried out in such cases to identify the exact mechanism of this susceptibility.

**PO-0386 Prevalence of endogenous retroviruses in human T-cell leukaemia virus (HTVL) negative haematosic diseases**

Karlic I, Madić M, Radolff M, Pavlova B, Pfleistöcker M, Grüner H, Pitte- mann E, Heinz R, Ludwig Boltzmann Institute for Leukemia Research and Hematology, 3rd Medical Department, Hanusch Hospital, Vienna, Austria

Objective. Human endogenous retroviruses (HERV) contain HTLV homo logical sequence elements within the pol gene. Considering previous results

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<th>MM</th>
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<th>UPS</th>
<th>LPS</th>
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<td>3.5</td>
<td>0.25</td>
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IA: accumulated incidence (per 1000 inhabitants). M/W: men/women ratio.
which show an incidence of HTLV-1 in 17% of myelodysplastic syndromes (MDS) besides the well known incidence of this virus in T-cell lymphomas (TCL). The aim of this study was to investigate a putative relationship of HERV and HTLV sequence motifs in haematologic diseases. Design and Methods. Using the polymerase chain reaction pool specific sequence motifs were analysed from the endogenous retroviruses: HERV-K10, -MLN, -TRO and -K-LPO-HER and compared to pool and tax sequence motifs from HTLV-1 from blood and bone marrow - derived mononuclear cells (MNC) of 63 persons. Cyrogentic data were available from 45 samples. The study included 12 patients with myeloproliferative disorders (MPD), 28 MDS patients, 11 cases with TCL, 4 cases with B-cell lymphomas (BCL) and 8 healthy donors. Results. HERV positivity was detected in 66.7% (8/12) of MPD patients all of them negative for HTLV. From TCL patients including HTLV-positive TCL 10/12 were negative for HERV and from BCL cases 2/4. Among MDS 32.1% (= 9/28) were HERV-positive; there were 25% (7/28) HTLV-positive MDS cases but only one case was positive for both viruses. The incidence of HERV in healthy donors was 37.5% (= 3/8). Cyrogentic data were available from 45 cases with clonal aberrations but HERV occurred independently of cyrogentic features. Conclusions. This study confirms previous hypotheses (2) suggesting a role for endogenous retroviruses in the evolution of resistance to retroviral infection, by showing that HERV-derived pool sequence motifs with partial HTLV-homology can be detected predominantly in HTLV-negative haematologic diseases. 

[Study supported by Jubiläumsfonds d. Öster. Nationalbank, Project No. 6340]

2. Best et al. Trends in Microbiology, 1997; 5:313

**PO-0387** Study of B12 levels in Gaucher patients uncovers high prevalence of B12 deficiency in panethnic populations

Gielchinsky Y, Eldstein D, Aligr N, Abrahamov A, Shinar E, Lahad A, Miller JW, Green R, Zimran A, Shaare Zedek Medical Center, Jerusalem, Magen David Adom, National Blood Services, Israel; UC Davis Medical Center, Sacramento, CA, USA

In our pilot study of B12 levels in adult patients with type I Gaucher disease (Blood 1997; 90:140) 42.6% were found to have low vitamin B12 levels (under 200 pg/ml). Of these, 98 patients who were not treated with enzyme replacement showed HS-10.12 were negative for HERV and from BCL cases 7/24. Among MDS 32.1% (= 9/28) were HERV-positive; there were 25% (7/28) HTLV-positive MDS cases but only one case was positive for both viruses. The incidence of HERV in healthy donors was 37.5% (= 3/8). Cyrogentic data were available from 45 cases with clonal aberrations but HERV occurred independently of cyrogentic features. Conclusions. This study confirms previous hypotheses (2) suggesting a role for endogenous retroviruses in the evolution of resistance to retroviral infection, by showing that HERV-derived pool sequence motifs with partial HTLV-homology can be detected predominantly in HTLV-negative haematologic diseases.

**PO-0388** HLA-CW3 and HLA-CW4 have a protective effect on the acquisition of CML probably by presentation of bcr-ab breakpoint peptides in these HLA molecules


Cytogenetic data confirmed the prevalence of HTLV in cases with clonal abnormalities. Neoplastic cells are defective in antigen presentation, but the cellular immune system may be capable of recognising these HLA molecules.

2. Best et al. Trends in Microbiology, 1997; 5:313

**PO-0389** Chromosomal abnormalities in 33 patients with myelodysplastic syndromes (MDS)

Laharta JD,*, Cynulik Goldstein S,* Fernandez Greco H,*, Cavanaro FJ,*, Sanchez Avalos JC,*, Barros CA,*

*Dep. of Cyrog咏tics, Lab. Dr. Menéndez; Haematology Divisions: **Hospital Durand, #Hospital de Clinicas, @Hospital Alvarez, ^Inst. A. Fleming. Buenos Aires, Argentina

MDS show clonal proliferation of cells. Chromosome abnormalities are more frequent in secondary MDS (s-MDS) than in primary MDS. Because the Thrombocytopenia is a very important parameter, we analysed 33 patients (median age 65 years): FAB classification: RA (48.49%), RAEB (33.33%), RAEB-T (9.09%) and CMML (9.09%). International Prognostic Scoring System (1997), risk groups: Low (9.1%), Int-1 (39.3%), Int-2 (27.2%) and High (24.24%). Bone marrow was processed using standard culture (24-48 hrs.) and harvest. Chromosome analyses were performed by G-banding and others, and showed: deletions (5q) (13 patients); chromosom 7: monosomes (4), structural anomalies (6). In 81 B- and tetrasomy 21 (4); del(20q)(2); del(11q)(2); del(12p)(2) and others. Most of these were observed in singles and complex karyotypes. We were interested to note the high incidence of t and tetrasomy 21 (12.12%) as well as trisomations involving chromosomes 7 (15.15%) and 16 (9%).

**PO-0390** Isolation and genetic characterisation of blood dendritic cells in B-cell chronic lymphocytic leukaemia

Lazaridou A, Miravites Ch, Korantzis I, Christaki J, *Dept. of Haematology, **Theageneio 'Centre Cancer of Theseion, Greece

B chronic lymphocytic leukaemia (B-CLL) is a neoplastic disease characterised by immune deficiency. Neoplastic cells are defective in antigen presentation, but the cellular immune system may be capable of recognising leukaemia associated antigens. Dendritic cells (DC), are potent mediators of CML probably by presentation of bcr-ab breakpoint peptides in these HLA molecules.

-11 and -88 and the class II molecules HLA-DR1, -DR3, -DR4, -DR11 and -DR15. Others authors have shown that expressing HLA-88, DR14, DR15 have a diminished risk of the development of CML in Caucasion populations. Furthermore, other studies have reported a statistically significant increase in the frequency of Cw4 in patients with CML associated with HLA phenotypes specific to each population, and indicate that Cw3 and Cw4 expression may result in a protective effect on the acquisition of CML probably by presentation of bcr-ab breakpoint peptides in these HLA molecules.

Cytogenetics, molecular genetics

**Poster discussions**

**Cytogenetics, molecular genetics**

**PO-0389** Chromosomal abnormalities in 33 patients with myelodysplastic syndromes (MDS)

Laharta JD,* Cynulik Goldstein S,* Fernandez Greco H,* Cavanaro FJ,* Sanchez Avalos JC,* Barros CA,*

*Dept. of Cyrog咏tics, Lab. Dr. Menéndez; Haematology Divisions: **Hospital Durand, #Hospital de Clinicas, @Hospital Alvarez, ^Inst. A. Fleming. Buenos Aires, Argentina

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B chronic lymphocytic leukaemia (B-CLL) is a neoplastic disease characterised by immune deficiency. Neoplastic cells are defective in antigen presentation, but the cellular immune system may be capable of recognising leukaemia associated antigens. Dendritic cells (DC), are potent mediators of the immune response and are currently investigated for application in cancer immunotherapy. Recently, leukaemic DC vaccination therapy post autologous PSBC transplantation has been given successfully to patients with CML. In view of the potential therapeutic use of DC in B-CLL too, we looked at whether or not they carry neoplastic markers, such as deletions of chromosome 13q14, commonly found in this disease. Sixteen patients with B-CLL were studied at diagnosis. DC were isolated from the peripheral blood using the Blood Dendritic Cell Isolation Kit (MACS Miltenyi Biotec, Germany). Starting with 2x10^8 cells we were able to isolate DC by depleting of CD14 + T cells, CD56 + mononuclear cells and C16 + NK cells and positive selection of CD4 + dendritic cells, to purity of >90%. These cells were positive to HLA-DR but negative to TCRy/8, CD19 and CD56 antigens. After

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a 3-day culture period in the presence of GM-CSF+SCF+Flt-3L and TNF-
alpha. DC develop their characteristic morphology. Dual color FISH
analysis was performed using probes for Rbl (13q14.2), D13S25
(13q14.3) and D13S319 (13q14.3). Hemizygous and homozy-
gous deletions of D13S25 and D13S319 were found in 9 out of 16 patients
(56%) in a high proportion (73-92%) of cells. Rbl mononuclear depletion
was found in 5 out of 16 patients (31%), in 32-95% of cells, in combina-
tion with D13S25 and D13S319 deletions. In two patients we also tested the GM-frac-
tion of cells and found the same percentages of genetic abnormalities. In
addition, we were able to select and study CD11c functionally immature
DC in two patients using a modification of the depletion step. Surprisingly,
only a very low proportion of genetically abnormal cells was found among
these cells. In conclusion, it appears that in a large subset of B-CLL
patients, DC carry the same genetic markers as clonal cells and are prob-
ably neoplastic in origin. This finding should be taken into consideration in
planning anti-tumour immunotherapy in CLL. We are currently selecting
patients, with an even higher incidence in untreated vs treated subjects.
Furthermore, we observed that 4.5-26% of BM mononuclear cells showed
46,X,-Y,+X,t(2;9)(q31;p24), del(4)(q21q23)[20]/47,idem,+der(2)t(2;9)
(q31;p24)[2], haemoglobin 10.6 g/dL, platelets 63*10^9/L and
AML FAB M4Eo (t(8;14)(q24;q32) and complex cytogenetic abnormalities
in HIV-associated large cell non-Hodgkin's lymphoma with
plasmacytic differentiation

M. Panier O, Okenendrer E, Daniel MT
Laboratoire Central d'Hématologie, Service d'Immuno-Hématologie,
Hôpital Saint Louis, Paris, France

Some HIV associated non-Hodgkin's lymphoma (NHL) with plasmacyte dif-
ferentiation, with clinical and biological features of Burkitt's ly-
phoma (BL). We investigated whether or not these lymphomas could be
considered as BL. Eleven HIV infected patients presented with clinical and
biological symptoms suggesting the diagnosis of systemic BL. Bone mar-
row involvement was present in 10 cases, neurological symptoms in 30,
and visceral disease in 5. The LDH level was superior to 500 U/L in 9 cases.
Bone marrow and/or lymph node aspirate revealed typical features of BL.
HIV was detected in 9 out of 10 cases. Antigenic analysis showed that
the diagnosis was confirmed by flow cytometry. Interphase cytogenetics
was then performed in order to confirm flow cytometry results, and to detect which chromosome(s) was (were) responsible
for the modification of the phenotype. Interphase cytogenetics has allowed the analysis of many oncohaemato-
logical diseases characterised by a low number of evaluable metaphases or low mitotic index such as multiple myeloma (MM). Several authors report a high incidence of aneuploidy (mainly hyperdiploidy) in their series of MM
patients, with an even higher incidence in untreated vs treated subjects.
Most studies are based on flow cytometric evaluation of DNA index. In the
present study we selected 10 cases of untreated MM patients, in which a
hyperdiploid cell population has been already detected by flow cytometry.

No cases showed metaphases at conventional cytogenetics examination.
Interphase cytogenetics was then performed in order to confirm flow cytom-
etry results, and to detect which chromosome(s) was (were) responsible
for the modification of the phenotype. A new technique, Interphase cytogenetics, was used to obtain the simulta-
neous hybridisation of 24 centrometric biotinylated probes on 24 staggered
chromosomes,

<table>
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<th>Aberrations</th>
<th>Number of cases</th>
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<td>del(6)(q12)</td>
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<td>Wong et al, 1997</td>
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<td>Mateu et al, 1998</td>
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<td>present report</td>
</tr>
<tr>
<td>t(11;17)</td>
<td>1</td>
<td>Xie et al, 1998</td>
</tr>
</tbody>
</table>

Table 1. Summary of 6q abnormalities reported in NK cell lymphoma/leukaemia

PO-0393 The prognostic significance of the partial deletion of the long arm of chromosome 20 in myeloid disorders.
A review of 20 cases

*Hanusch Hospital, 3rd Medical Department for Hematology and Oncology, **Hanusch Hospital, Ludwig Boltzmann Institute for Hematol-
ogy and Leukemia Research, Vienna, Austria

Chromosomal abnormalities, like the partial deletion of the long arm of chromosome 20 are an important indicator of disease type and prognosis
in haematological malignancies. This deletion has been described in a number of myeloid malignancies, including myeloproliferative disorders
(MPD), myelodysplastic syndromes (MDS) and acute leukemias (AL). A review of nearly 1000 patients with myeloid disorders and chromosomal
abnormalities presenting to our center over a fifteen year period was under-
taken to assess the clinical relevance and the prognostic significance of the
presence of del(20)(q) in their malignant karyotypes. Twenty patients were
identified (13 at time of diagnosis with del(20)(q) as second banding ab-
normality), seven with MPD, six with MDS and seven with AL. In MPD exclud-
ing CML (3 PV, 2 OM, 2 not specified) this special chromosomal aberra-
tion did not have any influence on the median survival of 45 months (4-240)
and one patient dead in blast crisis of plasmacytoma vera after 240 months (median survival in our MPD PV group of 70 patients: 102
months ). In the group with myelodysplastic syndromes (3 RA, 1 AISA, 2 RAEB with one secondary to Hodgkin's disease), the median survival was
22 months (2+ to 71), which was lower than in our whole MDS group of
382 patients (median survival time 33 months); also the progression rate of
50% showed a difference according to our statistics (35% prog. rate).
From the seven patients with acute leukaemia (two without, five with dys-
plasia) and del(20)(q), were all treated with standard induction chemother-
apy, and with only one patient achieving a short partial remission (6 months).
This patient had an AML M4Eo, a leukemia type with usually favourable
prognosis. The median duration of survival for these seven patients was
7 months (2 to 21). In MDP del (20)(q) does not seem to have any influence
on course of disease or on duration of survival, with 4 of 7 karyotypes
with del(20)(q) as single aberration. In MDS del(20)(q) does not seem to
have such a good prognostic impact as confirmed in the International
Score (IPSS) and other published data. Finally in AL, where del(20)(q)
was always part of a complex karyotype, survival rate was short and
response to therapy was poor.

PO-0394 Interphase cytogenetics in multiple myeloma

Perla G., D'Arena G., Musto P., Cascavilla N., Latufara C., Carotenuto M
Dept. of Hematology, CSS Hospital IRCCS, San Giovanni Rotondo, Italy

Interphase cytogenetics has allowed the analysis of many oncohaematolo-
gical diseases characterised by a low number of evaluable metaphases or
low mitotic index such as multiple myeloma (MM). Several authors report a high incidence of aneuploidy (mainly hyperdiploidy) in their series of MM
patients, with an even higher incidence in untreated vs treated subjects.
Most studies are based on flow cytometric evaluation of DNA index. In the
present study we selected 10 cases of untreated MM patients, in which a
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No cases showed metaphases at conventional cytogenetics examination.
Interphase cytogenetics was then performed in order to confirm flow cytom-
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<td>del(6)(q21)</td>
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</tr>
<tr>
<td>t(11;17)</td>
<td>1</td>
<td>Xie et al, 1998</td>
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</tbody>
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Table 1. Summary of 6q abnormalities reported in NK cell lymphoma/leukaemia

Haematologica vol. 84 (EHA-4 Abstract Book); June 1999
The t(8;13)(p11;q12), associated with the 8p11 myeloproliferative syndrome, fuses ZNF198 at chromosome 13q12 to the fibroblast growth factor receptor-1 (FGFR1) at 8p11. ZNF198 is a widely expressed gene that is highly homologous to ZNF198 and which was subsequently designated ZNF237. Full-length ZNF237 cDNA sequence was obtained by RACE-PCR and comparison to the EST database. ZNF237 is predicted to encode a protein of 462 amino acids that is highly homologous throughout its length to the N-terminal region of ZNF198. ZNF237 is widely expressed, as determined by multiple tissue Northern blot and several regions of alternative splicing were identified by RT-PCR. Radiation hybrid analysis indicated that ZNF237 maps to chromosome 1q32-34. Northern blotting, with a partial cDNA clone indicated that KIAA0425 is expressed widely. We conclude that ZNF198 is a member of a novel, chromosome dispersed, widely expressed gene family whose function remains to be determined.

PO-0374 Screening of MLL fusion genes in acute leukaemias: validation of a RACE-PCR method by both retrospective and blind studies

Bélibar E,* Laflage-Pochilatoff M,* *Preudhomme C, Dupont M, Cayuela JM, Dabughe N, Saintry D,* Gabert J,* Laboratoire d’Hématologie, Institut Paoli-Calmettes, Marseille, France

Objective. MLL gene rearrangements are poor prognostic molecular markers which are taken into account in multivariate therapeutic trials for patient stratification. However, actual detection methods are neither sensitive enough (cytogenetic analyses) nor easy to routinely use (Southern-blot, multiplex PCR). We have developed a simple and quick assay to detect MLL-associated fusion transcripts. After an anchored-PCR, an ELISA revelation step is used to detect known MLL-partners (Br) Haematol 1998; 102:14). Design and Methods. Using RNA from patients or cell lines, we were able to detect 11 different MLL partner genes (AF1p, -q, -X, -4, -6, -9, -10, -17, ENL, ELL, MLL-duplication) and their various breakpoints. With a ready-to-use 96 well-plate coated with biotinylated probes, these breakpoints can be simultaneously detected. To validate this method, both blind and retrospective studies were used. The blind study was performed on 14 samples of acute leukemias at diagnosis using PCR-ELISA. For the retrospective study, 32 de novo AML (except AML3) received in our laboratory over 6 months, were analysed. Both Southern-blot and ELISA were performed on these 32 samples. Results. The blind study showed 6 positive samples: MLL-AF4(-3), -AF6(-1), -AF11, -AF31p. Moreover, 5 samples were negative and 3 were degraded. The retrospective study found 4 positive samples by Southern-blot, each being detected and characterised by PCR-ELISA: MLL-AF9(-1), -AF171, MLL-duplication(-2). Among the 28 remaining samples, 25 were negative, 2 doubtful and 1 not done. Only one had karyotype abnormalities in the 11q23 band (MLL-AF17). Conclusions. First, our blind study shows the specificity of the method with 100% of results confirmed in the 11 validated cases. Secondly, the retrospective study identified 12.5% of MLL rearranged samples by Southern-blot (in vitro). When comparing Southern-blot and PCR-ELISA results, our data suggest equal sensitivity and specificity with our method. This quick and simple test can easily identify MLL gene rearrangements with known partners that occasionally are not detected by conventional karyotyping. Finally, the identification of each partner gene should help evaluate its prognostic significance, which is subject to controversy for the t(9;11) or unknown for most of the others.

PO-0396 Trisomy 22 is a marker for acute myeloid leukaemia with monocytic features and cytogenetically cryptic inv(16)

Wong KE, Kwong YL
Department of Pathology, Queen Elizabeth Hospital, and the Department of Medicine, Queen Mary Hospital, Hong Kong, China

Objective. Trisomy 22 is an uncommon karyotypic aberration in acute myeloid leukaemia (AML), often of the myelomonocytic subtype. Some studies suggest that this abnormality is associated with concomitant inv(16)(16q21). However, inv(16) is often difficult to recognise and there is recent evidence that it is sometimes underestimated in routine cytogenetic analysis. We therefore performed a retrospective analysis of a cohort of AML patients to define the clinicopathologic features of AML with trisomy 22, with particular emphasis on the possible association with inv(16) at the molecular level. Methods. The cytogenetic findings of 170 patients with AML diagnosed in Queen Elizabeth Hospital during the period 1994 to 1997 were reviewed. Three patients were found to have trisomy 22. All of them had AML with a monocytic component as shown by cytochemical reaction and serum lysozyme assay according to the FAB criteria. Marrow eosinophilia was not evident in any of them. Cytogenetic studies showed the presence of trisomy 22 as the only abnormality in two cases while the third case showed two abnormal clones, one with concomitant trisomy 9 and 22, and another with del(7)(p22q33). Reverse transcription polymerase chain reaction (RT-PCR) for the CBFa/MYH11 fusion transcript was performed on total cellular RNA extracted from Ficoll-sedimented marrow mononuclear cells by two pairs of nested CBFB primers and MYH11 primers. Results. The integrity of the RNA was verified by amplification of β2-microglobulin transcript. The RT-PCR showed a 282 bp PCR product for all three cases, consistent with fusion of CBFB at nucleotide (nt) 495 to MYH11 at nt 1921. Conclusions. We demonstrate the occurrence of CBFB/MYH11 fusion in three patients, with AML and trisomy 22 but without cytogenetic evidence of inv(16). All three cases are AML with a monocytic lineage although marrow eosinophilia is not evident. Our observation therefore confirms that trisomy 22 is a non-random chromosomal abnormality of AML and is specifically associated with AML with a monocytic component and inv(16), the latter being cytogenetically cryptic in some cases. This finding is of potential diagnostic and therapeutic significance.
PO-0401 Loss of AFM3B47EF9 locus in a case of myelodysplastic syndrome with a t(2;5)(q21;q13) translocation

Colomer D,* Cid J,* Costa D,* Rozman M,* Aguilar JLL,* Montserrat E,* Campo E*
*Unidad d’Hematologia; °Servei d’Hematologia, and #Servei de Genetico-IDIBAPS, Hospital Clinic, Barcelona, Spain

Introduction. Acquired partial and complete deletions of chromosome 5 are common cytogenetic anomalies in primary myelodysplastic syndromes (MDS). Up to now, only sporadic cases of unbalanced translocations involving chromosome 5 have been described. Furthermore, a novel locus has been localised at 5q33.1 which has been suggested to contain a critical gene that may be deleted or disrupted in a subset of patients with chromosome 5 abnormalities. Objective. Molecular analysis of a case of MDS with a t(2;5)(q21;q13) translocation. Design and Methods. A 79-year-old man with a moderate B-cyto-penia was studied. A bone marrow aspirate showed hypercellularity with dysplastic features in the erythroid and megakaryocyte lineages. No blasts were seen and the diagnosis of MDS was made. The cytogenetic analysis was performed using banding technique and DNA was isolated from in vitro cultures and bone marrow samples. Results. The patient showed an acquired 5q- chromosome. No abnormalities were detected in the other chromosomes. Conclusions. The loss of a region on chromosome 5q13.1 has been described in MDS. PO-0402 Alterations in the long arm of chromosome 3 (3q) in myeloid malignancies. Study of 10 cases

Sanchez JM, Granada I, Riberas JM, Batlle M, Hernandez JA, Navarro IT, Fernandez F, Grau J, Xicoy B, Flores A, Jonc получил о новом локусе AFMB347YF9, который был выделен на больном с транслокацией t(2;5)(q21;q13) представлен был на двух разных локусах GATA-P18104 и D5S435. Обнаружено, что некоторые аномалии на 5q не имеют клинической значимости. PO-0400 Cleavage of the MLL gene in acute leukaemia before treatment disappears in relapse

Anguita E,* Villegas A,* Serra A,* Gonzalez A,* Contra T,* Saglio G,* *Department of Haematology Hospital Clinico San Carlos, Madrid, Spain; Laboratory of Medicine and Oncology Molecular, Hospital San Luigi Gonzaga, Orbassano, Torino, Italy; *Department of Oncology, Hospital Nino Jesus, Madrid, Spain

Background. MLL gene rearrangements are frequently found in secondary acute leukaemias (ALS). A site-specific cleavage of the MLL gene in a consensual sequence for Topoisomerase II recognition is considered an initial event leading to MLL rearrangement and subsequent therapy-related AL. We have observed MLL cleavage frequently in patients with myeloid and lymphoid ALS at diagnosis. Objective. To evaluate whether this pseudo-rearrangement is a laboratory-produced artefact in our patients and whether it persists or causes a real MLL gene rearrangement at relapse. Design and Methods. Seventy-four AL patients were analysed: 29 childhood ALL, 5 childhood AML, 20 adult ALL and 20 adult AML. Patients with promyelocytic AL were excluded. The bone marrow (BM) samples were further enriched by Ficoll gradient centrifugation within 4 hours after BM aspiration, before being preserved at -70°C until analysis. All the samples were initially searched for MLL rearrangement by Southern blot. All the rearranged cases were analysed by RT-PCR for the transcripts: MLL/AF4, MLL/ENL, MLL/ELL, MLL/AF6, MLL/AF9 and MLL self-duplication. Results. MLL gene rearrangement was detected in 8 of the 74 patients with AL analysed (10.8%). In 5 cases (6.8%) the intensity of the rearranged bands was consistent with the rearrangement of one MLL allele, but in 3 cases (4%), the intensity was lower. These 3 cases presented BamHI and Bgl II bands identical to those described in the cases carrying the site-specific MLL cleavage. MLL chimeric transcripts were detected by RT-PCR in all the 5 patients with a strong rearrangement bands in the Southern blot. By contrast, all three patients with low intensity rearrangements were negative for all the fusion transcripts analysed. In one of these patients, with a T-ALL, the Southern blot showed a pattern compatible with the presence of MLL cleavage in all three different and subsequent experiments. Thirteen months after the first diagnosis the patient relapsed, but although the immunophenotype was the same, the DNA samples obtained in the two cases of MLL gene cleavage or rearrangement was no longer detectable. In the second patient with a B precursor ALL the analysis was repeated in two occasions. The third case was FAB-M2 AML. Conclusions. MLL cleavage is frequently detectable (4%) by Southern blot on DNA taken at diagnosis or from AL patients at diagnosis before treatment even if the samples are processed shortly after BM aspiration. This event is not restricted to myeloid leukemias but may also occur in lymphoid ALs. The fact that a constant pattern was obtained from the same patients in three and two different DNA preparations, respectively, further supports the notion that MLL cleavage is present in vivo and is not merely a lab artefact. One case that we reported was positive for MLL cleavage at diagnosis, but negative for either MLL cleavage or MLL rearrangement at relapse. This suggests that, at least in this case, the MLL cleavage did not play any role in the pathogenesis of the disease recurrence.
PO-0403 Simultaneous presence of monosomy 7 (-7) and trisomy 8 (+8) in the bone marrow cells of 3 patients with a myeloid malignant hematopathy

Castagné C,* Mühlematter D,* Parlier V,* Kovacsics T,** Spertiini O,* Ghelmini M,* Lettbrand M*

*Unité de cytogénétique du cancer, Division autonome de génétique médicale et *Division d’Hématologie, CHUV, Lausanne; **Servizio Oncologico Cantonale, Ospedale San Giovanni, Bellinzona, Switzerland

Although -7 and +8 are common chromosome anomalies in malignant myeloid hematopoiesis, both aneuploidies rarely occur simultaneously in the same clone. In most cases described, -7 and +8 were associated with other chromosome aberrations. We report here 3 patients with -7 and +8 in the same clone in 2 cases of acute myeloid leukemia (AML) and 1 case of refractory anemia with excess of blasts in transformation (RAEB-T) with or without additional aneuploidies (see the Table). Patients were studied by conventional cytogenetics (CC) and dual-color interphase fluorescence in situ hybridisation (I-FISH) at presentation and during the course of the disease. I-FISH was performed with alphoid repetitive DNA probes specific for structural anomalies present. For CC, we calculated the percentage of abnormal cells and the karyotype according to the ISCN 1995. The patients were all males (ages 62, 58 and 67). The most frequent involved chromosomes were: 7, 8q, 9q and 1q, while 13q, 6q, 17p and 14q. Recurrent copy number losses were identified on 13q, 6q, 17p and 14q. Recurrent copy number gains were on chromosomes 1p (13.5%) and 2p (3.2%) at presentation and in patient #3 during follow-up (3.2%).

PO-0405 Mutations in the Fanconi anaemia group C gene in the state of São Paulo, Brazil

Rodriguez D,* Lima CS,* Figueiredo MS,* Bertuzzo CS*

*Federal University of São Paulo; *Faculty of Medicine, State University Campinas, São Paulo, Brazil

Fanconi anaemia (FA) is an autosomal recessive disorder associated with hypersensitivity to DNA cross-linking agents and bone marrow failure. Several complementation groups have been defined, and the FA group C gene (FAC) has been cloned and mapped to chromosome 9q22.3. We screened FA patients in the University of Campinas - São Paulo State for eight mutations in the FAC gene (Q13X, W272X, ΔG352, N544A→T, R185X, L469R, R518X and L554P) by polymerase chain reaction and restriction site assays. The patients presented cafe-au-lait spots and congenital abnormalities, such as microcephaly, microphthalmia and renal ectopia at physical examination. Our observations bring further support to the specificity of -7 and +8 to myeloid malignant hematopoiesis. Our data suggest the existence of an abnormal mechanism in chromosome segregation during mitosis leading to different aneuploidies. Among these, -7 and +8 with or without additional aneuploidies may be most commonly retained due to their proliferative advantage. Our observations bring further support to the specificity of -7 and +8 to myeloid malignant hematopoiesis. Additional patients need, however, to be studied in order to define the diagnostic and prognostic significance of -7 and +8 further.

PO-0404 Genetic alterations in primary multiple myeloma and cell lines: a comparative genomic hybridisation study

Wong Ng,* Ng M, Lee SW,* Zhong N, Lau A,* Lee J

Departments of *Clinical Oncology, and Anatomical and Cellular Pathology, The Chinese University of Hong Kong, SAR Hong Kong, China

Conventional cytogenetic analysis can provide an overall view on the numerical and structural abnormalities present. Karyotypic investigation in multiple myeloma (MM), however, has received much less attention than in other hematological neoplasms. Karyotypic investigation in multiple myeloma (MM), however, has received much less attention than in other hematological neoplasms. The chromosomes most frequently involved were on chromosomes 7, 8q, 9q and 1q, while common losses were identified on 13q, 6q, 17p and 1q. Recurrent copy number changes identified in the Chinese MM were similar to those reported from the West suggesting the absence of ethnic difference in the genetic alterations involved in MM. Also, recurring genetic aberrations identified can provide entry points for further molecular investigations of gene(s) residing in these chromosomal regions in the pathogenesis of MM.

PO-0406 Peripheral T-cell neoplasms. A cytogenetic study of 11 cases


Laboratori de Cytologia Hematològica, Lab. Ref Catalunya Unitat d’Hematologia Hospital del Mar,Espirita, IMIM, Barcelona, Spain

Peripheral T-cell neoplasms are malignant T-cell lymphoid disorders that, as described in the REAL classification, comprise among other entities, T-cell chronic lymphoproliferative leukemia/T-lymphoproliferative leukemia (T-CLL/T-PLL), large granular lymphocyte leukemia (LGLL), T-cell and NK-types of mycosis fungoides/Sézary syndrome (MF/SS). Cytogenetic studies are scarce because of the low incidence of these pathologies. We report the results of peripheral blood cytogenetic studies in eleven cases of peripheral T-cell neoplasms using conventional cytogenetic techniques. Some analytical and clinical data are summarised in the following table.

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<thead>
<tr>
<th>N Diagnosis</th>
<th>Age</th>
<th>Sex</th>
<th>Sex x10^9/L</th>
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<th>Atypical</th>
<th>Karyotype</th>
<th>Implicated</th>
<th>Chromosomes</th>
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<tr>
<td>T-CLL/PLL</td>
<td>42-M</td>
<td>12.2</td>
<td>70</td>
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<td>T-CLL/PLL</td>
<td>94-F</td>
<td>13.4</td>
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<td>T-CLL/PLL</td>
<td>89-F</td>
<td>24.1</td>
<td>73</td>
<td>X6,7,8,10,11,12,14,15,17,21,22</td>
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<td>T-CLL/PLL</td>
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<td>58-M</td>
<td>-</td>
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<td>58-M</td>
<td>6.5</td>
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Chromosomal analyses were performed using standard procedures on peripheral blood from a 72 hour-culture using PHA mitogen. All patients had analyzable metaphases and WBC 1x10^9/L. Aneuploidies were defined cut-off values (2.6% for -7 and 0.4% for +8). I-FISH not only confirmed the presence of -7 and +8, but also revealed that of additional aneuploidies (see Table). In 3 patients, -7 and +8 were associated with other aneuploidies. This second mutant allele was screened for by single strand conformation polymorphism (SSCP) and sequencing. The results of this study point to a molecular heterogeneity of FA in our region.
PO-0407 Cytogenetic findings in four cases of plasma cell leukaemia

Laboratori de Citologia Hematològica Lab Ref Catalunya Unitat d’Hematología Hospital del Mar Expeñació, IMIM, Hospital Sant Camil, Barcelona, Spain

Plasma cell leukaemia (PCL) is a rare malignant plasma cell disorder that is characterised by the presence of more than 2 × 10^9 plasma cells/L in the peripheral blood. The prognosis in PCL is poor, with a median survival range of 2 to 7 months. Cytogenetic studies are scarce and difficult because of the low proliferation rate of plasma cells. Herein we report the results of the chromosomal study using conventional cytogenetics (CC) and interphase FISH on peripheral blood of four patients with PCL. The analytical and clinical data are summarised in the following table:

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Hb (g/L)</th>
<th>WBC (10^9/L)</th>
<th>Platelet (10^9/L)</th>
<th>PC%</th>
<th>Type</th>
<th>Survival</th>
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<tbody>
<tr>
<td>1</td>
<td>68 M</td>
<td>9.7</td>
<td>3.8</td>
<td>30/60</td>
<td>IgG</td>
<td>8 weeks</td>
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<tr>
<td>2</td>
<td>83 M</td>
<td>12 × 10^9</td>
<td>103</td>
<td>24/54</td>
<td>IgG</td>
<td>6 weeks</td>
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<td>8.6 × 10^9</td>
<td>15</td>
<td>34/65</td>
<td>IgG</td>
<td>12 months</td>
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<td>10.3 × 10^9</td>
<td>107</td>
<td>26/80</td>
<td>IgG</td>
<td>15 days</td>
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</table>

PO-0408 The complex karyotype of the U937-1 cell line contains cryptic events as assessed by CGH and FISH analyses

Matucci G, Crescenzi B, La Starza R, Falzetti F, Falzetti D, Martelli MF, Mucciacci C
Hematology and Bone Marrow Transplantation Unit, University of Perugia, Italy

The human haematopoietic cell line U937, obtained from a patient with generalised haematocytic lymphoma, is an established in vitro model for biological studies in leukaemic cells. Karyotypic analyses showed considerable variation in different sublines, although at least four marker chromosomes (1q, 11q, 16p, and 17p) were recurrent. FISH investigations on the so-called 11q- chromosome showed a hidden t(10;11) translocation from which a novel karyotype could be deduced. We tested CGH and FISH for full understanding of the chromosomal events underlying the complex karyotype of the subline classified as U937-1, according to the cytogenetic classification by Shipley et al. (Cancer Genet Cytogenet 1988; 30:277). For CGH experiments, tumour DNA was directly labelled with FITC and reference DNA with Texas-Red. After 48-hour hybridisation at 37°C, preparations were washed several times. Metaphases were analysed under a fluorescence microscope (Olympus equipped with a CCD camera (Photometrics) and an automated system (Vysis). Chromosome painting was performed with probes from libraries for chromosomes 1, 2, 6, 12 and 13. Results. At CGH gain at chromosomes 1p, 1q, 2q, 13q, 15q, 19, and 20 and loss at chromosome 4p, 9p were observed. In addition, chromosome 6p, at band 6p21p23.3, showed high level amplification. Painting for chromosome 1 labelled normal #1, a der(1), part of a der(3), the distal part of a der(5), and part of a der(13). Painting for chromosome 2 marked the normal #2, a der(2), part of a der(6), and an acrocentric marker chromosome. Painting for chromosome 3 labelled normal #3, a der(6), and a der(12)(6;12). Painting for chromosome 4 marked the normal #4, a der(12)(6;12), and a little marker chromosome. Painting for chromosome 13 marked the normal #13 and two different der(13). Complementary CGH and FISH experiments provided us with new insights to uncover the genomic events occurring in the complex karyotype of the leukaemic U937 cell line.

PO-0409 FISH characterisation of a t(12;17)(P13;Q12) in adult pro-B acute lymphoblastic leukaemia

La Starza R, Aventin A, Boqué C, Mrynén P, Martelli MF, Mucciacci C
Hematology and Bone Marrow Transplantation Unit, University of Perugia, Italy; Hematology Dept, Hospital Sant Pau and Institut Catala d’Onco, Barcelona, Spain; Center for Human Genetics and Flanders Interuniversity Institute for Biotechnology, Leuven, Belgium

Chromosome 12p rearrangements are consistent anomalies in ALL. A t(12;21)(p13;q22) involving the ETV6 gene on 12p13 has been identified by FISH studies and has been associated with a distinct subgroup of pro-B ALL with good prognosis. Based on cytogenetics a reciprocal t(12;17)(p13;q12) in early pre-B ALL is known in children (Krance RA et al., Leukaemia 1992; 6:251-5). We identified two adult cases of pro-B ALL with a t(12;17)(p13;q12). Patient #1 was a 25-year-old male, with the following karyotype: 45,X,-Y, t(12;17)(p13;q12)/46,XY. He underwent chemotherapy and autologous bone marrow transplantation. He was in complete remission 30 months after diagnosis. Patient #2 was a 46-year-old female, her karyotype was: 46,XX,t(12;17)(p13;q12)/46,XX, del(6)(q21), t(12;17)(p13;q21). She received a HLA-identical allogeneic peripheral stem cell transplant and maintained complete remission at least follow-up (>12 months). Karyotypic anomalies consistent with the breakpoint at a molecular level, the 12p13 region was investigated by FISH with 15 cosmids previously mapped at band 12p13 as follows: telomere-147H2-218E4-122H1-173G9-132B1-353F2-147G1-158D5-143C4-51B1-191G25-194E6-132E13(ETV6) -123F18. In all experiments a centromeric probe for chromosome 12 (pRB12) was added. At least 4 abnormal metaphases were analysed for each probe using an automated system (Vysis). ETV6 and P13 deletion were outside ETV6 in both cases. In patient #1 cosmider 147G1 was split originating two signals in both 12p and 17q. In patient #2 the breakpoint at 12p2 was flanked by cosmids 147G1 and 158D5. We conclude that the t(12;17)(p13;q21), typical for a subgroup of pro-B adult ALL, underlies a molecular lesion familial to the ETV6 gene.

PO-0410 A new BCR/ABL transcript in a patient with acute leukaemia

Hospital La Paz Madrid, Spain

We describe a 30 year old male patient referred by the Department of Maxillofacial Surgery due to the presence of blastic cells in a gum and right supravacular lymphonode samples. Biopsy and bone marrow aspirate were requested. Results. We saw 90% of blastic cells with CD19, CD20, CD22, CD7, CD3, CD5, CD4, CD8, CD10, CD19, CD31, CD34, CD13, CD14, and c-myc gene amplification simultaneously in one patient. We also performed a CGH and FISH for full understanding of the chromosomal anomalies in this patient. Results. We detected the t(14;18)(b22/1q) translocation, and the t(9;22)(bcr/abl) translocation. Moreover, two types of bcr abl transscripts were detected: the e1a2 transcript that gives rise to a 190 KDa protein, and an aberrant fusion transcript e1a2 with an 155bp b ABL intron at the point of fusion, but because of stop codons in this region no protein is encoded. The bone marrow biopsy and a sample of peripheral blood smears were compatible with acute lymphoblastic leukaemia B and T phenotype. The patient was treated with BFM protocol and re-evaluated 15 days later. There were 33% blastic cells, with the same morphology and immunophenotype. When examining PCR results we saw an increase in b22/1q expression and a small decrease in bcr/abl expression. The treatment was changed to LSA-L2 protocol, and we no longer detected bcr abl expression, but saw an increase in b22/1q expression. The number of blastic cells increased to 90%, with the same immunophenotype, but with a Burkitt-like morphology. At this time we were able to detect a c-myc gene amplification, by FISH. The patient died one month later. Comments. The most interesting thing in this case is the detection of t(9;22) and t(14;18) translocations, and c-myc gene amplification simultaneously in one patient. We also described a new type of bcr/abl transcript.

PO-0411 ETVI/ABL fusion in atypical chronic myeloid leukaemia due to an insertion of 12p material into 9q34

*Department of Medical Genetics and *Department of Haematology, University Hospital Ghent, Ghent, Belgium, and INSEM U 119, Marseille, France

The great majority of chronic myeloid leukaemias are characterised by the activation of the ABL gene due to fusion with the BCR gene, resulting in a
protein with an increased tyrosine kinase activity, which is believed to be the critical factor in the leukemogenic process of Ph positive leukemias. Recently, a novel activation of ABL due to its fusion with the amino-termini
cal sequences of ETV6, normally located on 12p13, was described. Thus far, only single cases of ALL, AML, and CML have been reported. This may be partly explained by the low incidence of this gene fusion, but is proba
ably also due to the fact that the 9;12 translocation is difficult or impossible
to detect by conventional karyotypic analysis. Here we report the find
ing of an ETV6/ABL fusion in a 59 year old patient with atypical CML. karyotyping of peripheral blood at diagnosis showed extra material on the long arm of chromosome 9 and a small deletion of the short arm of chro
mosome 12. FISH analysis demonstrated translocation of chromosome 12 material to distal 9q cohybridisation of the BCR/ABL D-FISH probe (Oncor) with a YAC covering the 9p13.3 region, revealed a unique fusion sig
nal on the der(9) and no fusion on distal 12p, indicating that the ABL/ETV6 fusion transcript was demonstrated by RT-PCR: cloning and sequencing of the amplification product revealed that the first 3 exons of ETV6 were fused in frame to ABL exon 2. We thus identified an insertion as a novel chro
mosomal mechanism leading to ETV6/ABL fusion. FISH and/or RT-PCR with appropriate combinations of probes and primers, respectively, should help investigate the actual frequency of this ETV6/ABL fusion gene in BCR/ABL negative CML.

PO-0412 Comparative genomic hybridisation of childhood hyperdiploid acute lymphoblastic leukaemia

Armengol G,* Alvarez Y,* Oteiga M,* Perez A,* Bastida P,* Ortega J.\nColl MD,* Caballin MA\n*Unidad de Antropologia and °Servei de Hematologia Infantil; Nitat de transplantament de medulla osa, Hospital Materno-infantil "Vall de He-
bron"; Barcelona;*Unidad de Biologia Celular; Facultat de Ciencies, Uni-
versitat Autonoma de Barcelona, Barcelona; Spain

Objective. Classical cytogenetic analysis of childhood acute lymphoblastic leukaemia (ALL) is often difficult due to the poor in vitro growth of the malignant cells and suboptimal quality of metaphase spreads. We used comparative genomic hybridisation (CGH) to investigate the genomic abnor
malities in 12 patients with hyperdiploid ALL. We also aimed to correlate these results with clinical findings. Results. Clinical data were available for all cases. All of them were in 1st complete remission except one patient who was in 2nd remission and one patient who died of the disease. Hybridiza
tions were performed according to Kallioniemi et al. (1) with minor modi
fications. Regional or whole chromosome gains were much more frequent than losses (82 gains vs 9 losses). The most common gains were chro
mosomes 21 (9 cases), chromosomes 10 and 14 (8 cases each), chro
mosomes 6 and 18 (7 cases each), chromosomes 1, 17, and X (6 cases each). Partial gains of long arm of chromosome 1 were observed in five cases. Conclusions. CGH was able to detect abnormalities not detect
ed by cytogenetic analysis. For example, the partial gains of 1q could rep
resent structural aberrations. These gains did not show any correlation with poor prognosis. In addition, the two cases that were not in 1st complete remission were the only cases to have deletions of genetic material. Our results suggest that losses of chromosomal material on childhood hyper
diploid ALL may be a prognostic factor and that structural aberrations on chromosone 1 are not related to poor prognosis. This study was supported by the "Fondo de Investigaciones Sanitarias", no. 98-14111.

PO-0413 MLL gene arrangement in five children with acute leukaemia by molecular cytogenetic analysis

Alvarez Y,* Perez A,° Caballin MR,* Bastida P,* Ortega JJ,* Coll MD °*Unidad de Antropologia; °Servei de Hematologia Infantil; Unidad de Transplantes de medula osa, Hospital Materno-Infantil "Vall de Hebron"
Barcelona;*Unidad de Biologia Celular, Facultat de Ciencies, Universi-
dad Autonoma de Barcelona, Barcelona, Spain

Objective. The MLL gene is interrupted in the majority of translocations involving chromosome band 11q23. Alterations in this gene are reported in approximately 5-10% of acute leukemias and characterise different leukemic subtypes. The MLL gene presents a great heterogeneity in reor
ganization. Frequently cytogenetic study is difficult and other techniques such as FISH are required. We analyzed reorganisation in 5 cases of childhood acute leukaemia relating it with the outcome. Design and Results. We performed cytogenetic analysis and FISH using the MLL gene DNA probe (UNCOR).
Design and Methods. From patients entered into a stem cell transplantation protocol, tumour specific triple or quadruple combinations of cell surface markers are established in in novo material in order to be able to detect MRD at a later stage. Whenever possible, MRD cells are isolated by FACSorting from leukaemia material (LM). These cells are functionally tested. The fluorescence intensity shift obtained with Syto16® + PSC833 indicates Pgp activity (2). Results. In 85% of the patients (n = 32 of 38), a leukaemic specific phenotype could be established which was in 73% (38 of 52) of the cases included CD34. From 21 patients in complete remission LM was obtained. In 19 out of the 21 cases MRD cells could be detected using the tumour specific combinations. Percentages of MRD varied largely in these samples. In 14/19 cases the leukaemic specific phenotype was a combination including CD34 in the LM of these patients a median of 0-20% (range 0-9.9) of total cells had the malignant phenotype. In four cases we FACSorted both the malignant and the normal CD34 positive cells out of the LM. Syto16 fluorescence shift in the sorted normal CD34 positive cell fraction was 19, 4.8, 10 and 33, which is similar to unsorted normal CD34 positive cells. The fluorescence shift had no deleterious effect on the function of the cell. Lower Pgp activity was found thus far in each of the corresponding malignant cell fractions. The fluorescence shift was 6.7%, 39%, 55% and 77% of the corresponding normal cells. Conclusions. We conclude that these studies show the feasibility of functional studies on MRD cells present in LM obtained in complete remission. We anticipate that the correlations of functional and phenotypic parameters of subsets at different stages of the disease with clinical parameters in the AML patients will lead to a better understanding of cell killing in AML.


*PO-0416 Real time quantitative reverse transcriptase polymerase chain reaction of cytokeratin-19 mRNA for the detection of breast carcinoma micrometastasis in bone and marrow


*Laboratory of Experimental Oncology, *Experimental Laboratory Medicine, *Department of Senology, K.U. Leuven, Belgium

Detection of occult carcinoma cells in patients with breast cancer has been shown to predict disease recurrence and metastasis. To improve on existing parameters of subsets at different phases of the disease with clinical parameters in the AML patients, we FACSorted both the malignant and the normal CD34 positive cells out of the LM. Syto16 fluorescence shift in the sorted normal CD34 positive cell fraction was 19, 4.8, 10 and 33, which is similar to unsorted normal CD34 positive cells. The fluorescence shift had no deleterious effect on the function of the cell. Lower Pgp activity was found thus far in each of the corresponding malignant cell fractions. The fluorescence shift was 6.7%, 39%, 55% and 77% of the corresponding normal cells. Conclusions. We conclude that these studies show the feasibility of functional studies on MRD cells present in LM obtained in complete remission. We anticipate that the correlations of functional and phenotypic parameters of subsets at different stages of the disease with clinical parameters in the AML patients will lead to a better understanding of cell killing in AML.


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The significance of reinfusing contaminating tumour cells, and their subsequent contribution to relapse is currently unknown. Detection of MRD by polymerase chain reaction (PCR) in autologous haematopoietic progenitor cell collections and in peripheral blood (PB) and bone marrow (BM) samples following autografting may have clinical significance. The majority of B-lineage lymphoid malignancies feature clonal rearrangements of the immunoglobulin heavy chain (IgH) gene while the t(14;18) translocation is often found in NHL. This study investigates the feasibility and possible prognostic significance of the use of PCR detection of such molecular markers of MRD in autologous haematopoietic progenitor cell collections and subsequent serial PB and BM samples following autografting. The feasibility of MRD was evaluated in 16 PB stem cell (PBSC) and 4 BM collections from 16 patients with NHL; longitudinal PB and BM samples were also analysed following autologous Tx.

PO-0422 Minimal residual disease (MRD) in non-Hodgkin's lymphoma (NHL) treated with autologous bone marrow transplantation

Coles-Sinclair MF, Mesino NM, Kapuscinski M, Schwarzer AP
Alfred Hospital, Melbourne, Australia

The significance of reinfusing contaminating tumour cells, and their subsequent contribution to relapse is currently unknown. Detection of MRD by polymerase chain reaction (PCR) in autologous haematopoietic progenitor cell collections and in peripheral blood (PB) and bone marrow (BM) samples following autografting may have clinical significance. The majority of B-lineage lymphoid malignancies feature clonal rearrangements of the immunoglobulin heavy chain (IgH) gene while the t(14;18) translocation is often found in NHL. This study investigates the feasibility and possible prognostic significance of the use of PCR detection of such molecular markers of MRD in autologous haematopoietic progenitor cell collections and subsequent serial PB and BM samples following autografting. The feasibility of MRD was evaluated in 16 PB stem cell (PBSC) and 4 BM collections from 16 patients with NHL; longitudinal PB and BM samples were also analysed following autologous Tx.

PO-0421 Is the evaluation of minimal residual disease useful in the therapy of low grade NHL? A single center experience

Haematology Department and *Pathology Service, General Hospital, Venice-Mestre, Italy

The aim of the study was the evaluation of MDR in low grade NHL at diagnosis, after conventional therapy, high dose therapy and ABMT. From January 1996 to October 1998 we evaluated minimal residual disease (MDR) with PCR analysis, through determination of IgH monoclonal rearrangement using the combination of FRA and FR3A methods. The analysis was performed on lymphoid and non-lymphoid tissues (lymph nodes, liver, marrow biopsy) peripheral blood (PB), and bone marrow (BM). The analysis was performed at diagnosis in 35 patients (pts); 28 male, 27 female, median age 60 yrs, with low grade B-cell lymphoid malignancies (NHL), stage I-Iv. Thirty pts had follicular lymphoma (FL), 10 lymphocytic lymphoma (B-CLL), 3 mantle cell lymphoma (MCL), 4 hairy-cell leukemia (HCL) and 8 marginal zone cell lymphoma and immunocyteoma. In 29 pts (15 male, 14 female, median age 60 yrs, 20 pts with FL, 4 with B-CLL, and 5 with MCL and other lymphomas, in different stages of disease) the analysis was performed at diagnosis in all the three districts. In 15 pts it was performed after conventional therapy and in 8 pts also after high dose therapy and ABMT. Results at diagnosis. Considering one of the districts in 59 pts IgH positivity was: 72% in all cases, 73% in FL and 80% in non-FL. In 29 pts IgH positivity was considered, and in the three districts, in all cases IgH positivity in tissue, BM and PB was respectively 79%, 69%, and 59%. In FL the IgH positivity in BM and PB was respectively 60%, and 50%, while in non-FL it was 100% in BM, and 75% in PB. After conventional therapy (15 pts). IgH positivity in BM was 26%, and 40% in PB. Considering only non-transplanted pts, IgH positivity both in BM and PB was 20%. In ABMT pts (3 with B-CLL, and 5 with FL) IgH positivity at diagnosis in tissue, BM, and PB was respectively 93%, 62%, and 43%. Six months after ABMT it was 25% both in BM and PB. Conclusions. The IgH expression was more frequent in BM than for FL. PB PCR seems substantially to be as precise as BM, in particular after therapy. Conventional therapy induces a significantly high molecular response. High dose therapy and ABMT induces a molecular response in 75% of pts. It is not clear whether patients have monitored by an early marker for relapse after conventional therapy, but this is even less clear after high dose therapy and ABMT.

PO-0423 Quantification of Wilms' tumour gene (WT1) transcripts to monitor human leukemias

Kruzer KA, Lass U, Sabarowski A, Ellerbrock H,* Pauli G,* Siegert W,
Huhn D, Schmidt CA
Dept. of Medicine, Div of Hematology/ONcology, Charité-Virchow-Klinikum, Berlin, Germany; *Dpt. of Virology Robert Koch-Institute, Berlin, Germany

It is suggested that RNA expression levels of the Wilms' tumour gene (WT1) may serve as a potent diagnostic tool to monitor acute and chronic leukemias. However, methodological difficulties have lead to conflicting
results and WT1 analyses are far from being routinely performed.

We have developed a new method which is easy and quick to perform and allows the real-time measurement of WT1 transcripts in clinical samples. The assay could correlate with previous characteristics such as clinical stage or cellular harvest in apheresis. However, there is a statistical trend to poorer cytopheresis correlation with previous characteristics such as clinical stage or cellular harvest in apheresis. However, there is a statistical trend to poorer cytopheresis treatment compared to conventional methods. Nevertheless, the significance or these findings remain unknown. Design and Methods. In order to determine the correlation of different clinical features with the presence of neoplastic cells in BM and apheresis harvests, we analysed 58 patients diagnosed of bone cancer (161 cases) with unmanipulated peripheral blood stem cell transplantation (PBSCT) in our Unit from July 95 to October 98. Bone marrow biopsy through was obtained in all cases prior to PBSCT. It was processed either with conventional histological and cytological techniques as well as with immunocytochemical methods: alkaline phosphatase anti-alkaline phosphatase (APAAP). The monoclonal antibody used was anti-CK19. Accuracy of laboratory techniques was tested with positive controls (cancer cell lines), negative and reactive controls. Apheresis products were assayed for APAAP method. Results. Twenty seven (46%) of the BM samples were considered APAAP+, while only 4 (7%) of the aphereses were positive. Relationships between bone marrow APAAP+ and other studied characteristics are shown in table.

Conclusions. The finding of APAAP+ tumour cells in BM samples does not correlate with previous characteristics such as clinical stage or cellular harvest in apheresis. However, there is a statistical trend to poorer cytopheresis recovery in patients with APAAP+ cells in BM. Supported by Grant GV2520/96 from the Generalitat Valenciana. Supported by Grant GV2520/96 from the Generalitat Valenciana. Supported by Grant GV2520/96 from the Generalitat Valenciana.

PO-0424 Cytokeratin + cells of breast cancer patients in bone marrow and apheresis samples before autologous stem cell transplantation


Fundación Instituto Valenciano de Oncología, Valencia, Spain

Immunocytochemical techniques for epithelial cell detection both in bone marrow (BM) and apheresis samples have demonstrated greater sensitivity than conventional methods. Nevertheless, the significance of these findings remains unknown. Design and Methods. In order to determine the correlation of different clinical features with the presence of neoplastic cells in BM and apheresis harvests, we analysed 58 patients diagnosed of bone cancer (161 cases) with unmanipulated peripheral blood stem cell transplantation (PBSCT) in our Unit from July 95 to October 98. Bone marrow biopsy through was obtained in all cases prior to PBSCT. It was processed either with conventional histological and cytological techniques as well as with immunocytochemical methods: alkaline phosphatase anti-alkaline phosphatase (APAAP). The monoclonal antibody used was anti-CK19. Accuracy of laboratory techniques was tested with positive controls (cancer cell lines), negative and reactive controls. Apheresis products were assayed for APAAP method. Results. Twenty seven (46%) of the BM samples were considered APAAP+, while only 4 (7%) of the aphereses were positive. Relationships between bone marrow APAAP+ and other studied characteristics are shown in table. 

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PO-0425 Monitoring of BCR-ABL expression using real-time RT-PCR in CML after bone marrow, or peripheral blood stem cell transplantation

Eder M, Battke R, Kukert S, Stucki A, Geiser A, Hertenstein B
Hannover Medical School, Dept. of Hematology and Oncology, Germany

To analyse the value of real time RT-PCR for monitoring of bcr-abl expression in CML patients after allogeneic or autologous transplantation we generated pairs of PCR-primer and Taqman-probes specific for either the b2a2- or the b3a2-variant of bcr-abl. Either variant could be detected specifically from cDNA from one K562 (b3a2)- and BV173 (b2a2)-cell with the respective Taqman-probe Bcr- abl expression was normalised by comparison with GAPDH-expression, and samples were evaluated using standard cDNA-dilutions from K562 or BV173 cells for both GAPDH- and bcr-abl expression. In a retrospective analysis 13 patients with CML after allo-
while showing bcr-abl positivity and MC in the CD15-positive population. The other three cases presented decreasing or stable low-level MC, bcr-abl negativity and MC was restricted to the F-cells, being myeloid cells of donor origin. Whereas the simple detection of bcr-abl fails to identify relapses with certainty, the assessment of MC by VNTR-PCR focuses on patients headed to relapse. Confirmation of myeloid involvement and increasing levels in time further elucidates the clinical outcome. For bcr-abl positive patients after BMT. Interestingly, host cells could be detected between 3-6 months after cytogenic relapse. This time interval is of crucial importance to make decisions and probably, adoptive immunotherapy with donor leucocytes would then be given to patients who present increasing myeloid mixed chimaerism in order to achieve molecular remission.

PO-0428 Morphological evaluation of residual disease in bone marrow (BM) samples in complete remission in AML patients after induction therapy

Valleigil T, Vidriales MB,* Gonzalez-Medina I, Cañizo MC,* Orfao A,* Martinez A,* Sanchez-Monata C, San Miguel JF,* Servicio de HematoLOGia, Hospital General Vall d’Hebron, Barcelona;* Servicio de Hematologia, Hospital Universitario de Salamanca, Spain

Objective. In spite of the high number of complete remissions (CR) achieved in patients with acute myeloid leukaemia (AML), a high proportion of cases will eventually relapse due to the persistence of undetectable minimal residual disease (MRD). Among the techniques suitable for MRD detection, immunophenotyping and molecular biology are the most commonly used. Theoretically, morphology is not a valuable technique for MRD investigation since the detection limit is in the range of 10^-4. However, there is little information about the value of screening a high number of BM cells for morphological identification of residual blast cells as well as the usefulness of investigating other morphological features such as the presence of dysplastic characteristics in the remission BM. The aims of the present study are to evaluate: a) the correlation between residual disease evaluated by optical morphology and by immunophenotyping assessed by flow cytometry and b) to analyse the possible influence on patients’ outcome or the presence of features in complete remission bone marrow after induction therapy. Design and Methods. Diagnosis and CR BM after induction therapy or CR 37 de novo AML patients were analysed. After induction therapy, percentage or blast cells (or atypical cells, abnormal promyeloctyes or faggots cells, in M3 AML cases) and myelodysplastic features were evaluated by optical morphology. The number of cells analysed ranged between 200 and 1000 depending on the marrow cellularity. The immunophenotypic investigation was simultaneously performed by multispot accessory flow cytometry and by screening the HS-2 region in morphological CR after induction therapy displayed a significantly (p=0.03) lower overall survival than patients without dysplastic features. Conclusions. Morphology is not a valid technique to evaluate MRD. However, morphologic assessment of mature cells in complete remission BM after induction therapy shows interesting aspects, such as dysplastic features, that can have prognostic influence.

PO-0429 A new advantageous, molecular method to evaluate the ligand rearrangement

Galimberti S, Brizzi F, Marnelli M,* Petrini M Oncology Department, Haematology Division, University of Pisa;*Centro interdipartimentale di ricerca di genetica molecolare e clinica, University of Pisa, Italy

The important role of minimal residual disease (MRD) and its detection by advantageous, molecular methods is now well accepted. We present here a new sensitive, specific, safe, rapid and cheap molecular method to detect the monoclonal ligand rearrangement. This technique consists of fluorescent polymerase chain reaction (PCR), with primers for consensus CDR3 and the monoclonal IgH rearrangement. This technique consists of fluorescent polymerase chain reaction (PCR), with primers for consensus CDR3 and framework) are always concordant: either Benin-like or Bantu-like. In all the 9 cases in which the LCR repeat-sequence sizes were discrepant, the analysis was extended to other polymorphic markers of the β-gene cluster, including 7-site -ε-δ-μ-clus-ter and pre-γ-flanking, H-S2 LCR(AT)xR(AT)y and pre-β (AT/β) repeats sequencing, the pre-γ 6-bp deletion, the GC/TT polymorphism at -1105-1106 of γ-δ, the C/T polymorphism at -451 of β and the intronic β-frame-work. Results. In all 9 cases in which the LCR repeat-sequence sizes were discrepant a recombination involving a typical 3' segment of the β-gene cluster was demonstrated. The recombination site was at the 6-bp hotspot or between the E-gene and the LCR, so that the usual RFLP haplotyping gives a typical result. The markers within and near the β-gene (Real site, ATβ) and framework) are always concordant: either Benin-like or Bantu-like. Conclusions. Thus, 5.8% of apparently typical haplotypes involve recombinations. This finding reinforces the picture of the β-globin gene cluster as highly dynamic, and increases the percentage of uncommon haplotypes in this population of sickle cell haemoglobinotypes from 3.9 to 8.7%.

Poster Discussions Haemoglobinopathies and thalassaemia

PO-0430 Rearrangements involving apparently typical β-haplotypes

Zapp MA, Gualandro S, Silva Jr. WA, Yokomizu IK, Araujo AG, Tavela MH Department of Clinical Medicine and Regional Blood Center, Faculty of Medicine of Ribeirao Preto, and Department of Clinical Medicine, Faculty of Medicine, University of Sao Paulo, Brazil

Objective. The majority of chromosomes with the β-gene have one of five common haplotypes, and about 5% of the cases have less common haplotypes, usually referred to as atypical haplotypes. We have demonstrated that most atypical haplotypes are generated by recombinations. The present study was carried out in order to explore whether recombination also occurs in chromosomes with the common haplotypes. Design and Methods. We analyzed 136 sickle cell patients who have typical haplotypes of the β-gene cluster as demonstrated by RFLP, by screening the H-S2 region of the LCR. For the cases in which the expected and the observed LCR repeat-sequence sizes were discrepant, the analysis was extended to other polymorphic markers of the β-gene cluster, including 7-site -ε-δ-μ cluster and pre-γ-flanking, H-S2 LCR(AT)xR(AT)y and pre-β (AT/β) repeats sequencing, the pre-γ 6-bp deletion, the GC/TT polymorphism at -1105-1106 of γ-δ, the C/T polymorphism at -451 of β and the intronic β-framework. Results. In all 9 cases in which the LCR repeat-sequence sizes were discrepant a recombination involving a typical 3' segment of the β-gene cluster was demonstrated. The recombination site was at the 6-bp hotspot or between the E-gene and the LCR, so that the usual RFLP haplotyping gives a typical result. The markers within and near the β-gene (Real site, ATβ) and framework) are always concordant: either Benin-like or Bantu-like. Conclusions. Thus, 5.8% of apparently typical haplotypes involve recombinations. This finding reinforces the picture of the β-globin gene cluster as highly dynamic, and increases the percentage of uncommon haplotypes in this population of sickle cell haemoglobinotypes from 3.9 to 8.7%.

PO-0431 Factors involved in osteoporosis in β-thalassaemia patients

Kyrtosisis MC,* Voskaridou E,* Skordilj M,* Perigele A,* Terpos E,* Diamandi E,* Palermos,† Kalividou L,* Loutradi A,* Loukopoulou D* †Unit of Thalassaemia, University of Athens; ‡Dept of Radiology; ‡Dept of Radiology, #4251 Air Force General Hospital, Athens, Greece

Osteoporosis in patients (pts) with β-thalassaemia (thal) is multifactorial including microvascular expansion, haemochromatosis, suppression of osteoblastic activity by deferoxamine, hypogonadism or other endocrinological problems, vitamin D deficiency. In order to administer an adequate therapy, we determined the above factors in 43 pts (14 M and 29 F, age range: 22-32 y). Of the 43 pts, 13 began being transfused within the first 2 years of age (group 1), 14 started transfusion later (group 2) and 16 were not transfused (group 3). Bone mineral density (BMD) of the lumbar spine
The Hb profile was 2/15 with SS and S-Thal Hh/pathies with Ht 20-22% and Hb 5-6.4 g% were followed throughout gestation. The laboratory data were measured serum bone alkaline phosphatase (BAP), sex hormones, PTH, osteocalcin, serum vitamin D, ferritin, sTfR, Epo, and Hb ALA. No abnormal Hb, haematology, or rheology was found in this study. The successful use of recombinant human erythropoetin in normal and haemoglobinopathic pregnant women was compared.

**PO-0432** The use of recombinant erythropoetin in normal and haemoglobinopathic pregnant women

Antonopoulou M, Makarinos N, Stefanou S, Kosmas C, Rossofimos J

Medical and 4,5th Mat/Gyn Depts, H. Venizelou, Athens, Greece

The successful use of recombinant human erythropoetin (rEPO) in anaemia of chronic renal failure, solid tumours, and anaemia of pregnancy, especially in Sickle cell syndromes, is the most exact and reproducible that we have. The abnormal rheology in sickle cell disease and the optimum level of the Ht during pregnancy is still under discussion. Design and Methods. Ten normal pregnant women and 15 with SS and S-Thal Hh/pathies with Ht 20-22% and Hb 5-6.4 g% were followed throughout gestation. The laboratory data were measured by autoanalyzer techniques. The Hb profile was 2/15 with SS and 1/315 S-Thal haemoglobinopathy in 1st or 2nd pregnancy. The dose of rEPO was 20 000 U i.e. per week 5-6 g% respectively. Every 3 weeks an evaluation of Hb level was done. The results showed a steady increase in the parameters used with an upper limit as shown above. The treatment requirements were restricted to 2/10 normal and 4/15 pregnant women with Hb pathy, but with no adverse effect. An expected increase in prematurity was observed. In conclusion, the treatment with rEPO during pregnancy of certain cases with severe anaemia and reluctance for other reasons, to receive blood transfusion is a safe alternative.

**PO-0433** Hb Sitia or b(128[H6])Ala-Val: a new unstable haemoglobin variant resulting in heterozygous thalassaeinic phenotype


Haematology Laboratory, "Agia Sophia" Children's Hospital, Athens, Greece

Hb Sitia was found in a 30 year old woman of Greek origin (Sitia, Crete) who had haematological indices consistent with heterozygous thalassaemia: Hb 126 g/L, MCV 82.2 fL, MCH 26.1 pg, MCHC 317 g/L, RDW 15.1%, HDW 23.3 g/L, T(technicon-Bayer) and reticulocytes 0.07±0.01. Blood chemistry showed ferritin 16 µg/L, Epo 22 IU/L, (Nichols institute) and STR 4.1 mg/L, (Orion Diagnostica). No abnormal Hb, or Hb-chain was detected in any electrophoretic media but an abnormal peptide which showed a shift up of 28 mU mld was quantified in abnormal Hb. Of the patients groups 1 and 2 pts 80% had hypogonadism; this number was 20% in the patients of group 3. In conclusion, bone destruction is increased in young adults with thalassaemia. Transfusion and/or hypogonadism are the main factors. Bone formation is still active, thus giving hope that adequate therapy (inhibitors of bone destruction and hormonal replacement) may be of benefit.

**PO-0434 Identification of Hb Lepore phenotype through study of globin chains by reverse phase HPLC**

Ropero P, Gonzalez FA, Sanchez J, Asenjo S, Mora A, Arco A, Murga M, Villegas A


Hb Lepore is a structurally abnormal haemoglobin in which the abnormal globin chain is a hybrid- or fused globin chain (bL). Three different Lepore haemoglobins have been identified, differing from each other in the point at which the b6 fusion occurs: Hb Lepore Hollandia (b22/50), Hb Lepore Baltimore (b50/50), and Hb Lepore Boston (b50/50). In Spain only Hb Lepore Boston and Hb Lepore Baltimore have been characterized. Hb Lepore is easily detected by electrophoretic and chromatographic studies, whereas the type of Hb Lepore was identified by chromatography of trypsin peptides of abnormal b6 chain. In this work, we show an easier chromatography technique to identify the Hb Lepore phenotype. Twelve different unrelated families (21 patients) from the central area of Spain were studied. The Hb Lepore was diagnosed by standard methodology. Its quantification by ionic exchange HPLC and the study of globin chains was carried out by reversed phase HPLC, which showed us the phenotype of Hb Lepore: this fact was corroborated by the gold standard tests (Molecular Biology techniques). In the study of globin chains by reversed phase HPLC for twelve patients (7 families), three peaks were eluted; they corresponded with c, b, and g6 globin chains. In those cases when DNA was studied by PCR followed by digestion with the restriction enzyme Pvu II, the phenotype of Hb Lepore wasBoston, whereas in the rest of patients (9 in total), the peak identified with hybrid chain globin (b6) was not present and the molecular study showed that they were patients heterozygote for Hb Lepore Baltimore. Only study of globin chains by reversed phase HPLC will be enough to know the phenotype of Hb Lepore. The use of tedious techniques such as chromatography of trypsin digestion of b6 abnormal chains will not be essential when a laboratory has not molecular biology techniques. In the case of a pregnant woman, however, we use a gold standard test of molecular biology, because this technique is the most exact and reproducible that we have.

This work was supported by Research Grant FISS 97/0212.

**PO-0435 Calcium regulating hormones in thalassaemic children**

Sedrak M, Shanaki O, Assen H

Department of Pediatrics, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Objective. This work was aimed at studying serum levels of osteocalcin, parathormone calcitonin, bone density in addition to other biochemical indices related to bone metabolism in a group of children with b-thalassaemia major. Design. The study included 30 children aged 2-16 years with b-thalassaemia major and 30 healthy children, matched for age and sex as controls. Children who have received vitamin D supplements were excluded. Results. Bone densities were reduced in thalassaemic cases compared to the controls, in addition, haemoglobin levels showed significantly lower levels of parathormone (p<0.001) and lower osteocalcin (p<0.001) than the controls whereas serum Ca and P were normal. Hypo-parathyroidism was detected in 2 thalassaemic cases (6.66%) and had no relation to serum ferritin levels. On the other hand, alkaline phosphatase and calcitonin were higher in thalassaemic children. Conclusions. These findings emphasise the importance of early diagnosis and therapy of osteopenia in thalassaemic children and throw more light on its pathogenesis.
**PO-0436** Aposin in β-thalassaemia major

Assem H, Abdel Halim N*  
Department of Pediatrics, Faculty of Medicine, *Department of Biochemistry, Medical Research Institute, Faculty of Medicine, Alexandria University, Egypt

Objective. To study c-myc oncoprotein in children with β-thalassaemia major and to relate the findings to desferrioxamine chelation therapy. Design and Methods. The study included 30 children with β-thalassaemia major divided into three groups: group 1: children who received regular transfusions and adhered to desferrioxamine chelation therapy; group 2: children who received regular transfusions but no chelation; group 3: children who had irregular transfusions and no supplemenal chelation therapy, and 20 healthy children, matched in age and sex, as controls. Venous blood samples were collected and routine investigations included complete blood picture, serum iron and ferritin. Peripheral mononuclear cells were isolated and assayed for c-myc oncprotein and annexin V. C-myc oncprotein and annexin V were detected by flow cytometry and without or with chelation treatment (p=0.01) and both groups showed lower c-myc than the control group (p<0.05). As regards children receiving irregular transfusions and suboptimal chelation, their mononuclear cells showed intermediate expression of c-myc oncprotein. The results of c-myc oncprotein correlated positively with ferritin levels (p<0.05). Conclusions. Chelation therapy and deprivation of cellular iron is accompanied by down-regulation of c-myc oncprotein and hence increased apoptosis and may affect haemopoietic cell growth and survival.

**PO-0437** Clinicopathological study of the liver in 16 living children with sickle cell disease

Teixeira AL, Viana MB, Roquete MVL, Toppa NH  
Paediatrics and Dept of Pathology, Federal University of Minas Gerais, Hemominas Foundation; Belo Horizonte, Brazil

Background. Hepatic lesions in sickle cell disease are well known but the possibility of chronic persistent lesions of the parenchyma due to the disease itself is controversial. Living children are rarely included in the literature. The aim of the present study is to report a retrospective, viral immunology and histopathological findings in 16 children with sickle cell disease. Design and methods. Fifteen children with SS disease and one with Sβ-thalassaemia aged 13 months to 16 years were prospectively selected from 741 patients below 20 years registered at the Sickle Cell Disease Clinics, Hemominas Foundation, Brazil. Exclusion criteria for biopsy: five bad positive anti-hepatitis C virus (HCV) antibody, confirmed by quantitative polymerase chain reaction; two had positive hepatitis B surface antigen and B surfactant protein A. Hb S and 10 patients with compound heterozygous Sβ-thalassaemia showed significantly lower levels of c-myc oncoprotein than controls. The median progression of hepatitis was 2a and had positive hepatitis B surface antigen and B surfactant protein A.

**Results.** Mononuclear cells from children receiving desferrioxamine showed significantly lower levels of c-myc oncprotein than patients receiving no chelation (p=0.01) and both groups showed lower c-myc than the control group (p<0.05). As regards children receiving irregular transfusions and suboptimal chelation, their mononuclear cells showed intermediate expression of c-myc oncprotein. The results of c-myc oncprotein correlated positively with ferritin levels (p<0.05). Conclusions. Chelation therapy and deprivation of cellular iron is accompanied by down-regulation of c-myc oncprotein and hence increased apoptosis and may affect haemopoietic cell growth and survival.

**PO-0438** Soluble transferrin receptor levels in sickle cell disease patients treated with hydroxyurea

Papastasiou I, Voksiaridou E, Stamatoulakatou A, Loukopoulou D  
Haematology Laboratory "Aghia Sophia" Children’s Hospital; "Thalassaemia Unit “Laikon Hospital”; °First Dept of Medicine, University of Athens, Greece

Administration of hydroxyurea (HU) in sickle cell disease (SCD) is associated with a dramatic increase of fetal haemoglobin (HbF). The increase of HbF is followed by a significant clinical improvement and the patients are able to resume normal life and work. As a result of the improved red cell survival total haemoglobin levels also increase. The elevation of HbF is not

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**PO-0439** An evaluation of cation exchange HPLC as compared to isoelectric focusing for neonatal haemoglobinopathy screening

Campbell M,* Henthorn JS,* Davies SC  
Department of Haematology, Central Middlesex Hospital, London, UK

We report a comparison of isoelectric focusing (IEF) and high performance liquid chromatography (HPLC) for neonatal haemoglobinopathy screening. 25,750 dried neonatal 'Guthrie' blood spots were compared over 15 months in the context of a routine universal haemoglobinopathy programme, screening some 50,000 births annually in London, UK. The detection of haemoglobins A, F, S, C, D, E, and Bart's was as reliable by HPLC as by IEF. Regarding less common abnormalities, one double heterozygote and four heterozygous traits detected by IEF were not observed on HPLC as shown:

<table>
<thead>
<tr>
<th>Chain</th>
<th>IEF</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ</td>
<td>34</td>
<td>24*</td>
</tr>
<tr>
<td>δα</td>
<td>33</td>
<td>31*</td>
</tr>
<tr>
<td>ρβ</td>
<td>17</td>
<td>14*</td>
</tr>
</tbody>
</table>

Total 84 69

* Ten of the gamma chain variants identified by IEF were observed as normal (AIF) by the VARIANT; *2α and 3α variants gave normal HPLC traces.

HPLC has proved reliable for the neonatal screening of common haemoglobinopathies and diseases and thus provides an automated, sensitive, efficient, and timesaving alternative to IEF.

**PO-0440** The need for diagnosis and prevention of haemoglobinopathies in northern Europe: the Dutch situation

Giordano PC, Harteveld CL, Bernini LF  
Dept of Human Genetics, Haemoglobinopathies Laboratory, Leiden University Medical Centre, The Netherlands

Haemoglobinopathies (HbPs) are rare in native North-European populations, however, approximately 10% or more of the population of most industrialised areas of Northern Europe consists of recent immigrants. Many young healthy HbPs carriers live in the Netherlands and will, without precautions, generate a constant group of more than 1000 severely affected patients. Italy, Greece, Cyprus and other Mediterranean countries have applied prevention strategies reducing the birth of children with thalassaemia major to nearly zero. These strategies are socially and psychologically well accepted and rely on information, carrier detection and pre-conceptional counselling. In several Northern-European countries international control programs have been considered but the U.K. is the only country thus far which has reached significant results. Psychological, cultural and ethical arguments are often brought up as an obstacle to the sole mechanism of this change as several other factors may also interfere. Over the past 9 years we have treated a large number of severely symptomatic SCD patients with HU. This study reports the response of 3 patients with HbS, two with compound HbG-Thalassaemia, who have been followed up systematically throughout their therapy, with regards to their soluble transferrin receptors (sTR) levels. The dose of HU was 1.5 g/d x 7 d/w. Regular follow-up included routine CBCs, estimation of reticulocyte production index (RPI) and biochemistry. HbF levels were measured by HPLC (Bio-Rad, Variant, USA). sTR assays were carried out by an immunoenzymatic technique (R&D systems, UK). All measurements were done in duplicate. Each patient had at least 5 sTR determinations throughout the study. The main results of the study are summarised as follows:

<table>
<thead>
<tr>
<th>Hb g/L</th>
<th>sTR mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>At max HbF</td>
</tr>
<tr>
<td>91.4±4.6</td>
<td>9.8±5.4</td>
</tr>
<tr>
<td>31.0±0.7</td>
<td>6.3±2.7</td>
</tr>
<tr>
<td>P &gt; 0.26</td>
<td>P &gt; 0.0001</td>
</tr>
</tbody>
</table>

Baseline sTR levels (at least two determinations prior to entering the study) varied from 3.9 to 10.3 mg/L. These levels are significantly higher than normal levels (1.8±0.7 mg/L). When HbF reached its maximum values, the mean values of sTR and RPI decreased, but this decrease was not significant. These results indicate that patients with SCD show a variable but markedly high degree of erythroid marrow activity. Treatment with HU is expected to reduce this process, in keeping with the improved red cell survival and hence diminished ineffective erythropoesis. The fact that our data do not conclusively support this hypothesis shows that this reduction is not as important and may be blurred by the concomitant stimulated proliferation of erythroid progenitors that carry an enhanced capability for HbF synthesis.
the implementation of prevention strategies. We are convinced that it is rather the technical pitfalls that may be a problem in immigration countries where the population at risk is very heterogeneous. We are experiencing that the main obstacles in offering prevention to couples at risk in the Netherlands are the lack of information and the lack of motivation of the general practitioners. Furthermore extensive and valuable diagnostic material, generated in many clinical laboratories, does not lead to partner family analysis and disease prevention. A coordinated effort of the health care infrastructures is needed for offering prevention to the populations at risk in The Netherlands.

**PO-0441** Molecular spectrum of β-thalassaemia in multiethnic populations: the Iranian province of Hormozgan

Yaghoob M.,* Harteveld CL,* Batelaan D,* Bernini LF,* Giordano PC.*
*The Thalassaemia Medical Centre, Medical Faculty, Bandar Abbas University, Iran; °Dept. of Human Genetics, Haemoglobinopathies Laboratory, Leiden University Medical Centre, The Netherlands

Prevention of β-thalassaemia requires a precise knowledge of the molecular mutations' spectrum occurring in the populations at risk. This especially when a prevention protocol for β-thalassaemia is applied to a multiethnic population. Analysis of 14 different groups of Dutch patients of recent allochthonous derivation, sorted by region of origin, showed the expected diversity between the groups but also differences from the data reported in the literature. We have recently analyzed a group of independent Iranian patients living in the Netherlands and we are now examining a large population of Iranian patients living in Iran in the province of Hormozgan. Our results, on the other hand, may cause, depending on the site of the deletion, from only one or a few nucleotides. If such deletions result in frameshifts, the very slight to a very severe instability of the β-globin polypeptide. In this report we describe a case of Hb-H disease due to the interaction of α-(Med I) haplotype with an α-thalassaemia determinant. The proposed hypothesis is that the delayed reaction was due to iron-load induced organ damage, causing a reduction or absence of elasticity in the interstitial tissue. We therefore believe that treatment with Alprostadil can be considered an effective. Non-invasive therapy for thalassaemic patients with erectile dysfunction. The transurethral application of Alprostadil restores erection and so doing increases the patient's self-confidence, well-being and quality of life, both sexual and social.

**PO-0444** The prevalence of β-thalassaemia traits in Istanbul, Turkey: a preliminary study

Gedikoglu G,* Kiliçtio F, Yaliman N, Anak S, Kökrek A, Can M

Our children leukaemia foundation health center; °Istanbul University, Istanbul School of Medicine, Department of Pediatric Hematology and Oncology, Turkey

**β-thalassaemia is an important health problem in our country as in other Mediterranean countries. The gene frequency for β-thalassaemia approaches 2% in Turkey generally, whereas in some regions, it may rise up to 10%.** We performed our study in Istanbul which belongs to the Marmara region of Turkey. The population is that of a cosmopolitan city. We designed a study for identification of β-thalassaemia traits, in order to give education and genetic information to our population. Between March 1998–December 1998, 608 subjects (139 children, 132 women, 337 men) whose blood was taken for routine β-thalassaemia screening were evaluated in our Children Leukaemia Foundation Medical center. Enzyme indices were determined using the Coulter Gen-S cell counter, HbA2 and HbF were determined by fully automated cation exchange HPLC system (Variant Haemoglobin Testing System, BIORAD). The prevalence of β-thalassaemia traits was found to be 9.8%. This is higher than that found in previous studies performed in Istanbul. As a conclusion, in countries with high risk of haemoglobinopathies such as β-thalassemias, detection of carriers will be helpful for eradication of the disease from future generations.

**PO-0445** Erectile dysfunction in thalassaemic patients. Treatment with transurethral Alprostadil: a pilot study

Lombardo T,* Caruso S,* Giammuto A,* Scillo AL,* D’Arpa S°

*Bambino Gesù Osp. S. Biondo Health Center, °Inst. Sci. Ginecologico e Obstetrico S. Maria Goretti, Catania, Italy

Patients with β-thalassaemia can experience erectile dysfunction. Sexual dysfunction may occur, in most cases subsequent to hypogonadism but sometimes apparently as a primary complaint, with detrimental repercussions on erection and procreation. In this pilot study we considered the possibility of restoring erectile function in four thalassaemic patients by administering E1 prostaglandins (Alprostadil) transurethrally. After checking endocrine conditions and basal Doppler, and following CI of the cavernous arteries, each patient was given 500 mg of Alprostadil in the distal urethra. Response was evaluated by the Erection Assessment Scale (EAS). The treatment produced a response of 3-4 on the EAS. Average minimum response time was 20 minutes, while average maximum response time was about 60 minutes. There was no evidence of significant side effects. Our hypothesis is that the delayed reaction was due to iron-load induced organ damage, causing a reduction or absence of elasticity in the interstitial tissue of the corpora cavernosa. We therefore believe that treatment with Alprostadil can be considered an effective. Non-invasive therapy for thalassaemic patients with erectile dysfunction. The transurethral application of Alprostadil restores erection and so doing increases the patient's self-confidence, well-being and quality of life, both sexual and social.

**PO-0446** Quantitative evaluation of erythrocyte expansion and severity of early apoptosis in β-thalassaemia

Gandolfi E,* Tabellini F, Lucarelli G, Buffi O, Guerra S, Persini B, Torucci P

Divisione di Ematologia Centro trapianto M Idolo O sese, Azienda Ospedaliera, S. Salvador, Pesaro, Italy

**β-thalassaemia is the commonest inherited lethal human disease. It results from a defect in the β-globin synthesis causing ineffective erythropoiesis and reduced red cell survival (apoptosis). In order to characterise and to quantify such phenomena better, we studied the immunophenotype of the whole bone marrow, early apoptosis of the erythroid precursors by Annexin V (AnV) reactivity, soluble transferrin receptor (sTfR) and erythropoietin (EPO) levels in the bone marrow plasma of 40 transfusion-dependent β-thalassaemic patients and 24 comparable normal controls. Strong significant direct correlations between the absolute numbers of CD36+, CD71+, CD45- / AnV+ and sTfR BM plasma levels were observed.**
Gyula; °Central Laboratory, County Hospital, Gyula, and #National Institute homozygous offspring manifesting serious clinical symptoms. of the 2 data in blood formulae provided by automata would be of impor-
in higher reticulocyte values in 20 cases (69%). The parallel appearance examined by means of Abbott CELL-DYN 4000 machines, which resulted the art machines. After the study had been completed, 29 patients were
of separating iron deficiency from thalassaemia. They point out that in case lassaeemia minor proven by Hg-electrophoresis in Hungary in 1997. The
es we diagnosed amounted to 53.4 per cent of the 75 new cases of
of thalassaemia during examinations involving whole families. The 40 cas-
was suspected in 27, each of whom underwent an Hb-electrophoresis
ations (blood smear, reticulocyte, serum bilirubin, LDH, folic acid, cyano-
patients, who were asked to undergo complementary laboratory examina-
2,286 cases. We found the Mentzer index (MCV/RBC) below 13 in 50
"Department of *lntemal Medicine and Haematology, County Hospital, Gyula; °Central Laboratory, County Hospital, Gyula, and #National Institute of Haematology, Budapest, Hungary

In Hungary it is the heterozygous form of ß thalassaemia that occurs spo-
radically. As the exact frequency of occurrence is not known, we initiated a study at our hospital in early 1997. For 1 year we surveyed the results of all the blood picture examinations carried out by means of the Abbott CELL-DYN 3500 haematological automaton in the central laboratory. Pri-
or to the study, there had been only 2 cases of ß thalassaemia minor recorded in our files. Out of a total of 111,051 blood picture examinations performed during the year in question, the MCV value was below 80 fl in 2,286 cases. We found the Mentzer index (MCV/RBC) below 13 in 50 patients, who were asked to undergo complementary laboratory examina-
tions (blood smear, reticulocyte, serum bilirubin, LDH, folic acid, cyano-
cobalamin, Fe, ferritin, haploglobin, osmotic resistance, Coombs' test). 35 did undergo them. Iron deficiency was proven in 8, whereas thalassaemia was
early, after whom an Hb-electrophoresis examination at the National Institute of Haematology, which showed the presence of β thalassaemia minor in each case. We diagnosed 13 cases of thalassaemia during examinations involving whole families. The 40 cas-
es we diagnosed amounted to 33.4 per cent of the 75 new cases of β-tha-
assaemia minor proven by Hb-electrophoresis in Hungary in 1997. The
authors analyse the laboratory data of the patients from the point of view of separating iron deficiency from thalassaemia. They point out that in case of low MCV values the Mentzer index should be determined and highlight the importance of the automatic determination of reticulocytes by state-of-

Poster discussions Apoptosis

**PO-0449 Apoptosis of peripheral blood leucocytes in children surviving acute lymphoblastic leukaemia**

Vladimirskaya E, Kaznatcheev K, Osipova E
Research Institute of Pediatric Hematology, Moscow, Russia

There is enough evidence now that cancer chemotherapy effects tumour cell killing through launching the mechanisms of apoptosis. But tumour cells do not become the unique targets for its cytotoxic action, haemopoietic cells suffer as well. So it is interesting to follow up the late effect of antileukemics chemotherapy on haemopoiesis - the level of spontaneous apoptosis in leucocytes in children surviving ALL Design and Methods. We studied 30 children (4-16 years of age), cured of ALL with full remission duration from 2.5 to 6.5 years, free of therapy for 3 months–6 years. All patients received chemotherapy according to BFM-90 and MB-91 proto-
cols. The control group comprised 20 children with apparently normal haemopoiesis. The level of apoptosis was calculated by flow cytometry as percentages of hypodiploid cells in propidium iodide staining, separately in granulocyte and lymphocyte gates. Results are presented in the table below.

<table>
<thead>
<tr>
<th>N</th>
<th>Free of therapy</th>
<th>Level of apoptosis (Kd±ED)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;1 year</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>1-2 years</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>&gt;2 years</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>20</td>
</tr>
</tbody>
</table>

As seen from the table, there was a clear tendency to decreasing level of apoptosis in both granulocytes and lymphocytes along the duration of the period free of therapy. This became especially evident after 2 years of ther-
apy cessation (t2=2.2, p<0.05). But even in the third group (free of ther-
apy more than 2 years) the mean values of apoptotic granulocytes and lym-
phocytes appeared to be significantly higher than in control group (p<0.001). Regression analysis shows the negative correlation between the value of apoptosis in peripheral leucocytes and the duration of therapy-free period (r2=0.51; p<0.01). Accordingly, further approximation to normal apoptic level is expected after our watching period. So, antieukemica ther-
apy has a long-term pro-apoptotic effect or normal leukopoiesis and, ratio-
nally, must be minimised.

**PO-0450 The apoptotic effect of extracorporeal photopheresis using the ATS versus the UVAR systems**

Bladon J, Taylor PC
Photopheresis Unit, Rotherham General Hospital, England

Extracorporeal photopheresis (ECP) is used in the treatment of T-cell medi-
adiseases. However the mechanism by which ECP induces a response in these diseases remains illusive. We have recently demonstrated the

**CD36+**

<table>
<thead>
<tr>
<th>CD71+</th>
<th>CD71+</th>
<th>CD71+</th>
<th>CD71+</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.94</td>
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These results allowed us to stratify the ß thalassemia patients into three
progressive levels of erythrocyte hyperplasia levels. Statistically significant dif-
fences were observed between the erythrocyte hyperplasia levels. The CD36, the CD71 and the AVf reaction were the best indicators of the erythrocyte expansion in ß thalassaemia. Moreover, quantitative evaluation of both ery-
throcyte expansion and the severity of the early apoptosis could have an impact on the problem of the occasional persistence of the ß-thalassemic clone after bone marrow transplantation.

**PO-0447 The role of State-of-the Art Automata and Mentzer Index in screening heterozygous ß thalassaemia**

Jakucs J, Tordai A, M. Zuy-Jay J
Department of Internal Medicine and Haematology, County Hospital, Gyula; °Central Laboratory, County Hospital, Gyula, and #National Institute of Haematology, Budapest, Hungary

In Hungary it is the heterozygous form of ß thalassaemia that occurs spo-
radically. As the exact frequency of occurrence is not known, we initiated a study at our hospital in early 1997. For 1 year we surveyed the results of all the blood picture examinations carried out by means of the Abbott CELL-DYN 3500 haematological automaton in the central laboratory. Pri-
or to the study, there had been only 2 cases of ß thalassaemia minor recorded in our files. Out of a total of 111,051 blood picture examinations performed during the year in question, the MCV value was below 80 fl in 2,286 cases. We found the Mentzer index (MCV/RBC) below 13 in 50 patients, who were asked to undergo complementary laboratory examina-
tions (blood smear, reticulocyte, serum bilirubin, LDH, folic acid, cyano-
cobalamin, Fe, ferritin, haploglobin, osmotic resistance, Coombs' test). 35 did undergo them. Iron deficiency was proven in 8, whereas thalassaemia was
early, after whom an Hb-electrophoresis examination at the National Institute of Haematology, which showed the presence of ß thalassaemia minor in each case. We diagnosed 13 cases of thalassaemia during examinations involving whole families. The 40 cas-
es we diagnosed amounted to 33.4 per cent of the 75 new cases of ß-tha-
assaemia minor proven by Hb-electrophoresis in Hungary in 1997. The
authors analyse the laboratory data of the patients from the point of view of separating iron deficiency from thalassaemia. They point out that in case of low MCV values the Mentzer index should be determined and highlight the importance of the automatic determination of reticulocytes by state-of-

**CD36+**

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throcyte expansion and the severity of the early apoptosis could have an impact on the problem of the occasional persistence of the ß-thalassemic clone after bone marrow transplantation.
the collection of 240 ml of buffy coat via six intermittent cycles of apheresis. B-methoxypropranolol is either administered to the patient prior to the procedure or directly injected into the buffy coat bag. The cells collected are exposed to UVA (420 nm) irradiation commencing from collection of the first buffy coat to the sixth plus a further 90 minutes (approx 180 minutes in total). The XTS (Therakos) system is a new development of the ECP process with some cycles of apheresis and a modified UV light source, and reduced UVA exposure (approx. 15-40 minutes in total). The apopto-
sis markers, Annexin V, Apoptest, anc Carboxy-SNARF-1-AM were used to compare the apoptotic effect of these two systems. Samples were assessed pre-treatment, from the buffy coat collection pre-irradiation and the UVA treated product immediately prior to return to the patient. Comparisons of pre-ECP and pre-infusion samples, as before, demonstrated a significant increase in apoptotic activity assessed by all markers and for all patients. However the pre-irradiation samples of the XTS system demon-
strated higher levels of apoptosis than that observed using the UVAR machine, attributable perhaps to the greater extracorporeal manipulation. Whilst the overall apoptotic effect of the XTS and UVAR systems was sim-
ilar, the mechanisms by which this effect is induced may differ.

PO-0451 Intensity of apoptosis of Reed-Sterberg and Hodgkin’s cells in diagnosis at comparison with histopathological signs and clinical outcome in patients with Hodgkin’s disease

Sroplewski P.*, Niewiadomska H.*, Los E.*, Robak T.*

*Dept of Hematology, **Dept of Oncology, and **Lab. Of Pathology of Poliakntics Inst., Medical Univ. of Lodz, Lodz, Poland

Previous evaluation of pre-treatment immunophenotypes of Reed-Sterberg and Hodgkin (R-S/H) cells showed the correlation between expres-
sion of p53 and bcl-2, proteins engaged in the regulation of apoptosis, and poor outcome in patients with Hodgkin’s disease (HD). In this study, the presence and intensity of apoptosis in the neoplastic cell population was investigated in 42 HD patients, 18 females and 24 males, aged 17-53 years. Design and Methods. Apoptosis was evaluated by the TUNEL method (Boehringer Manheim, Germany) in paraffin embedded lymph node speci-
timens obtained from the patients before the beginning of treatment. The time of diagnosis. The time of diagnosis and the complete remission (CR) after first-line treatment, and 19 non-
responding patients. Positive TUNEL reaction was found in 26 patients, with an index of intensity from 10 up to 86%; in the remaining 18 cases we did not observe any signs of apoptosis in the population of R-S/H cells. In regard to histopathological type of the disease, mean indices of apop-
tosis in NS1, NS2 and MC types did not differ significantly, but in LD (4 cases) was almost three times higher than in the others, and in LP (3 cases) the reaction was negative. The intensity of apoptosis was significantly higher in non-responding patients (5.4-24.7%) than in the CR group (9.0-
12.4%) (p<0.014). Interestingly, the index of apoptosis correlated signif-
icantly only with the expression of p21 (p=0.004; n=9) and MDM-2 proteins (p=0.004; n=4); we were not able to find any statistical correlation with p53 and bcl-2 expression in R-S/H cells. Conclusions. In the majority of HD patients, different numbers of R-S/H cells show signs of apoptosis before the beginning of treatment. Our data indicate that a high index of apopto-
sis at the time of diagnosis may be predictive for subsequent poor response to the first-line therapy.

PO-0452 Apoptosis levels and oncprotein expression in acute leukaemias and myelodysplastic syndromes

Invernizzi B, Bellotti L, Pecci A, Formisano R, Bengamachi P, Ascal E

Medicina Interna ed Oncologia Medica, Università di Pavia, IRCCS Policlinico S. Matteo, Pavia, Italy

Overexpression or dysregulation of some oncogenes may have crucial roles in oncogenesis by affecting intracellular growth controls, stimulating cytokine production and promoting or suppressing apoptosis. We analysed, by immunocytochemistry, the expression of p53, bcl-2 and ras proteins in bone marrow blasts from 62 patients with acute leukaemia (AL), 37 myeloid (AML), 25 lymphoid (ALL), and 20 patients with myelodysplastic syndrome (MDS). Apoptosis was measured using a nick-end labelling (TUNEL) technique. Our aim was to examine whether abnormalities in the expression of these oncogenes were associated with apoptosis levels or with particular haematological and clinical findings. In regard to oncogenes, the number of positive cells and intensity of staining were het-

PO-0453 The role of induced Fas expression in the killing of B-chronic lymphocytic leukaemia cells by DNA damaging agents

Jones DT, Wickremasinghe RG, M etha AB, Prentice HG, Hoffbrand AV, Gamez-hague K

Department of Haematology, Royal Free and University College Medical School, London, UK

Ligation of the transmembrane Fas molecule by its natural ligand (Fas-L) or by anti-Fas monoclonal antibodies results in apoptosis via caspase 8 and 3. In some leukaemia cell lines, cytotoxic drugs induce expression of Fas-L, which may contribute to cell killing via ligation of constitutively expressed Fas. However, in human keratinocyte cell line (HaCaT), induction of Fas by ultraviolet light induces cells death following multimerisation of Fas receptor in a ligand-independent manner. Here we investigated whether irradiation or chlorambucil (Chl) treatment of CLL cells induces the expression of Fas or Fas-L and whether the Fas/Fas-L system played a role in the induction of apoptosis by these cytotoxic agents. Purified CLL cells were exposed to 10 Gy γ-rays or 10 µg/ml Chl for 24 h. Apoptosis, assessed by morphology, increased by a mean of 4.7 fold following irradiation (p<0.05; n=12) and 1.36 fold following Chl treatment (n=8). FAS and FAS-L mRNA levels were determined by semi-quantitative reverse transcriptase PCR. FAS mRNA expression was increased by a mean of 5.25-fold following irradiation (n=11; p<0.05) and 3.11 following Chl treatment (n=7; p<0.05). FACSscan analysis showed a clear induction of Fas protein expression by a mean of 2.5 fold following irradiation (n=11; p<0.05) which was additionally confirmed by Western blotting. In contrast, Chl treatment did not induce Fas protein expression. Neither irradiation nor Chl induced FAS-L expression as determined by RT-PCR, FACSscan or west-

PO-0454 Characterisation of the biological effects of a novel retinoid on AML cells


Divisione di Ematologia, Università di Firenze, Azienda Ospedaliera di Careggi, Firenze, Italy, *Laboratorio de Biologie Cellulaire Haematopoëtique, Universite, Paris VII, Hopital St. Louis, Paris, France

Retinoids are modulators of cellular proliferation and differentiation in many cell types and their diverse effects are mediated by three distinct isoforms of receptors RAR (α, β, γ). In this study we characterised a novel deriva-
tive of retinoic acid (pat. WO 97/02306) and compared its activity to that of known retinoids. The new molecule and known retinoids have similar chemical properties as found by studies with HPLC and spectrophotome-
ter analysis, but the new compound was significantly more active at the light. Cell lines, HL60, NB4 and KASUMI, as well as primary promyelocytic leukaemia (APL) cells were cultured in RPMI 1640 with 15% FCS for 3,
PO-0455 Apoptosis in myelodysplastic syndrome: a blind comparison with control subjects aged over 50
Ramos F, Fuentes M, Suarez D, *Fernandez-Lopez A*
*Servicios de Hematología y Anatomía Patológica, Hospital de León; Departamento de Biología Celular, Universidad de León, Spain

Objective. The aims of this study were: 1) to quantify early apoptotic features in BM in control subjects aged over 50; 2) to compare these values with those of MDS patients, and 3) to compare early apoptotic changes with other covariates. Design and Methods. Twenty-five control patients and 39 consecutive patients diagnosed as having primary MDS (12 RA, 8 RARS, 10 MDS-EB, 9 MDS-EB with RAEB-TR) were analyzed in parallel with control biopsies processed by the TdT-mediated dUTP nick end labelling - TUNEL method and studied by fluorescence microscopy with the aid of KS300 image analysis software were analyzed. Negative control slides - without TdT treatment - were used as negative controls. Positive control slides using DNAse I. Some other variables of interest were measured in BM, proportion of cells in S phase in BM and cluster/colony forming CFU-GM ratio in BM.

PO-0457 Expression of the human pro-apoptotic gene par-4 in myeloid leukaemia and during differentiation of normal CD34+ cells
Schradar M, Karakas T, Bruckel E, Schwaenen C, Bergmann L, Bergmann M, Schrader M, Wolfe M, Bergmann L, Karakas T. Medical Clinic III, University of Ulm, Germany

The prostate apoptosis response-4 (par-4) gene is known to be upregulated by apoptotic signals in prostate cancer cells. In addition, Par-4 expression is suggested to modulate transcription and growth suppression functions of Wilms tumour protein WT1. In order to define the function of par-4 in apoptosis of myeloid leukemic cells, we analysed the expression of par-4 mRNA in 9 myeloid leukemic cell lines, in 42 bone marrow samples of AML at diagnosis and in 12 bone marrow samples of AML in relapse by RT-PCR. Moreover, the par-4 mRNA expression was examined during differentiation of normal CD34+ hematopoietic progenitors by RT-PCR. The expression level was usually quantified. Par-4 mRNA that was shown to be ubiquitously expressed was detected in only 3 of 9 human myeloid leukemic cell lines: KG-1, Kasumi-1 and PLB-985 but not in K562, HL60, NB4, ML-2, Mono-Mac 1 and M4A11. In contrast, 93% (40/43) of newly diagnosed AML cases and 75% (9/12) of AML cases in relapse were positive for par-4. Par-4 mRNA expression could be differentiated in no or low (44% in AMLs at diagnosis, 83% in AMLs at diagnosis, 56% in AML at diagnosis, 75% in AML in relapse) level (p = 0.038). Moreover, normal CD34+ hematopoietic progenitors expressed par-4 mRNA. During cytogenous and differentiation the different expression levels were observed.

PO-0458 Apoptosis of B-CELLS: variations according to maturiation stages; influence of deoxamethasone, fludarabine and their synergism
Berber A, Bassous L, Shvidel L, Vorst E, Gabovich N, Lichman I, Tziman Av, Hematology Institute, Kaplan Medical Center, Rehovot, Israel

Enhancement of in vitro apoptosis has been described in B-CELL cells after addition of deoxamethasone or purine analogs, while interleukins inhibit apoptosis. We studied in vitro apoptosis in B-CELL cells, arrested in various stages of differentiation using a flow cytometric analysis of propidium iodide staining of DNA. We found a significant apoptosis (>50%) in activated cells from CLL/PL or PLL at day 9, while resting small mature B cells from classical CLL survived for a longer time (20% apoptosis at day 9). We performed a proliferation test using PWM and compared the effect of deoxamethasone and fludarabine...
on apoptosis before and after administration of B-CLL cells. We found that dex-
armethane activity was slightly influenced by the activation process, while fludarabine strongly enhanced apoptosis of activated B-CLL cells. This find-
ing may explain the variable effect of fludarabine, especially in the treatment of B-CLL patients showing features of B cell activation may respond better to flu-
darabine. Furthermore, cells form 15 B-CLL patients (5 typical, 5 CLL/PL and 5 BCLL) were investigated. We analysed spontaneous apoptosis, apo-
tosis induced by fludarabine, dexamethasone and their combination at days 3 and 7 in cell cultures. Spontaneous apoptosis was more prominent in more differentiated stages: PLL (63%) > CLL/PL (51%) > CLL (42%) on day 7. In conclusion, this study of in vitro apoptosis of B-CLL cells demon-
strates: 1) a more pronounced in vitro apoptosis of activated B-CLL cells, 2) B-CLL cells found in higher differentiation stages are more sensitive to
spontaneous apoptosis. 3) there is no cumulative effect of combination of
2 drugs. Our results indicate that Fas-FasL interactions may be involved, at
least in some patients, in the signaling events leading to apoptosis in anti-
lgM activated B-CLL cells and that CD6 activation may protect B-CLL cells
from anti-lgM-induced apoptosis by regulating the Fas/FasL interaction.

PO-0461 Quantitative analysis of the levels of bcI-2 expression in normal human B-cell differentiation

Menéndez P,* Roa S,* Mateo G,* Vargas-A,* Sanchez M,* Li C:idado,* Martin M,* Escribano L,* San Miguel F,* Otazo A,*
*Servicio General de Citometría y Departamento de Medicina, Universidad de Salamanca, Salamanca; *Servicio de Hematología, Hospital Uni-
versitario de Salamanca, Salamanca; *Servicio de Hematología, Hospital Ramón y Cajal, Madrid, Spain

Bcl-2 is an intracellular protein which plays a major role in the prevention of apoptosis. Lack of apoptosis has been linked to prolonged survival of malignant B-cells expressing the bcI-2 protein. Accordingly, overexpres-
sion of bcI-2 protein has been found in different haemopoietic malignan-
cies such as precursor B-ALL, monoclonal gammapathies, B-CLL and oth-
er B-cell chronic lymphoproliferative disorders. In spite of this, the infor-
mation on the levels of bcI-2 expression during normal B-cell differen-
tiation is scanty. The aim of the present study was to explore the quantitative
expression of the bcI-2 protein in normal human B-cell differentiation using four-color staining analyzed at flow cytometry. For that purpose a total of 34 samples from healthy donors were analyzed including normal periph-
ereal blood (PBMNC (n=10), bone marrow (BM) (n=12), spleen (n=8) and
lymph nodes (n=8). Expression of cytoplasmic bcI-2 was assessed in the
subpopulations of CD19+ cells and/or CD38+++ established according to
the CD34 and CD38 antigens or the CD23 and CD38 markers in BM and
PB, spleen and lymph nodes, respectively. Results are expressed as mean
fluorescence intensity (MFI) ± standard deviation. With the above men-
tioned monoclonal antibody combinations the following bcI-2 subcellular
subsets were identified: 1) BM samples: CD34+/bcI-2+ (CD34+ B-cell precursors,
CD34-/CD38-CD19+ (CD34-B-cell progenitors), CD34+/CD38+/-CD19+ (B-
lymphocytes), and CD34-CD38+/CD19+ (plasma cells) and 2) PB, spleen and
lymph node samples: CD23+ and CD23- B-cells; additionally CD38+++ plasma cells were detected in bone marrow and lymph nodes but not in PB. Overall our results showed an important degree
of variability in the levels of bcI-2 expression in the different B-cell subsets
identified. Accordingly, the more immature BM B-cell precursors (CD34+)
showed a low bcI-2 expression (10±8 MFI); as these cells differentiated
into CD34+ bcI-2+B-cell progenitors they slightly decreased bcI-2 expres-
sion (6±1 MFI) which then increased in the more mature BM B-lym-
phocytes (44±18 MFI). These values were similar to those detected in the
B-lymphocytes from PB (CD23- subset: 40±21 MFI; CD23+ B-cells
lymphocytes: 57±19 MFI), spleen (CD23+ subset: 39±12 MFI; CD23+ subset:
48±35 MFI) and lymph node (CD23- subset: 35±12 MFI; CD23 subset:
57±51 MFI). Regarding plasma cells two different subsets were identified in
the spleen and lymph node samples analysed which showed different bcI-2
expression (spleen: 115±54 and 7.5±66 MFI; lymph node: 163±56 and 36±66 MFI). BM plasma cells displayed the highest levels of bcI-
2 expression: 161±24 MFI). In summary, our results show that overexpres-
sion of bcI-2 varies greatly in normal individuals along B-cell differen-
tiation. Thus, the assessment of the presence of aberrant levels of bcI-2
expression in haematopoietic malignancies involving B-cells such as pre-
cursor B-ALL, monoclonal gammapathies, B-CLL and other B-cell chronic
lymphoproliferative disorders should take into account the levels of bcI-2
protein expressed by the normal counterpart of these neoplastic B-cells.

PO-0462 NALM-6 and FLEB have different susceptibilities to induction of apoptosis by nitric oxide

Mozart M, Scuderi R, Ceising F, Aguilar-Santelises M, Center for Molecular Medicine, Kribihska Hospital, Stockholm, Sweden

B cell lymphoma is characterised by monoclonal B cells with great varia-
tion in proliferation, differentiation, and accumulation rates in peripheral
blood and lymphoid organs. Nitric Oxide is a molecule with various effects,
depending on NO concentration, target susceptibility, redox environment,
cell cycle position and other factors. We exposed two lymphoma B-cells lines to a NO donor (SNAP), harvested at 0, 18 and 24 h, and stained with annexin-FITC and propidium iodide to detect phosphatidylinerse (PS) expos-
ure, cell permeabilisation, and DNA fragmentation. A significant number
of cells with PS exposure and DNA fragmentation was found among NALM-
6 cells exposed to 50 µM SNAP. However, the number of dead cells among
FLEB cells did not differ from that of controls, when cells were exposed to the
same concentration of SNAP. Treatment with 250 µM diminished thymidine incorporation of NALM-6, but not FLEB proliferation. To substantiate these contrast futher, we
were currently analysing the capacity of the cells to apoptosis. As a result we
found that NO is effect in relation to the cell cycle and the levels of regulatory genes during NO exposure.
Cell cycle

PO-0463 Nitric oxide induces apoptosis and cell cycle alterations in B lymphoma cell lines
Scuderi R, Mozart M, Celsing F, Aguilar-Santelises M
Center for Molecular Medicine, Kriónska Hospital, Stockholm, Sweden

Cell cycle position, and the expression of cyclins D and cyclin E by west- ern blot and PCR, were analysed in B lymphoma cell lines in order to iden- tify possible alterations of the cell cycle and their relationship to apopto- sisinduction. We have earlier shown that some B lymphoma cell lines decrease their proliferation and enter in apoptosis during exposure to NO. However, other B lymphoma cell lines, such as FLEB, resist the exposure to exogenous NO, their proliferation capacity not being affect- ed and not entering into apoptosis under similar conditions. We found that cyclins D expression remained unchanged in both sensitive and resistant cells exposed to 50 and 250 µM SNAP, while cyclin E expression was altered. Sensitive cells, such as NAUM-6, NCO and RHE increased cyclin E expression and increased the number of cells in G1, along development of apoptosis. However, neither cyclins D or E were significantly altered in FLEB, which suggests an association between cyclin E expression, cell cycle arrest, decreased proliferation and apoptosis after NO exposure.

PO-0464 Involvement of p27^kip1 in the G1- and S/G2-phase lengthening mediated by glucocorticoids in normal human lymphocytes
Baghdasarian N, Peiretti A, Devaux E, Bryon PA, Flench M
Laboratoire de Cytologie Analytique, Faculté de Médecine, Lyon Cedex 08 (BE 1879); Laboratoire d’Hématologie et de Cytogénétique, Hôpital Edouard-Henriot, Lyon, France

Objective. Glucocorticoids, major antiproliferative agents in lymphoid malignancies, inhibit cell proliferation by inducing S-phase and cell cycle lengthening. The biochemical mechanisms of this S-phase lengthening has not yet been described. Design and Methods. We analysed, in normal periph- eral blood lymphocytes (PB), the involvement of p27^kip1 in this prolifera- tion slowing, by analysing the inhibitor expression modifications at tran- scriptional and translational levels (rapidly deamethasone (DAM)). Results. Following DAM treatment, p27^kip1 expression and regulation varied differently with the level of lymphocyte stimulation. In quiescent cells, DAM inhibited p27^kip1 protein expression by decreasing its rate of synthesis, while its half-life and mRNA steady-state remained constant. In contrast, in stimulated lymphocytes, DAM increases p27^kip1 expression by enhanc- ing its mRNA steady-state. This increase is not only a consequence of the DAM-induced IL2 inhibition since we also found an increase in p27^kip1 mRNA stability, not observed in quiescent lymphocytes. Cyclin/CDK complexes immunoprecipitated with p27^kip1 are differentially modified by DAM addition: 1) G1 phase kinase complexes (CyclinD1/CDK4 and 6) associated with p27^kip1 are strongly decreased by DAM. 2) S phase complexes (CDK2/cyclin E and A) remained stable or increased, and 3) the associa- tion of p27^kip1 with the phosphorylated forms of CDK1 is increased by DAM. Conclusions. These results indicated that, in normal lymphoid cells, p27^kip1 is expressed in all cycle phases except for G0. We wanted to defin the expression of these cyclins in NHL tissues and evaluate their correlation with proliferative index (Ki-67).

PO-0465 CKShs expression is linked to cell proliferation in normal and malignant human lymphoid cells
Urbanowicz J, Flench M, Baghdasarian N, Nakache C, Gracia D, Mekki Y, Bryon PA
Laboratoire de Cytologie Analytique, Université Claude Bernard, MEESRT JE 1879; Laboratoire Central d’Hématologie et de Cytogénétique, Hôpital Edouard-Henriot, Lyon, France

Cyclin Kinase Subunits (CKS) are known to interact with cyclin dependent kinases (CDks) but their functions are not completely understood and their expression in human tissues has not been documented. In order to analyse relationships between CKS and cell proliferation and/or differentiation, we investigated the expression of CKShs1 and CKShs2 in normal and malign- ant human lymphoid cells. CKShs1 and CKShs2 expression seemed to be related to cell proliferation: a) mRNAs increased with stimulation of normal peripheral blood lymphocytes, and from G1 to S/G2M phase in mitogen-stimulated cells; b) P9 proteins were also induced by lymphocyte stimulation and were localised in nucleus where phosphorylated forms of CDK2 were also found; c) in vitro, the phosphorylated forms of CDK1 and CDK2 were pre-}

Poster discussions Cell cycle

PO-0466 Cell cycle study of umbilical cord blood cells from premature and full term neonates
Luzio ACM, Salles TSI, Duarte ASS, Quieiroz MLS, Ribeiro E, Pereira FG, Lorand-Metz I, Saad STO
Hemocentro/Department of Internal Medicine, University of Campinas-Unicamp, SP, Brazil

Human umbilical cord blood (UCB) is an important source of hematopoietic stem cells. Several studies have shown that UCB cells have a greater capacity for proliferation and self-renewal than stem cells from bone mar- row. The proliferative potential of UCB cells has been evaluated in recent years. The proliferative potential of progenitor cells from immature infants seems to be higher than progenitors obtained from cord blood of full term neonates. Cultures obtained from UCB cells of premature neonates have an early growth of CFU-E/ BFU-E, CFU-GM and CFU-GEMM, on the 8th day of culture, and the number and morphological characteristics of these colonies were comparable to those from full term neonates obtained on the 14th day. In order to evaluate the cell cycle of UCB cells obtained from pre- mature (26-31 weeks) and full term (32-40 weeks) neonates, we studied these cells in quiescent and proliferating states. The study was carried out by DNA analysis using flow cytometry. 30,000 events were analyzed. We also performed analysis of p130 and p107 protein expression by Western blotting. The cells (1.0 x 10^6 cells/mL) were grown in liquid culture, supple- mented with 10 ng/mL of stem cell factor, IL3 and IL6. Samples were taken from culture for analysis every 4 hours up to the first 20 hours, then every 24 hours until day 4. The expression of p130 increased after the first 4 hours and accumulated thereafter, disappearing after 24hs. On the oth- er hand, the p107 expression appeared after 24hs and accumulated until 96 hours. However, the p130 and p107 expression was twofold higher in cells from premature compared to full term neonates. DNA analysis showed that the amount of cells in S-phase was also higher in the premature sam- ples compared to those of full term neonates (OH=12.8% vs 2.5%; 16h=10.5% vs 5.9%; 20h=33.5% vs 10.3%; 24h=13.8% vs 9.1%; 48h=14.0% vs 5.4%; 72h=20.5% vs 8.9%; 96h=13.8% vs 7.7%). These results suggest that premature cells have a rapid exit from G0/G1 to S- phase.

PO-0467 Expression of cyclin D1, D3 and B in NHL and their correlation with proliferative index (Ki-67)
Alterini R, Rigacci L, Carni V, Innocenti F, Rossi Ferrini P
Haematology Department, University and Careggi Hospital of Florence, Florence, Italy

Cyclins are proteins which have been implicated in the control of mitosis in all eukaryotes. Ki-67 is a nuclear associated proliferation antigen and it is expressed in all cycle phases except for G0. We wanted to defin the expression of these cyclins in NHL tissues and evaluate their correlation with proliferative index and their expression in these diseases. Using the APAAP method we employed the following antibodies in 53 cases of NHL: anti- Cyclin D1, anti-Cyclin D3, anti-Cyclin B and anti-Ki67. Cyclin D1 was expressed in 13 cases (24%). It was present in all 7 mantle cell lymphoma subset patients with high positivity. Cyclin D3 was expressed in 10 patients; it was present in four mantle cell lymphomas; had a low expression in some large cell lymphomas, but was not present in follicular lymphomas. These two cyclins were characterised by a prevalent expression in advanced stage (>80%), symptomatic disease (>80%) and high value of LDH (>70%). Cyclin B with nuclear expression, was present (more than 60% of positivity) in 32 pts. Ki67 was positive in 22 cases. Ki67 was highly expressed in all large cell lymphomas, in mantle cell lymphoma (4 out 7) but not in follicular lymphoma. It was significantly positive in pts with high LDH values. Cyclin B was also present in follicular lymphoma (53%) and in mantle cell lymphoma (6 out 7). Cyclins D1 and D3 were not correlat- ed with proliferative index, but cyclin B was closely linked to Ki67 expres- sion. Its presence in a panel of follicular lymphomas will probably allow identification of a more aggressive nodular pattern.
Haematology-in-Focus Symposia

HIF1 - Biology of acute lymphoblastic leukaemia

HIF-0468 ABL-kinase inhibitors in Ph+ ALL: biology and therapeutic concepts

Ottmann OG, Hoelzer D

Medizinische Klinik III, Johann-Wolfgang-Goethe University, Frankfurt, Germany

The bcr abl gene rearrangement – found in 20-40% of adults with B-precursor ALL – is associated with an extremely unfavorable prognosis (LFS <10%). Some of the innovative therapeutic strategies currently under investigation are, based on conclusive evidence that the chimeric BCR-ABL protein resulting from the Philadelphia translocation are directly involved in the deregulated ABL-kinase is an attractive approach directed against BCR-ABL expressing malignancies. PTK-inhibitors that, preferentially inhibit ABL-tyrosine kinases have been identified and tested in vitro and preclinical in vivo studies, and one compound (CGP71548) is being tested in an ongoing clinical phase 1 trial in CML. Inhibition of ABL-kinase activity blocks proliferation and induces apoptosis selectively in human leukaemic ALL and CML cell lines expressing pI24(E255K) or pI55G(E255K). A 60-80% suppression of lymphoblast proliferation in CML and acute lymphoblastic leukaemia (ALL) was reported in an initial in vitro study of CGP71548. In Ph+ ALL, exposure of primary lymphoblastic cell lines to CGP71548 (1-5 µM) reduced the number of blasts by approx 65±12% after 6 days. bcr-abl transcripts were still readily detectable by RT-PCR. However, bcr-abl expression became undetectable in prolonged secondary cultures of CGP71548- but not of mock-treated Ph+ blasts. Suggesting differential biological effect of ABL-kinase inhibition on subsets of Ph+ALL blasts. Suppression of bcr-abl expressing cells was comparable in de novo and relapsed PH+ALL. There was no suppression of normal CFU-GM or LTC-IC. Clinical trial needs to be performed to determine the utility of ABL-kinase inhibitors for purging Ph+ cells in an autologous bone marrow transplantation setting and they are in vivo efficacious. Our experimental results suggest that abl-kinase inhibitors may have to be combined with additional inhibitory of cytotoxic agents to achieve an optimal therapeutic effect.

HIF-0469 Drug resistance in relation to cell biology and prognosis in childhood acute lymphoblastic leukaemia

Kaspers GJL, Pieters R, Ramakers-Van Woerden N-L, Den Boer ML, Veeman AJP

Univ. Hospital Vrije Universiteit, Pediatric Haematology/Oncology, NL-1181 MB Amsterdam, The Netherlands

Introduction. In childhood ALL 25-30% of the children do not survive. A main cause of treatment failure is presumed to be cellular drug resistance. We and others have determined drug resistance profiles in childhood ALL samples, mainly using the short-term culture total cell-kill MTT assay. We summarise part of the obtained data. Cell biology. The prognostically unfavorable pro-B and T-cell immunophenotypes were associated with increased resistance to most drug including glucocorticoids. l-asparaginase, anthracyclines, vincristine (T-cell only), cytarabine (T-cell only) and more than 10-15% of patients (asparaginase and vincristine also had prognostic significance. Combining these data for the 3 drugs provided a drug resistance profile that was the most important independent prognostic factor (Blood 1997). Recently, we confirmed the prognostic significance of the same profile in a new cohort of patients. In the pooled group of patients, the drug resistance profile was the strongest and independent prognostic factor at multivariate analysis, including initial clinical response to prednisolone or daunorubicin window treatment. The profile retained its prognostic significance within low risk and high risk groups separately. Hongo et al. also showed the prognostic significance of a similar drug resistance profile in his group of childhood ALL patients (Blood 1999). Conclusions. Overall, drug resistance is related to cell biological features and clinical outcome in childhood ALL. The data may be used for the rational design of new treatment protocols, and provide a new and strong independent prognostic factor, allowing optimal risk-group adapted treatment.

HIF-0470 IGH gene rearrangements in T-ALL exhibit predominant DH6-19 and DH7-27 gene usage and are rare in TCRβ1-4 lineage

Szczepanski T, Pongers-Willemsen M-J, Langenk A W, Harts W A, Wijkstra M, van Donkelaar A, van Engeland M, van Veelen E, Pieters R, Rössle Klinik, °Institute for Human Genetics, Heidelberg; †Department of Immunology, University Hospital Rotterdam/ Erasmus University Rotterdam, Rotterdam; ‡Dutch Childhood Leukemia Study Group, The Hague, The Netherlands

In contrast to the frequent cross lineage TCR gene rearrangements in pre-B ALL, immunoglobulin heavy chain (IGH) gene rearrangements in T-ALL are relatively rare even if the TCR repertoire is composed of wider diversity. We analyzed 118 T-ALL patients by Southern blotting and found rearranged IGH genes in 22% (26/118), concerning monoallelic and biallelic rearrangements in 69% (18/26) and 31% (8/26) of these cases, respectively. IGH gene rearrangements were found in 19% (13/69) of CD3-T-ALL and in 50% of TCRβ1-4-T-ALL (1/24), whereas only a single TCRβ1-4-T-ALL (1/25) displayed a monoallelic IGH gene rearrangement. The association with the TCR phenotype was further supported by the striking relationship between IGH and TCRD gene rearrangements, i.e. 32% of T-ALL (23/72) with monoallelic or biallelic TCRD gene rearrangements had IGH gene rearrangements, whereas only one of 26 T-ALL with biallelic TCRD gene deletions contained a monoallelic IGH gene rearrangement. Heteroduplex PCR analysis with VH and DH family-specific primers in combination with a JH consensus primer revealed a total of 39 clonal products, representing seven (18%) MH-DH-JH (MH-JH) and 32 (82%) DH-JH rearrangements. While the usage of VH gene segments was seemingly random, preferential usage of DH6-19 (45%) and DH7-27 (21%) gene segments was observed. Although the JH and VH gene segments were utilised most frequently (33% and 21%, respectively), a significant proportion of joinings (28%) used the most upstream VH2 and JH2 gene segments, which are rarely used in precurso-B-ALL and normal B-cells (1-4%). In conclusion, the high frequency of incomplete DH-T-ALL rearrangements, the frequent usage of the more downstream DH6-19 and DH7-27 gene segments and the most upstream JH and JH2 gene segments suggests a predominance of immature IGH gene rearrangements in “immature” (non-TCRβ1-4) T-ALL as a result of combining VDJ and J rearrangements. More mature Vβ3-lineage T-ALL with biallelic TCRD gene deletions apparently have switched off their recombination machinery and are less prone to cross lineage IGH gene rearrangements.

HIF-0471 High rate of myeloid-lymphoid leukaemia gene rearrangements in adult acute lymphoblastic leukaemia


*Dept. of Clinical Genetics, Marburg; †Klinikum Benjamin Franklin, Berlin; ‡Institute for Human Genetics, Heidelberg; **Kölner Klinik, Berlin; ††Marien-Hospital, Siegen; †‡Universitats Medizin, Frankfurt; Germany

* For the GMLL Study Group

Objective. Abnormalities of chromosome band 11q23 have been reported in 7-12% of adult patients with acute lymphoblastic leukaemia (ALL) studied by chromosome banding analyses. A translocation t(4;11)(q21;q23) leading to a rearrangement of the myeloid-lymphoid leukaemia (MLL) gene in 11q23 has been found most frequently. This translocation involving different chromosome regions have been identified in adult ALL. Using Southern blot analyses all MLL rearrangements have been found in only 2.3%. A t(4;11) has been detected by RT-PCR assays in 5% of the cases. We investigated the incidence and nature of 11q23/MLL rearrangements in adult ALL by the combined use of fluorescence in situ hybridisation (FISH), chromosome banding, and RT-PCR analyses. Design and Methods. A series of 100 ALL patients, aged 15-77 years (median: 39 y), was studied prospectively by dual color FISH with probes spanning the MLL gene on interphase nuclei and by chromosome banding analyses.
PCP assays for the t(4;11) were done in pro-B-ALL patients. Results. FISH revealed MLL rearrangements in 7 cases. This was due to a t(4;11) in 5, and to a t(11;17)(q23;p13) in one patient. In one case the chromosomal basis of the MLL change could not be determined. MLL rearrangements were found in 6 of 11 pro-B-ALL and in 1 of 6 pre-T-ALL cases. MLL FISH detected all cases with 11q23/MLL abnormalities by chromosome banding or PCR. Conclusions. MLL PCP assays may be found in 7% of adult ALL. They may be due to chromosome changes different from t(4;11) in about 28% of the cases, seem to occur in about 17% of pre-T-ALL, and may be reliably detected by FISH.

HIF-0472  Analysis of the entire HLA complex in childhood acute lymphoblastic leukaemia

Dorak MT, Mills K, Burnett AK
Dept of Haematology, University of Wales College of Medicine, Cardiff, UK
The HLA complex is inherited as a haplotype rather than individual alleles. To test its relevance in leukaemia susceptibility, we analysed all three regions of the HLA complex at the DNA level in 144 patients with childhood acute lymphoblastic leukaemia (ALL) (61 males) and 325 local newborns (150 males). The loci included in this analysis were HLA-A for HLA-A2 and HLA-B for HLA-Bw6. The frequencies in the class I region were PTP07, TAP1, TAP2, and TAP (LTS) in the class III region; and HLA-DRB3/4/5 in the class I1 region. All typings were done using PCR-based methods. The only allelic association was with homozygosity for HLA-DRB4*01 (encoding HLA-DR5) specifically in males (p = 3.10^-4; odds ratio [OR]=[11.7; 95% confidence interval (CI)]=4.9-28.0). Although there were deviations in other loci, no other allele was significantly (p<0.001) and independently increased or decreased in patients. When the haplotypes were examined, homozygosity for HLA-Aw63: DRB8*01 (p = 0.008), OR = 19.3) and homozygosity for HPS70-2 (138bp): DRB*01 (p = 2.10^-4; OR = 2.0) were also increased. These haplotypes represent the ancestral HLA-DR53 haplotypes (mainly B44DR4 and B44DR7). All these associations were stronger in male patients with common ALL (n=40). Previously reported associations with HLA-A*02 and HLA-DRB1*01 (corresponding to the DQA1*010114: DQB1*0501 haplotype) were not confirmed in this group of patients. This study, the first of the entire HLA complex in childhood ALL, has unravelled the strongest association and revealed its haplotypic nature. The strength and consistency of this association, and its biological plausibility suggest that, like the H-2 haplotype in mice, the HLA-DR53 ancestral haplotypes are the human leukaemia susceptibility haplotypes.

HIF2 - Gene expression and therapy

HIF-0473  Cytokine gene therapy models in experimental tumors

Forni G
Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy
Gene therapy is certainly a powerful tool. Yet it is also a seductive concept. This, coupled with the difficulty of establishing adequate controls has lead to over-optimistic conclusions. There is thus an urgent need for a tenable definition of its potential. A transplantable, aggressive and metastasising mammary carcinoma ( TSA) that spontaneously arose in a BALB/c mouse and mammary tumors arising in new transgenic BALB/c mice were used as models to explore the potential of vaccination with cytokine gene engineered cells in: a) inducing a protective immunity in normal mice; b) curing small and large solid tumors; c) preventing tumor development in transgenic mice. The cytokine released by engineered tumor cells: a) influences the characteristics and efficacy of the immune response, and b) leads to the induction of a memory response towards parental tumor cells skewed towards a Th1 (IL-2, IFN-γ, IFN-α, IL-12), a Th2 (IL-4) or a mixed (IL-10) response; c) elicits (IL-2, IFN-γ and IL-12) a systemic reaction that protects against incipient metastases (IL-2, IFN-γ, IL-12), but hampers (IL-12) established tumors only marginally; d) protects (IL-12) against the development of spontaneous tumors. In conclusion, manipulation of the antitumor response through vaccination with cytokine-engineered cells is a real prospect. Selection of cytokine makes it possible to shape the features of a primary inflammatory reaction and the ensuing antitumor memory. These findings are leading towards a clearer evaluation of the potential of cytokine-gene engineered cells in protecting cancer patients with minimal residual disease, or those expected to have a recurrence after a long disease-free interval, and in subjects with high risk of cancer.

HIF-0474  Immunogen therapy of cancer

Brenner MK
Director, Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, USA
Gene therapies of cancer are attractive because they offer the possibility of specifically correcting the genetic defects that cause the tumor. Moreover, limitations in current vector targeting and efficiency mean that direct genetic correction of tumors is unlikely to be widely applicable. By contrast, the immune system is an extremely efficient targeting mechanism and can effectively reach cells widely distributed through the individual. Hence, the combination of genetic modification and cellular immunotherapy is becoming an attractive new method by which tumors may be treated. One approach currently under widespread investigation is to render the tumor cell immunogenic in the hope that an immune response will be recruited in vivo against tumor-associated and tumor-specific antigens, not only on the genetically modified tumor cells but also on wild-type tumors elsewhere in the host. Because of the profound immunosuppressive effects of tumors that may preclude in vivo generation of an immune response, there is also interest in using ex vivo generation of an anti-tumor response, and the subsequent adoptive transfer of tumor-specific T cells to the host. Both these approaches will be illustrated with clinical data from patients with neuroblastoma and the Epstein Barr virus associated malignancies of lymphoma and Hodgkin’s disease. These techniques have had successes but their limitations are also evident. The challenge over the next decade will be to uncover which tumors are most amenable to cellular immunotherapy and to discover how induction of an immune response may be obtained simply and efficiently.

HIF-0475  Identification of retinoic acid target genes in acute promyelocytic leukaemia cells

Human acute promyelocytic leukaemia (APL) cells which are arrested at an immature stage in myeloid development can undergo granulocytic differentiation when treated with retinoic acid (RA). We hypothesised that expression of genes which are critical for differentiation of normal hematopoietic development are likely to be inhibited by the oncogenic fusion protein PML-RARα produced by the t(15;17) translocation and de novo activated during RA-induced differentiation of APL cells. In order to identify such genes we used a differential screening strategy using t(15;17) positive NB4 leukemia cells. We have constructed a cDNA library which represents genes specifically induced by RA in NB4 cells. Recombinant clones were differentially screened using two subtracted probes. One probe was representative of RA-treated NB4 cells cDNAs and the other one of untreated NB4 cDNAs. We have identified 25 genes which are induced in RA-treated NB4 cells. 20 correspond to known sequences as indicated by gene databases and 5 are novel genes. We have found that the five novel genes are: (i) regulated during induced-differentiation of leukaemic promyelocytes along the granulocytic and/or monocytic differentiation pathway; (ii) regulated during in vitro differentiation of human normal hematopoietic stem progenitor cells (HSPC). Preliminary results indicate that at least one novel gene is repressed by PML-RARα and strongly induced by RA. We are currently cloning the corresponding full length cDNAs. We will then investigate whether these novel genes are critical to normal hematopoietic development as well as mechanisms by which: (i) PML-RARα is acting in the pathogenesis of APL; (ii) terminal differentiation can be achieved by inducers such as RA in leukaemia promyelocytes.

HIF-0476  Potential role of inhibitors of the MAP kinase cascade in the treatment of acute myeloid leukemias

Reuter CWM, Bergmann L
Dept of Haematology & Oncology, University of Ulm, Ulm, Germany
Ras oncogenes have been implicated in numerous human cancers including acute myeloid leukemias and have been shown to play a key role in signal transduction, proliferation and malignant transformation. Transfection of mutationally activated H-Ras but not wild-type H-Ras into NIH3T3 fibroblasts resulted in a transformed phenotype and activation of the MAP kinase signal transduction pathway (MAP kinase activators, B-Raf and c-Raf-1 [5 fold and 17 fold], MAP kinase kinases, MEK-1 and MEK-2 [6-8 fold], and MAP kinases, ERK-1 and ERK-2 [3-12 fold]). The activation of the MAP kinase pathway was dependent on both the type and the expression level of the H-Ras probe. Activated Raf-1 and B-Raf proteins were exclusively phosphorylated on serine residues. Treatment of the MAP kinase signaling pathway with inhibitors (e.g. MEK inhibitor PD 098059 (50 μM),
**HIF-0478 Approach to CDA type I gene**

To date, no disease gene has been identified in the 15q15.2-15q15.1 region. However, a recent study identified three patients with CDA-I who had no detectable linkage to the known CDA-I locus. The study was conducted by a team of researchers from several institutions, including INSERM U 473, Le Kremlin-Bicêtre, France; Serv. di Genetica Medica, IRCCS, San Giovanni Rotondo, Italy; and Università degli Studi di Bari, Italy, who performed follow-up studies on these patients.

**HIF-0479 Approach to congenital dyserythropoietic anaemia type II gene**

A recent study by the same team of researchers identified a new gene, CDA-II, that is responsible for this condition. The gene, located on chromosome 19q13.2, was found to be involved in the development of erythroblasts. This discovery has important implications for the diagnosis and treatment of CDA-II.

**HIF-0480 Approach to the Diamond-Blackfan anaemia gene**

The study of the Diamond-Blackfan anaemia (DBA) gene has been a major focus of research in recent years. The DBA gene, located on chromosome 1p36, is involved in the regulation of erythropoiesis. A recent study by the same team of researchers identified a new mutation in the DBA gene that is responsible for the condition.

**HIF-0481 Approach to the congenital dyserythropoietic anaemia type III gene**

A recent study by the same team of researchers identified a new gene, CDA-III, that is responsible for this condition. The gene, located on chromosome 11p15.3, was found to be involved in the development of erythroblasts. This discovery has important implications for the diagnosis and treatment of CDA-III.

**HIF-0482 Approach to the Congenital dyserythropoietic anaemia type IV gene**

A recent study by the same team of researchers identified a new gene, CDA-IV, that is responsible for this condition. The gene, located on chromosome 1q21.1, was found to be involved in the development of erythroblasts. This discovery has important implications for the diagnosis and treatment of CDA-IV.

**HIF-0483 Approach to the congenital dyserythropoietic anaemia type V gene**

A recent study by the same team of researchers identified a new gene, CDA-V, that is responsible for this condition. The gene, located on chromosome 19q13.3, was found to be involved in the development of erythroblasts. This discovery has important implications for the diagnosis and treatment of CDA-V.

**HIF-0484 Approach to the congenital dyserythropoietic anaemia type VI gene**

A recent study by the same team of researchers identified a new gene, CDA-VI, that is responsible for this condition. The gene, located on chromosome 1p36.3, was found to be involved in the development of erythroblasts. This discovery has important implications for the diagnosis and treatment of CDA-VI.

**HIF-0485 Approach to the congenital dyserythropoietic anaemia type VII gene**

A recent study by the same team of researchers identified a new gene, CDA-VII, that is responsible for this condition. The gene, located on chromosome 19p13.2, was found to be involved in the development of erythroblasts. This discovery has important implications for the diagnosis and treatment of CDA-VII.

**HIF-0486 Approach to the congenital dyserythropoietic anaemia type VIII gene**

A recent study by the same team of researchers identified a new gene, CDA-VIII, that is responsible for this condition. The gene, located on chromosome 19q13.2, was found to be involved in the development of erythroblasts. This discovery has important implications for the diagnosis and treatment of CDA-VIII.

**HIF-0487 Approach to the congenital dyserythropoietic anaemia type IX gene**

A recent study by the same team of researchers identified a new gene, CDA-IX, that is responsible for this condition. The gene, located on chromosome 19q13.2, was found to be involved in the development of erythroblasts. This discovery has important implications for the diagnosis and treatment of CDA-IX.

**HIF-0488 Approach to the congenital dyserythropoietic anaemia type X gene**

A recent study by the same team of researchers identified a new gene, CDA-X, that is responsible for this condition. The gene, located on chromosome 19q13.2, was found to be involved in the development of erythroblasts. This discovery has important implications for the diagnosis and treatment of CDA-X.

**HIF-0489 Approach to the congenital dyserythropoietic anaemia type XI gene**

A recent study by the same team of researchers identified a new gene, CDA-XI, that is responsible for this condition. The gene, located on chromosome 19q13.2, was found to be involved in the development of erythroblasts. This discovery has important implications for the diagnosis and treatment of CDA-XI.

**HIF-0490 Approach to the congenital dyserythropoietic anaemia type XII gene**

A recent study by the same team of researchers identified a new gene, CDA-XII, that is responsible for this condition. The gene, located on chromosome 19q13.2, was found to be involved in the development of erythroblasts. This discovery has important implications for the diagnosis and treatment of CDA-XII.
HIF-0481 Mutations in the DC1K gene cause X linked dyskeratosis congenita and skewed X inactivation patterns in carriers

Masson PJ, Knight SW, Heiss NS, Vulliamy Tj, Stavrides G, Poustaka A, Dokalet I
Department of Haematology, ICSM, London UK and DKFZ, Department of Molecular Genome Analysis, Im Neuenheimer Feld, Heidelberg, Germany

Dyskeratosis congenita (DC) is an inherited bone marrow failure syndrome. The X-linked form of the disease is caused by mutations in the DC1K gene in Xq28 which encodes a ubiquitously expressed 58kD protein, dyskerin, which is homologous to a yeast protein that plays an essential role in ribosome formation. We have collected 88 DC families and determined the causative mutation in 34 of them by SSCP and sequencing. A single missense mutation Ala 533 Val is present in 15 unrelated families and in 6 of these the mutation is a de novo event. Altogether there are 17 different missense mutations, a splice site mutation, a single amino acid deletion and a deletion at the 3’end of the gene that removes the last 22 amino acids. This spectrum of mutations suggests that the defective protein may retain some activity and that complete lack of dyskerin would be lethal. In every family except one, heterozygous females exhibit a skewed X chromosome inactivation pattern, whereby the active X chromosome in peripheral blood cells is always the same and is the one harbouring the normal DC1K gene whenever this could be determined. Thus in 34 families for which the mutation is known the mothers’ DC1K was undetermined in 7, skewed in 18 and random in 7 of which 7 are known to have low dyskerin levels. This suggests that cells expressing the defective dyskerin have a growth disadvantage. Failure to find mutations in the other families may be due to the fact that the disease is caused by mutations in noncoding regions of DC1K or at other loci. Eight of these families have an affected female member suggesting the underlying mutation is autosomal. Mothers of affected boys from the remaining 26 families that have only affected males were tested for XCIP mutations. In 11 cases the pattern was undetermined and in 14 cases it was random and in only one case was the XCIP skewed. We conclude that DC1K mutations causing DC are mainly missense mutations, that XCIP analysis is a useful but not perfect way to define involvement of the DC1K locus and to determine carrier status and our data implicate a locus other than DC1K in a significant number of DC cases.

HIF-0482 Molecular basis of glucose-6-phosphate dehydrogenase (G6PD) deficiency in a population of Comorian origin

Martinez di Montemuros F, Badens C, Lena-Russo D, Fiorelli G, Cappellini MD
Centro Anemie Congenite, Ospedale Maggiore Policlinico IRCCS, Dipartimento di Medicina, Universita di Milano, Italy, Centro des Hémostologies, CERGM, Faculte de Medecine, Marseille, France

G6PD deficiency is characterised by a wide biochemical and molecular heterogeneity. To date approximately 400 biochemical variants have been described, grouped into 5 classes according to the level of residual enzyme activity and clinical manifestations. By contrast, only some 100 molecular defects have been identified as being responsible for these variants, showing a lower than expected genetic heterogeneity. The highest G6PD deficiency gene frequency (up to 25%) is found in tropical Africa, in the Middle East, in tropical and subtropical Asia, in some Mediterranean areas, and in Papua New Guinea. Among the most common G6PD mutations, G6PD A- is widespread all over Africa, in the West Indies, and among black Americans. G6PD Mediterranean is polymorphic in all countries surrounding the Mediterranean sea but it is also widespread in the Middle East, including Arab countries and Israel, and it accounts for almost all G6PD deficiency in Kurdish Jews. The Comoro archipelago is located in the Indian ocean and groups 4 islands, Comoro islands were originally populated by Bantu Africans coming from the Mozambic coast (G6PD allele frequency 10-15%) and more recently by immigrants from the Arabian peninsula (G6PD allele frequency 15-26%). In the last fifteen years, France has become the main hospital for Comoros migrants with in excess of 30,000 DC1K gene carriers in Marseille. From preliminary studies, the prevalence of G6PD deficiency among this population is around 5%, but no data on the molecular basis of the defect are presently available. In this study we analysed the G6PD gene in 11 deficient subjects (9 males and 2 females) originating from the Comoro islands and living in Marseille with a G6PD activity comprised between 5% and 23% of normal values. DNA analysis was carried out by PCR amplification of the G6PD coding sequence, followed by restriction endonuclease digestion. The results show that G6PD A- is widely represented in all the subjects studied. One of the female subjects has co-inherited G6PD A- and G6PD Mediterranean in a state of double heterozygosity according to the low enzyme activity observed (10% of normal). Family studies revealed that she inherited the G6PD A- allele from the father (together with 376 G>A allele typical of A+ variant) and the G6PD Mediterranean allele (together with the G1311 C>T polymorphism) from the mother who has Arab ancestry and also lacks both the A- and A+ variants. This is the first description of co-inheritance of the two worldwide most common G6PD variants fitting well with the mixed Arabian-African population of the Comoro islands.

HIF4 - Thrombophilia

HIF-0483 New causes of inherited thrombophilia

Baglin T
Department of Haematology, Addenbrooke’s NHS Trust, Cambridge, UK

Until resistance to activated protein C (APC resistance) was recognised in 1993 less than 15% of patients with a positive family history of thrombosis were found to have a genetic factor. Co-inheritance of FV Leiden, FV Mutation and PAI-1 prophenolase bridge) is the only other mutation described. Mutations affecting other critical proteins in the haemostatic network have been reported in small numbers of patients. Examples include those affecting thrombomodulin and TPII. The effects of more common polymorphisms such as the haemostatic gene (C282Y) mutation and the VV ACE gene polymorphism require clarification, as provisional reports suggest they are significant risk factors. The relative risk of venous thrombosis associated with these mutations is being evaluated in our large case-control study.

HIF-0484 Genetic factors causing thrombophilia: a role in arterial thrombosis?

Rosendaal FR
Department of Clinical Epidemiology and Thrombosis and Haemostasis Research Unit, Leiden University Medical Center, Leiden, The Netherlands

Abnormalities in the clotting system are associated with venous thrombosis. This is particularly so for abnormalities in the coagulation and the procoagulant pathways, whereas the role of the fibrinolytic system is obscure. Abnormalities increasing the risk of venous thrombosis are deficiencies of protein C, protein S and antithrombin, factor V Leiden (factor V 1691 A>G), factor II 20210 G>A, high levels of factor VIII. Because of their high prevalence, the last three are most important. The role of the clotting system in arterial disease is much less pronounced. This is most likely due to the major effect of other risk factors, notably those causing atherosclerosis (smoking, hypertension, hypercholesterolemia, diabetes mellitus), none of which affect the risk of venous thrombosis. The predominant role of these factors in the etiology of arterial cardiovascular disease may obscure the role of clotting factor abnormalities. One of the approaches to study the role of clotting factor defects in arterial disease is to study young individuals, in whom atherosclerotic disease may have progressed less, and clotting factor abnormalities may stand out more clearly to affect the risk. Data will be presented on myocardial infarction and stroke in young adults, and on the role of clotting factor defects in these individuals, which will subsequently be compared to studies in older age groups. A second approach is to study individuals with clotting factor deficiencies and look for a potential protective effect. Data will be shown on the occurrence of myocardial infarction in patients with homocysteinemia (deficiency of factor VII or factor IX).

Finally, an effect of clotting on arterial disease may be mediated by an effect of thrombosis, on atherosclerosis, or on both. Data will be presented where these effects are disentangled.
Objective. Inherited and acquired thrombophilic states are associated with an increased risk of deep vein thrombosis, but whether they are also risk factors for superficial vein thrombosis is uncertain. We assessed the risk conferred by thrombophilic states in patients with a first episode of superficial vein thrombosis. We also evaluated the role as risk factors of circumstances situations known to predispose to venous thromboembolism, such as surgery, trauma, prolonged immobilization, oral contraceptive use, and pregnancy/puerperium. Design and Methods. We investigated 218 consecutive patients with previous episodes of superficial vein thrombosis, after exclusion of patients with venous veins, malignant or autoimmune disorders, and 537 healthy individuals. In 133 of the 218 patients superficial vein thrombosis was the only thrombotic manifestation, whereas it was followed by deep vein thrombosis in the remaining 85. The G1691A mutation in the factor V gene, the G20210A mutation in the prothrombin gene, deficiencies of the normally occurring inhibitors of coagulation (antithrombin, protein C, protein S) and the antiphospholipid syndrome were searched for. Results. The prevalences of each thrombophilic state was higher in patients than in controls. The odds ratio for superficial vein thrombosis was 6.0 (95% CI, 3.2-11.2) in patients with the G1691A factor V mutation, 4.4 (95% CI, 2.9-6.2) in those with the G20210A prothrombin mutation, and 12.9 (95% CI, 3.6-45.6) in those with deficiencies of the naturally occurring inhibitors of coagulation taken together. Risks did not substantially change when patients who also developed deep vein thrombosis were excluded. Among the circumstantial risk factors, pregnancy, puerperium was the most frequently associated with superficial vein thrombosis, being present in 39% of women. Conclusions. Our findings indicate that inherited thrombophilic states are associated with an increased risk of superficial vein thrombosis. Hence, a laboratory search for these alterations is recommended in patients with superficial vein thrombosis, because it allows identification of patients at high risk of deep vein thrombosis in whom antithrombotic prophylaxis is particularly warranted.

HIF-0486 Low plasma levels of vitamin B6 are independently associated with an elevated risk of deep-vein thrombosis

Cattaneo M, Lecchi A, Lombardi R, Buccioni P, Maruzzi PM
Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, IRCCS Ospedale Maggiore, University of Milan, Italy

Objective. High plasma levels of total homocysteine (tHcy) before and after an oral methionine loading (PML) are associated with an elevated risk of deep-vein thrombosis (DVT). In the present case-control study we investigated whether plasma levels of B vitamins, which are involved in Hcy metabolism, are associated with an elevated risk of DVT. Design and Methods. We compared 352 patients with previous episodes of DVT of the lower extremity (M/F, 173/179; median age, 42y, range 10-76y) with 547 healthy controls (M/F, 228/319; 47y, 15-79y). The plasma levels of folate, vitamin B12 and vitamin B6 were measured. The following risk factors of DVT were also looked for: resistance to activated protein C; antiphospholipid syndrome; and vitamin B6 were measured. The following risk factors of DVT were also looked for: resistance to activated protein C; antiphospholipid syndrome; and vitamin B6 were measured.

Results. The prevalence of high fasting and/or PML tHcy levels was higher in cases (57/352, 16%) than those in controls (43/547, 8%). OR, 2.3, 95% CI, 1.43.7. The mean ±SEM plasma levels of folate, vitamin B12 and vitamin B6 were 6.4±3.8 mg/ml, 437±228.5 pg/ml and 36.1±32.8 nmol/L in patients and 6.7±3.6, 420.8±240.4 and 40.6±34.3 in controls (p<0.05 for all). tHcy correlated negatively with all vitamins. The table shows the odds ratios (95% CI) for DVT associated with quartiles of folate, vitamin B12 and vitamin B6.

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<th>Vitamin</th>
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<td>Folate</td>
<td>0.7 (0.4-1.1)</td>
<td>0.5 (0.3-0.9)</td>
<td>0.8 (0.5-1.3)</td>
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<tr>
<td>Vitamin B12</td>
<td>0.8 (0.5-1.2)</td>
<td>0.7 (0.4-1.0)</td>
<td>0.7 (0.5-1.1)</td>
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<tr>
<td>Vitamin B6</td>
<td>2.0 (1.3-3.2)</td>
<td>1.9 (1.2-3.0)</td>
<td>0.9 (0.6-1.5)</td>
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</tbody>
</table>

*adjusted for age, sex, fasting and PML tHcy, B vitamins, and other risk factors for DVT.

Conclusions. The study confirms that high tHcy plasma levels are associated with an elevated risk of DVT and correlate negatively with plasma levels of folic acid, vitamin B12 and vitamin B6. Moreover, it shows that low vitamin B6 levels, in addition to a link with tHcy, are independently associated with an elevated risk for DVT.
Acute myeloid leukaemia in the older patient presents specific problems due to the significantly greater co-morbidity that is seen in older individu- als. The clearance and metabolism of pharmacokinetic drugs is differ- ent from those in younger and middle age individuals and generally bone marrow reserve, liver function, renal function, cardiac function, pulmonary and other organ functions are decreased. Only in recent years have spe- cific trials been designed to develop treatment for this patient category. These studies have been both directed at the design of more effective induction chemotherapy, and at improving the supportive care. During recent years the EORTC and HDGVON cooperative groups have completed two studies in more than 800 patients 60 years and older: AML-9 and AML- 11. AML-9 is an example of a study that compared mitoxantrone Ara-c with daunomycin Ara-c induction. The AML-11 study addressed the question of using GM-CSF as an adjunct to daunomycin Ara-c induction chemotherapy. Both studies also addressed the question as to whether low- dose Ara-c post-induction treatment would be of benefit. The results of these studies will be presented in the context of other recently completed studies on the use of induction chemotherapy and the use of haemopoietic growth factors for support in older patients. A major challenge for the future will be the identification of patients with variable risks. This would allow early discontinuation of treatment in patients with a low probability of treatment success and the continued therapeutic efforts in favourable risk individuals e.g. in the interest of the quality of life of these individuals. Several recent studies have revealed that poor performance status, high blood cell counts cytogenetic abnormalities, and multigland resistance pheno- type, represent powerful prognostic factors for outcome.

**HIF-0492 Reducing the intensity of the conditioning regimen for allo- genetic haemopoietic stem cell transplantation (HCT) in elderly patients: comparison of three different schedules**


Department of Haematology, Ospedale San Martino, Genoa, Italy

We have explored different doses of thiopeta (THIO) and cyclophosphamide (CY) in the conditioning regimen of 80 patients with haematologic malignancies. The dose (expressed as mg/kg) was 15 THIO+150 CY (n=29, median age 41, median follow up 538 days), 15 THIO+120 CY (n=25, median age 43, median follow up 442 days), and 10 THIO+100 CY (n=26, median age 53, median follow up 448 days). The latter regimen was designed for patients aged 45-60: the median age was 41 (22-52), 42 (21- 50) and 53 (45-60): age of patients in the THIO10+CY120 regimen was signifi- cantly (p<0.001) higher than in the other two. The stem cell source was peripheral blood (PB) (n= 51) or bone marrow (BM) (n= 29) which was infused without manipulation. The ratio BM/PB was distribute as follows: 4/5, 14/11 and 11/13 respectively. Donors were HLA iden- tical siblings and GVHD prophylaxis consisted of conventional Cyc-MTX. The distribution of disease (p>0.06), and disease status (p=0.5) was not sta- tistically different in the 3 groups. The time to 0.5x109/l of neutrophils was 14, 14, 16 days respectively (p=ns). Platelet counts on days +20, +50, +100 were also comparable. Elderly patients receiving the THIO10+CY100 regi- men had less, although not significantly so, acute and chronic GVHD, when compared to the other two regimens (p=0.5). Chronic GVHD was more severe in patients over 45 years receiving PB grafts (p=0.4). Transplant related mortality in elderly patients was 2 (8%) vs 2 (14%) in the THIO15+ CY120 and 9 (31%) in the THIO15+ CY150 groups (p=0.09). Twenty-two patients of 24 survive in the elderly group in the THIO15+ CY120 and 48% in the THIO15 + CY150 (p=0.003). This study suggests that it is possible to allo graft patients between the age of 45 and 60 with a mild conditioning regimen: the long term outcome has to be determined.

**HIF-0493 The cytogenetic challenge for older patients with haematological malignancies**

Harrison CJ

Leukaemia Research Fund Cytogenetics Group, Department of Haema- tology, Royal Free and University College Medical School, London, UK

Haematological malignancies, in particular acute myeloid leukaemia (AML), are primarily diseases of the elderly. Overall median survival in this age group is short and many are resistant to treatment. An accurate insight into prognostic factors at diagnosis is vital to patient management. Cytogenet- ics is an independent prognostic factor (i.e. higher risk) which is good, inter- mediate and poor risk groups, which can guide the choice of therapy and prediction of response. The proportion of patients in the poor risk catego- ry is increased in the elderly in both myeloid and lymphoid malignancies. In acute lymphoblastic leukaemia (ALL) the Philadelphia chromosome (Ph) is strongly associated with poor risk and the incidence increases expo- nentially with age. Specific probes for fluorescence in situ hybridisation (FISH) allow the accurate detection of this abnormality ensuring that patients are given the appropriate treatment. In myeloid malignancies the most frequently observed poor risk karyotypes are complex and involve loss or deletion of chromosomes 5 and 7. Multiple colour FISH techniques have proved to be helpful in increasing the characterization of these chro- mosomal abnormalities and have the potential to identify new recurring chromosome abnormalities of prognostic significance.
HIF6 - Hodgkin's disease

HIF-0496 Advanced, resistant and relapsed Hodgkin's disease (HD): what is new

Dietl V, Sieber M, Ruediger U, Josting A for the German Hodgkin's Lymphoma Study Group (GHSG)

Klinik 1 für Innere Medizin, Universität zu Köln, Köln, Germany

Although HD is generally curable, the treatment results of advanced, resistant and relapsed HD appear to be inadequate. With the previous generation of chemotherapy regimens, remission rates of 80% in advanced stages have been reported, but still 30-50% of these patients relapse and less than 25% of those in first relapse can be cured. Therefore, efforts have been made to optimise the therapeutic strategies for these patients. In order to test the concept of dose intensity, the GHSG designed the new BEACOPP chemotherapy regimen. This regimen is a basic dose variant and escalated dose variant. In a large randomised multicentre trial the escalated variant of BEACOPP showed a considerable advantage in FFTR compared to the basic dose variant and the CHOP/VACOPP chemotherapy regimen. Since the introduction of ABVD in the treatment of HD, this is the first time, that a new chemotherapy regimen has shown significantly increased efficacy in a large randomised trial. In the treatment of resistant or relapsed HD improved outcomes have been reported from selected centres with high-dose chemotherapy (HDC) and stem cell transplantation. Randomised trials, with the intention of comparing conventional treatment strategies with HDC are, however, rare. The GHSG conducted a randomised trial (HDR-1) to investigate the role of HDC in chemosensitive relapses. Conventional salvage chemotherapy (four cycles of DEXA-EBAM) was compared to a HDC strategy (2 cycles of DEXA-EBAM followed by HDC) in patients with relapsed HD. Although, a preliminary analysis of this trial suggests a superiority of the HDC-arm, overall survival remains unsatisfactory for both treatment strategies. Therefore, more effective salvage strategies are needed. Sequential administration of high-dose single agents, double transplantations, new drugs and immunotherapy are now under investigation and these approaches will be discussed.

HIF-0497 Serum IL-10 elevation and failure-free survival of Hodgkin's disease treated with radiotherapy


Houston, Rochester, USA, and Milan, Italy

Objective. The malignant cells of Hodgkin's disease (HD) derived from germinal center B-cells, are often latently infected by EBV, and secrete cellular IL-10. IL-10 stimulates the growth of B-cells, rescues cells from apoptosis by increasing bcl-2 levels, and inhibits T-cell growth. Serum IL-10 levels are often elevated in patients with HD and are associated with inferior failure-free survival (FFS) after treatment with ABVD or equivalent regimens. Therefore we decided to investigate the relationship between pretreatment serum IL-10 levels and FFS in HD patients treated only with RT. Design and Methods. Pt's from MD Anderson, Mayo Clinic, and Instituto Tumori were included if treated only with RT, if age > 16 years, and if pretreatment serum was available. Serum IL-10 levels were determined with an ELISA specific for the cellular form of the protein. PS were determined by review of all records, and updated in 12/98. Results. We identified 57 pts, 27 males, 2 with B-symptoms, and median age of 30 years

Summary. For a long while it has been recognised that Hodgkin's disease (HD) is heterogeneous in terms of morphology and clinical course. In 1966 at the Kiel conference, agreement was reached on the histological classification of HD. Four sub-types were distinguished: (i) lymphocyte predominance (LPHD), (ii) nodular sclerosis (NSHD), (iii) mixed cellularity (MCHD) and (iv) lymphocyte depletion (LDHHD). In 1994 the International Lymphoma Study Group (ILSG) proposed combining nodular sclerosis, mixed cellularity, lymphocyte-depleted and the term classic Hodgkin's disease in order to emphasise the close relationship between these histotypes and their difference to lymphocyte predominance. This difference does not only concern the immunophenotype of the Hodgkin-and Reed-Sternberg (HRS) cells but also the progression of the disease. LPHD is a very indolent lymphoproliferation and it is not yet clear as to whether patients with this HD entity benefit from polychemotherapy. In contrast, immediate treatment with polychemotherapy is essential for all types of classic HD because otherwise the disease's course is fatal. The ILSG also proposed distinguishing a diffuse lymphocyte-rich HD (DNLHHD) form as a further subtype of classic HD. Within the last three years it has become evident that among classic HD there is not only a diffuse but also a nodular lymphocyte-rich category (NLCHD). The distinction of these lymphocyte-rich forms appears important because they are often confused with LPHD. Criteria to diagnose NLCHD and to distinguish it from the nodular form of LPHD will be discussed in the lecture. A further proposal of the ILSG was to distinguish a type of anaplastic large cell lymphoma (ALCL) that shares features with HD. It was called anaplastic large cell lymphoma, Hodgkin's-like (ALCL-HD-like). However, recent studies about the occurrence of the NPM-ALK fusion protein consisting of parts of nucleophosmin and ALK kinase caused by the t(2;5) showed that the ALCL-HD-like is in most cases distinct from the T-type ALCL but probably related to classic HD. Therefore the ILSG, in agreement with the WHO committees, now proposes allocating this borderline form of tumour to classic HD rather than to ALCL. It has also been agreed that this borderline tumour form should not be listed as an established lymphoma type in the new WHO lymphoma classification. In the last three years convincing evidence has been provided that all types of Hodgkin's disease represent a clonal outgrowth of lymphoid cells most commonly related to the B-cell system. Therefore at the WHO steering committee meeting in Paris 1995 Karl Lennert suggested changing HD oncology. On the basis of the data and suggestions mentioned, the following classification of Hodgkin's disease is proposed for the new WHO lymphoma classification system:

**Hodgkin's lymphoma**

<table>
<thead>
<tr>
<th>Nodular lymphocyte predominant</th>
<th>Classical Hodgkin's lymphoma</th>
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<tr>
<td>± diffuse areas</td>
<td>nodular sclerosis</td>
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<tr>
<td></td>
<td>lymphocyte-rich (nodular or diffuse)</td>
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<td></td>
<td>mixed cellularity</td>
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<td>lymphocyte depletion</td>
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**HIF-0495 Early stage Hodgkin's disease (HD); radiotherapy, chemotherapy or both?**

Radford JA

Department of Medical Oncology, Christie Hospital, Manchester, UK

The therapeutic goal in early stage HD is to achieve a high cure with the lowest possible incidence of late effects of treatment on cardiac, pulmonary and gonadal functions and incidence of second malignancy. In Manchester a strategy of minimal initial therapy (MIT) has been tested in a randomised pilot study activated in 1989. Since then 125 patients with HD have been randomised to receive either limited field RT alone (n=62). CT consisted of doxorubicin 35 mg/m2 iv weeks 1 and 3, cyclophosphamide 350 mg/m2 iv week 1, etoposide 100 mg/m2 po weeks 1-4 then tailored to zero over ten days and prophylactic ketoconazole/co-trimoxazole. Following CT in the combined modality arm, 36 patients achieved CR, 6 patients CR uncertain, 15 patients PR and 4 patients were not assessed (1 additional patient refused CT and received RT alone). After subsequent RT, 56 patients were in CR, 3 patients in CR uncertain, 2 patients in PR and one was not assessed. In the RT alone arm, 51 patients achieved CR, 6 patients CR uncertain, 3 patients PR, 1 patient failed to respond, 1 patient was not assessed and one was not assessable. Four patients experienced significant toxicity with CT; 2 developed infections requiring iv antibiotics, 1 required treatment for grade 3 mucositis and one had an exaggerated skin reaction to subsequent RT. All made a full recovery. After a median follow-up of 3.8 years, there have been 25 progressions (RT alone arm 21, CT and RT arm 4), 6 deaths due to HD (RT alone arm 5, CT and RT arm 1), 1 death due to high grade NHL (CT and RT arm; diagnosis confirmed at relapse and presentation), 2 second grade malignancies (AML 1 in each arm) and one sudden death (RT alone arm). Actuarial event-free survival (EFS) at 5 years is 56% for the RT-alone arm and 90% for the combined treatment arm (p<0.001). There is no significant difference in 5 year survival between the two arms of the trial (RT alone 89%; CTRT 94%). Although these results show that in CD I/IIA HD, four weeks of neo-adjuvant CT before limited field RT is sufficient to produce a marked reduction in the number of subsequent progressions longer follow-up is required to determine whether MIT of this type is ultimately associated with improved survival and fewer late effects.

**HIF-0494 Hodgkin's disease: classification and biology**

Stein H

Institute of Pathology, Benjamin Franklin Hospital, Free University, Berlin, Germany

For a long while it has been recognised that Hodgkin's disease (HD) is heterogeneous in terms of morphology and clinical course. In 1966 at the Kiel conference, agreement was reached on the histological classification of HD. Four sub-types were distinguished: (i) lymphocyte predominance (LPHD), (ii) nodular sclerosis (NSHD), (iii) mixed cellularity (MCHD) and (iv) lymphocyte depletion (LDHHD). In 1994 the International Lymphoma Study Group (ILSG) proposed combining nodular sclerosis, mixed cellularity, lymphocyte-depleted and the term classic Hodgkin's disease in order to emphasise the close relationship between these histotypes and their difference to lymphocyte predominance. This difference does not only concern the immunophenotype of the Hodgkin-and Reed-Sternberg (HRS) cells but also the progression of the disease. LPHD is a very indolent lymphoproliferation and it is not yet clear as to whether patients with this HD entity benefit from polychemotherapy. In contrast, immediate treatment with polychemotherapy is essential for all types of classic HD because otherwise the disease's course is fatal. In both HD, in agreement with the WHO committees, now proposes allocating this borderline form of tumour to classic HD rather than to ALCL. It has also been agreed that this borderline tumour form should not be listed as an established lymphoma type in the new WHO lymphoma classification. In the last three years convincing evidence has been provided that all types of Hodgkin's disease represent a clonal outgrowth of lymphoid cells most commonly related to the B-cell system. Therefore at the WHO steering committee meeting in Paris 1995 Karl Lennert suggested changing HD oncology. On the basis of the data and suggestions mentioned, the following classification of Hodgkin's disease is proposed for the new WHO lymphoma classification system:
(range 16-74). Ann Arbor Stage was I in 22, II in 31, and III in 4 pts. Histology was nodular sclerosis in 32, mixed cellularity in 5, lymphocyte predominance in 17, and unclassified in 3 pts. Anemia was seen in 13, inguinal involvement in 3, low serum albumin in 3, high serum LDH in 7, and serum β₂-microglobulin > 2.5 mg/dL in 4 pts. Pretreatment serum IL-10 was high (> upper limit of normal) in 22 pts. With median follow-up of 42 months for the survivors, the projected 5-year FFS for all pts was 65%. For those with high vs. normal IL-10 it was 48% vs. 78%, respectively (p = 0.01 by logrank). Conclusions. In this group of RT-treated HD pts who had serum available, elevated serum IL-10 was associated with inferior FFS, even in the absence of B-symptoms. Additional work is needed to confirm this in other patient populations and to determine whether serum IL-10 levels may be useful in predicting relapse after RT. The relationship between serum IL-10 levels, EBV infection of HD cells, and the cell(s) secreting IL-10 is under investigation.

**HIF-0498 High dose therapy with autologous stem cell transplantation in Hodgkin’s disease in first relapse: experience of the Spanish GEL TALMO group**


Two hundred and thirty-one patients (pts) diagnosed as having Hodgkin’s disease (HD) and autografted in first relapse were reported to the Spanish GEL TALMO group on behalf of 135 patients (pts) who had serum available, elevated serum IL-10 was associated with inferior FFS, even in the absence of B-symptoms. Additional work is needed to confirm this in other patient populations and to determine whether serum IL-10 levels may be useful in predicting relapse after RT. The relationship between serum IL-10 levels, EBV infection of HD cells, and the cell(s) secreting IL-10 is under investigation.

**SS1 - Chronic myeloproliferative disorders - Biology**

**SS-0499 Fragile sites containing region (FSR) involvement in 5q deletions: a marker of genomic instability?**


*Department of Haematological Medicine, King’s College of Medicine, London, UK*  
*Department of Biomedical Sciences, Haematology Section, Università di Ferrara, Italy

Fragile sites are specific regions of chromosomes that are prone to breaks, deletions and rearrangements when exposed, in vitro, to carcinogens. A concordance has been found between the involvement of FSR and non-random abnormalities detected in cancers, leading to the hypothesis that FSR may play a mechanistic role in the recurrence of chromosomal abnormalities. In a series of 101 patients with myeloid malignancies and 5q deletions (5q- syndrome, n=14; RA/RARS, n=9; RAEB/RAEB-t, n=2; M2, n=27; other myeloid malignancies, n=9), we found that 69.7% of the breakpoints on 5q were associated with FSR. In 80% of the cases, 5q31 in 28.3%, 5q35 in 19.8%, 5q15 in 8.4% and 5q21 in 0.8%. In 41.6% of the patients deletions involved 2 FSR, while in 47.5% and 10.9% one or no FSR was involved, respectively. The involvement of 2 FSR was associated with complex karyotypic evolution of the chromosome and a more frequent involvement of FSR in other chromosomal abnormalities (p<0.001), while no difference was observed for non-FSR. No association was found between specific cytogenetic abnormalities and the involvement of FSR in 5q deletions even though a trend was observed for the presence of trisomies and/or deletions on chromosomes other than chromosome 5. Our data suggest that the factors which drive FSR breakage could also be involved in some of the observed chromosomal abnormalities. These FSR are prone to breakage and recombination when a cell become genetically unstable. There is some evidence that this could occur through a dysregulation of genes involved in DNA synthesis or DNA mismatch repair and that exposure to genotoxic agents may accelerate DNA instability by targeting FSR. These observations could explain the higher incidence of 5q deletions and additional chromosomal abnormalities among patients with myeloid malignancies and a history of occupational exposure to myelotoxic substances. The association between FSR involvement and karyotype complexity suggests that FSR involvement could predict an aggressive disease. Therefore, we propose that genomic alterations at FSR such as deletions may be sensitive indicators for a higher instability of the genome and disease progression in a subset of patients affected by myeloid malignancies. However, in the final analysis, this hypothesis can only be proved when these FSR are cloned and characterised in a clinical setting.

**SS-0500 Tyrosine phosphorylation of CD34 regulates progenitor cell adhesion and proliferation: defective CD34 function may play a role in chronic myeloid leukaemia**

Gordon MY, Marley SB, Grand FH, Lewis JL, Nguyen DX, LeMarer N, Lloyd S, Goldman JM

*LRF Centre for Adult Leukaemia, Department of Haematology, Imperial College School of Medicine, Hammersmith Campus, DuCane Road, London, UK

The function of CD34, a transmembrane sialomucin expressed by human haemopoietic progenitor cells, is poorly understood. Its structure suggests it may act as a cell adhesion and signalling molecule. We used a quantitative aggregation assay and found that 70±2% (mean±SD; n=7) of CD34-positive cells (purified by MiniMACS technology) in human bone marrow aggregate when incubated with the CD34 monoclonal antibody QBEND 10. Moreover, cells from patients who were being treated at the time of the experiment exhibited higher levels of aggregation. The aggregation status of CD34 was the only prognostic factor influencing FFS, while a short first CR, ASCT with active disease and the use of TBI reduced OS. In summary, the best candidates for ASCT after a first relapse of HD are those in CR after salvage CT with a long first CR and the best conditioning regimen appears to be high-dose CT.

**SS-0503 Tyrosine phosphorylation of CD34 regulates progenitor cell adhesion and proliferation: defective CD34 function may play a role in chronic myeloid leukaemia**

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Aggregation of p210 transduced cells was 30% that of untransduced controls. This deficiency was counteracted by IFN-α when aggregation increased to 60% of control values (p<0.001). We conclude that CD34-mediated cell binding is an important regulator of normal progenitor cell proliferation, is abnormal in CML, probably as a result of p210 expression, and may be a target for the therapeutic activity of IFN-α.

SS-0501 Frequent deletion of hSNF5/INI1, a component of the SWI/SNF chromatin complex, during progression of CML
Kulkarni S, Grand F, Chase A, Goldman JM, Gordon M, Cross NCP
Department of Haematology, Imperial College School of Medicine, Hamersmith Hospital, London, UK

During routine two fusion FISH analysis of patients with blast crisis of chronic myeloid leukemia (CML), we observed YAC 29G7D7, which is distal to BCR at 22q11, failed to hybridise to the 9q + derivative chromosome in 3/11 (27%) of cases. This deleted region is close to hSNF5/INI1, a gene that encodes a widely expressed component of the SWI/SNF chromatin remodeling complex and is known to suffer biallelic mutations in malignant rhabdoid tumors. To determine whether hSNF5/INI1 is also deleted in patients with CML, we performed FISH analysis with a specific cosmid probe. Deletion of hSNF5/INI1 on the 9q+ chromosome was found in 9/25 (36%) of cases in blast crisis (lymphoid, n=3; myeloid, n=6). For the 3 of these 9 patients for whom material was available prior to transformation, deletions were also seen in chronic phase indicating that they are early events. Analysis of additional 21 patients in chronic phase revealed heterogeneity of deletions of hSNF5/INI1 in 5 (24%) cases. BM samples obtained from our ET patients or from normal donors tested failed to demonstrate the presence of BCR-ABL transcripts in any of the 25 samples.

A recent report suggested the presence of BCR-ABL transcripts in the bone marrow aspirates of patients with chronic myelogenous leukemia (CML). We failed to detect by more sensitive techniques in these patients or even in normal donors; b) whatever the frequency of this anyhow rare event, and considering the natural clinical history of ET patients, we did not observe cases with the 9q+ derivative chromosome.

Essential thrombocythemia (ET) and polycythemia vera (PV) are chronic myeloproliferative disorders (MPD) characterized by a high incidence of thrombo-haemorrhagic events. Abnormalities of platelets and red cells have been extensively described, however no studies have been conducted on the status of activation of circulating polymorphonuclear leucocytes (PMN), in these disorders. PMN can be involved in haemostasis and are implicated in thrombogenesis and endothelial damage. The aim of this study was to evaluate, in a group of patients with MPD (28 ET and 25 PV), the PMN activation status (CD11b antigen expression, plasma elastase levels and PMN elastase levels), and the relationship between PMN activation markers and plasma markers of hypercoagulation (TAT, Fd+2 and D-dimer) and endothelial perturbance (thrombomodulin (TM) and von Willebrand Factor antigen (vWF:Ag)). The results showed the occurrence of PMN activation in the MPD patients compared to a control group of healthy subjects (C). CD11b expression by PMN cell membrane was statistically higher in MPD patients than in controls (C), while vWF:Ag levels were also greater in MPD patients compared to controls (C).

Hematology Division, Ospedali Riuniti, Bergamo; and *Consorzio Mario Negri Sud, Santa Maria Imbaro, Chieti, Italy

SS-0503 Neutrophil activation and haemostatic changes in patients with essential thrombocythaemia and polycythaemia vera
Hematology Division, Ospedali Riuniti, Bergamo, and *Consorzio Mario Negri Sud, Santa Maria Imbaro, Chieti, Italy

Essential thrombocythemia (ET) and polycythaemia vera (PV) are chronic myeloproliferative disorders (MPD). CD11b and CD18 expression, plasma elastase levels and PMN elastase levels, and the relationship between PMN activation markers and plasma markers of hypercoagulation (TAT, Fd+2 and D-dimer) and endothelial perturbance (thrombomodulin (TM) and von Willebrand Factor antigen (vWF:Ag)). The results showed the occurrence of PMN activation in the MPD patients compared to a control group of healthy subjects (C). CD11b expression by PMN cell membrane was statistically higher in MPD patients than in controls (C), while vWF:Ag levels were also greater in MPD patients compared to controls (C).

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SS2 Non-Hodgkin’s lymphoma – Biology

SS2-0507 API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with MALT lymphomas

*Center for Human Genetics, Flanders Interuniversity Institute for Biotechnology, K.U. Leuven, Belgium; †Department of Oncology and Hematology, University Hospital Eppendorf, Hamburg, Germany

Malignant marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) is the commonest subtype of lymphoma arising in extranodal sites. The t(11;18)(q21;q21) appears to be the key genetic lesion and is found in approximately 50% of cytogenetically abnormal low-grade MALT lymphomas. We demonstrate that the API2 gene, encoding an inhibitor of apoptosis also known as c-IAP2, HAPl and MIHC, and a novel gene on 18q21 named MLT, are recurrently rearranged in the t(11;18). In both MALT lymphomas analysed, the breakpoint in API2 occurred in the intron separating the exons coding respectively for the baculovirus IAP repeat (BIR) domains and the caspase recruitment (CARD) domain. The breakpoints within MLT differed but the open reading frame was conserved in both cases. In one case, the translocation was accompanied by a cryptic deletion involving the 3′ part of API2. As a result the reciprocal transcript was not present, strongly suggesting that the API2-MLT fusion is involved in the oncogenesis of MALT lymphomas. The effects of expression of the fusion gene and the potential role of the 11q deletion on apoptosis in model systems are under investigation. The molecular cloning of the API2-MLT fusion allows the development of RT-PCR and FISH-based diagnostics. The frequency of the t(11;18) and its eventual association with a specific subtype of marginal zone B-cell lymphoma is being analysed.

SS2-0506 Cytogenetic findings in a series of 32 patients with splenic marginal zone B-cell lymphoma

Laboratori de Citologia Hematologica, |Lab. Ref. Catalunya, Unitat d’Hematologia, Hospital del Mar|Espania, IMIM, Barcelona|CICECHG, Spain

Introduction. Splenic marginal zone B-cell lymphoma (SMZBCL) is a recently recognised entity of which the clinical, morphological and immunohistological characteristics are well established (Harris et al, 1994). Nevertheless uncertainty about the genetic features do still exist. SMZBCL has a very peculiar characteristic and a well known histologic picture. We have studied 32 SMZBCL patients, combining conventional cytogenetics with the in situ hybridisation (ISH) technique. Design and Methods. We studied 32 cases of SMZBCL. The diagnosis was ascertained in all cases after studying splenomegaly specimens morphologically and immunologically. Cytogenomic analysis was carried out on lymphoid cells from peripheral blood, spleen and lymph nodes. Phosphomammagustin and phosphorytate were used as mitogens. FISH was performed with a chromosome 12-specific alpha satellite DNA probe to detect trisomy 12, chromosome 3-specific alpha satellite DNA probe to detect trisomy 3, and in situ suppression hybridisation of DNA from chromosomes 1, 3, 5, 7, 8, 11, 13, 14, and 16 (the chromosomes preferentially involved in patients with NHL). Results and Discussion. Cytogenomic abnormalities were found in 21132 patients (66%) being identified in 13 as a complex anomaly. The most frequent recurrent abnormalities were: del (3), del (7q) and involvement of chromosomes 1, 3, 7 and 8. No patients showed t(11;14). The most observed chromosomal breakpoints were: 1;2q (2), 3p23 (3), 7q22-23 (7), 8q24 (2) and 13q14 (2). The confirmation of 7q involvement in larger series might suggest that this anomaly could be a genetic marker of SMZBCL. An outstanding finding is the low incidence of trisomy 3 (19%) compared to the incidence in patients with MALT lymphoma. Our findings support the interpretation that considers SMZBCL as a distinct lymphoproliferative disorder.
SS5 - Acute lymphoblastic leukaemia


The molecular characterisation of the angiogenic phenotype associated with acute lymphoblastic leukaemia (ALL), thereby identifying targets for therapeutic intervention. Design and Methods. The presence of angiogenic modulators on the BM plasma from ALL patients was evaluated by both molecular (ELISA, Western Blot, immunofluorescence) and functional (endothelial cell proliferation and three-dimensional organization) analyses. BM plasma was obtained from BM diagnostic samples, and compared with BM plasma obtained from disease-free individuals. Results. Bone marrow (BM) plasma from ALL patients induced the formation of tubular-like structures of autologous or allogeneic BM endothelium in a gel rich in extracellular matrix proteins. The formation of such structures occurred as early as 4 hours after exposure to the BM plasma and could be mimicked by the use of rhuVEGF and bFGF. The presence of these modulators of angiogenesis in the BM plasma was confirmed.

Conclusions. The expression of these angiogenic modulators in BM plasma may have a major impact in the treatment of ALL.

SS-0510 Correlation between hepatitis C virus infection and clinico-pathological data in 239 overt NHL patients


Background and Objective. Recent epidemiological studies suggested an association between hepatitis C virus (HCV) and B-lymphoproliferative diseases, including non-Hodgkin's lymphomas (NHL) and mixed cryoglobulinaemia (MC). The association between HCV and NHL shows geographical heterogeneity: a prevalence of HCV + between 5% and 42% was reported in studies from Italy, whereas in Great Britain this association was not confirmed. We studied the prevalence of HCV infection in a series of NHL cases and correlated virological findings with clinico-histological features.

Methods. One hundred patients from the same area, affected by other onco-haematological diseases, for the presence of HCV-RNA sequences (by RT-PCR) and of cryoglobulins. Histological diagnosis was made according to the REAL classification. Their sera were tested by ELISA and RIBA for the presence of anti-HCV antibodies. Results. HCV antibodies were present in 19.2% (46/239) of NHL patients and were documented before the diagnosis (≤1 years) in most of the positive cases. The prevalence of HCV infection in the general population in Italy varies between 0.8% and 2.8%; in our control group this prevalence was 4%. Of interest, the highest rate of HCV+ was found in marginal zone lymphomas (31.8%) (in particular in non-gastrintestinal MALTomas) and lymphoplasmacytoid lymphomas (28%). Primary extranodal localisation of NHL was significantly more frequent in HCV-positive patients (51.1% vs 26.4%, p = 0.005). Saliary glands, tonsils, liver and spleen were frequently involved. Patients with HCV-infection were significantly older (84% over 50 years vs 66%, p = 0.05). None of the 14 T-NHL were HCV+. Viral RNA was found in 92% of tested patients, cryoglobulins in 76.1% of the HCV-positive patients. Conclusions. Our findings strengthen the hypothesis of a pathogenetic link between HCV and B-NHL in our geographical area and suggest an association with particular histotypes and primary extranodal localisation of NHL.

SS-0511 Analysis of ETV6 abnormalities in ALL: incidence, alternative spliced forms and MRD value


Haematopathology Department, Royal Free Hospital, London, UK.

The t(12;21)(p13;q22) translocation resulting in the fusion of the ETV6 and AML1 genes, occurs in 20-25% of paediatric acute lymphoblastic leukaemia (ALL). It is associated with a good prognosis and consequently the identification of the fusion product has prognostic implications. The aim of this project was threefold: (1) to assess the frequency of the fusion gene in a random group of childhood ALL patients; (2) to identify alternative forms of ETV6/AML1 transcripts; (3) to make use of the fusion product for the investigation of minimal residual disease (MRD) in 6 ALL patients (23 bone marrow samples analysed). Total RNA from 61 paediatric ALLs was tested using primers for ETV6 (exon 5) and AML1 (exons 3-6) using two rounds of RT-PCR. Results. ETV6/AML1 fusion was detected in 23 of 61 patients (37%). In all patients the fusion product was between ETV6/E5 and AML1/exd. In only 2 of 15 cases loss of either exon 4 or exon 5 of AML1 (1 case each) could be confidently detected in alternative spliced products. In one patient at least, t(12;21) appears to be a subclonal event. MRD analysis showed disappearance of detectable signal at 1 month in 3 of 4 de novo ALL and in 1 of 2 patients in relapse. These patients remained negative for between 10 and 22 months after presentation. However, in 2 patients (1 de novo and 1 in 1 relapse) a clinical relapse was preceded by negative tests, 4 and 3 months earlier. Conclusions. ETV6/AML1 is the most frequent chromosomal abnormality in acute lymphoblastic leukaemia. The breakpoints appear to be consistently found downstream of ETV6/exon 5 and upstream of AML1/exon 2. Finally, the predictive value of this fusion product for MRD is in doubt until confirmed by larger studies.

SS-0512 Angiogenic phenotype of acute lymphoblastic leukaemia: implications for the design of novel therapeutic strategies

Veiga JP, Sallan SE, Nadler LM, Cardoso AA.

Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA; “Abeel Salazar” Institute for Biomedical Sciences, Oporto University, Oporto, Portugal.

Objective. The molecular characterisation of the angiogenic phenotype associated with acute lymphoblastic leukaemia (ALL), thereby identifying targets for therapeutic intervention. Design and Methods. The presence of angiogenic modulators on the BM plasma from ALL patients was evaluated by both molecular (ELISA, Western Blot, immunofluorescence) and functional (endothelial cell proliferation and three-dimensional organization) analyses. BM plasma was obtained from BM diagnostic samples, and compared with BM plasma obtained from disease-free individuals. Results. Bone marrow (BM) plasma from ALL patients induced the formation of tube-like structures of autologous or allogeneic BM endothelium in a gel rich in extracellular matrix proteins. The formation of such structures occurred as early as 4 hours after exposure to the BM plasma and could be mimicked by the use of rhuVEGF and bFGF. The presence of these modulators of angiogenesis in the BM plasma was confirmed.

Conclusions. The expression of these angiogenic modulators in BM plasma may have a major impact in the treatment of ALL.

SS-0513 Use of highly sensitive FISH method to detect double BCR/ABL fusion signals in adult acute lymphoblastic leukaemia

Mancini M on behalf of the GIMEMA Cooperative Study Group Hematology, Department of Cellular Biotechnology and Hematology, University “La Sapienza”, Rome, Italy

Although t(9;22) is a well-recognized chromosomal abnormality associated with acute lymphoblastic leukaemia (ALL), its role in ALL has been so far investigated in small series of selected patients and in single cases with variant Ph translocations. In such studies, probes for the BCR probes in chronic myelogenous leukaemia is well documented, its role in ALL is unclear. While the presence of double BCR/ABL fusion signals in adult ALL patients is still under investigation, the use of a highly sensitive FISH method to detect double BCR/ABL fusion signals in adult ALL cells will likely have a major impact in the treatment of ALL.
FISH, the number of false positive cells decreased dramatically. In fact, the percentage of cells with a coincidental fusion signal found in normal controls was only 1.2% and the cut-off level to avoid false positive result was fixed at 3% (mean±SD). The results were compared with conventional cytogenetics (CC) and RT-PCR in order to evaluate the reliability and specificity of this FISH method for the detection of the Ph translocation. Four of the 34 cases studied (11%) proved not evaluable by FISH because of the extremely small size of the nuclei, probably due to bone marrow cell damage related to the overnight sample dispatch. In the remaining 30 samples, interphase D-FISH detected a BCR/ABL fusion in 9 (30%) with a high percentage of rearranged nuclei (median 55% range 48-84%). CC was carried out successfully in 21/30 cases (70%) and a classical t(9;22) was identified in 6 (20%); of the 3 remaining cases with a BCR/ABL rearrangement by D-FISH, 2 were not evaluable and 1 had an apparently normal karyotype. RT-PCR was performed in 26/30 cases and a BCR/ABL fusion gene was found in 7 (27%); all these cases were also positive by FISH. One case with a t(9;22) by CC, confirmed by D-FISH, proved repeatedly negative by RT-PCR. Of the 3 remaining cases negative by D-FISH, interphase D-FISH emerges as a very reliable tool for the detection of BCR/ABL in ALL patients at diagnosis. Its sensitivity is clearly higher than that of CC and CC proved superior to RT-PCR in cases with unusual BCR/ABL breakpoints.

SS-0514 GIMEMA trials for adult (>12 yrs) acute lymphoid leukaemia

Mandelli F, Annino L. on behalf of the GIMEMA Cooperative Study Group Hematology, Department of Cellular Biotechnology and Hematology, University "La Sapienza", Rome, Italy

The 1st trial, ALL 0183, was designed to test its feasibility and the efficacy of randomised post-CR therapy in a variety of even small hospitals. The whole treatment lasted 14 mos (Mandelli et al., 1988). From 1/1983 to 4/1986, 379 pts – median age 31 yrs – entered: 79.3% were CRs, 13.1% refractory and 7.4% ID. Median OS was 21 mos, median CR length 20 mos: 10 yrs DFS estimate was 25%.Of 284 CRs, 195 relapsed: 125 in pts of randomised post-CR therapy in a variety of even small hospitals. The rate (p=0.001), DFS (p=0.0003), EFS (p=0.0001). In the 4th trial, ALL0288, active from 1/1988 to 4/1994, was designed to verify the impact of 7 day PDN pre-treatment on a large cohort of pts, the efficacy of a randomised 4 vs 5(Cy+)-drug induction on CR rate, and the impact of randomised post-CR therapy-maintenance(M) vs v’s consolidation(CHM) (Mandelli et al., 1993). Of 794 pts, 778 (median age 27 yrs) were evaluable for response: CR rate was 82% (81% in Cy+, 83% Cy-), 11% of pts were resistant and 7% ID. Median CR duration was 28 mos, 8 yr CCR was 33%, median DFS was 24 mos, 9 yr DFS was 29%: DFS comparison 7/86 pts (3 deaths, 4 exclusions from the trial). Toxic death rate in this latter pts median DFS was 29 mos.

SS-0515 Fractionated cyclophosphamide plus IVAP as five-drug regimen for acute lymphoblastic leukaemia

Badalona, Spain

Objective. To analyse the preliminary results of the ongoing multicenter prospective randomised protocol PETHEMA ALL-93 for HALL patients. Design. Induction (5wk): vincristine (VCR), daunorubicin, prednisone, asparaginase (ASP), cyclophosphamide (CFM). Intensification: 3 cycles of monthly intensive chemotherapy including VCR, dexamethasone, mitoxantrone, CFM, high-dose ASP, methotrexate (MTX) and cytarabine, as well as teniposide and mercaptopurine (MP). Patients with HLA-identical sibling received ALL0-HCT, and the remaining were randomised to AUTO-HCT or to the same intensive chemotherapy followed by bone marrow or peripheral blood stem cell transplantation (MP, MTX) for 2 yr. Characteristics of the series. 23 hospitals, 118 evaluable pts, 72 males, age (±SD) 25±13 yr (range 1-50), 95 adults, WBC (±SD) 106±151 (0.5-822), ALLI 41, ALL2 77, early pre-B 32, common+pre-B 44, T 42, MyALL 50. Cytogenetics (n=107): normal 35, no translocation 35, t(9;22) 12, t(4;11) 1, t(8;14) 1. Documented infections and extra-haematological toxicity occurred at comparable rates (48%/50% and 48%/56% respectively), with infections being more frequent in pts undergoing an HCT.

Conclusions. The preliminary results of the ongoing PETHEMA ALL-93 trial for HALL patients are promising. They confirm the feasibility of the protocol.

**Intention-to-treat analysis. In brackets, 95% CI. °Medians (months).**
We have previously established the importance of diagnostic cytogenetics as a key independent prognostic factor in children and younger adults with AML (MRC AML10), providing the framework for a stratified treatment approach to this disease, adopted in the subsequent MRC AML 12 trial. On the basis of complete remission (CR) rate, relapse risk (RR) and overall survival (OS), 3 prognostic groups were defined: AML associated with t(8;21), t(15;17) or inv(16) predicted a relatively favourable outcome. Whereas, in patients lacking these favourable changes, the presence of a complex karyotype (≥5 unrelated abnormalities), t(5q), -7 or abnormal 3p was linked with a relatively poor prognosis. The remaining groups were found to have an intermediate prognosis. Here, we sought to determine whether these cytogenetic risk groups are also predictive of outcome in older adults with AML, through analysis of cases entered into MRC AML 11. Karyotype analysis was successful in 922 patients (90%) (median 66 years), including 208 with 2+ AML. The most frequent abnormalities were as follows: complex (13%), del(7q)- (13%), del(5q)- (12%), +8 (10%), +18 (7%), -17q(10%), +21 (3%), whilst in 48% a normal karyotype was reported. Adverse risk cytogenetic abnormalities were more common in older patients (AML11, 19%; AML10, 10%), whilst favourable risk aberrations were less frequent (8% vs 23%). Cytogenetic risk groupings defined in AML10 retained their predictive value amongst older patients with AML. CR rate amongst the intermediate cytogenetic risk group in AML11 was 60%, with 22% patients defined as having resistant disease (RD); RR and OS at 3 years were 76% and 15%, respectively. The group with favourable cytogenetic abnormalities was found to have a superior CR rate (72%, p<0.001), reflecting relatively low levels of RD (7%, p<0.001). Furthermore, RR in this group was lower (54%, p<0.001) compared with superfi cal OS (39%, p<0.001). Conversely, patients with adverse cytogenetic abnormalities were found to have an extremely poor prognosis: only 29% achieved CR because of high rates of RD (53%), RR was 92% associated with superior OS at 3 years of 7%. These differences remained significant when stratified by age, type of leukaemia (de novo/+2) and WBC at presentation. These findings highlight the importance of karyotype as a critical determinants of outcome in older patients with AML, and provide a rationale for the development of stratified treatment approaches based on diagnostic cytogenetics in this age group in future studies.

**SS-0518** Fluorescent in situ hybridisation analysis of the short arm of chromosome 12 in myeloid malignancies

Bilhou-Naboka C, Bernard Ph, Ghegnerjei A, on behalf of the Groupe Français Cytothérapie Hématologique (GFC)-Laboratoire d’Hématologie, Université Victor Segalen Bordeaux 2, France; Center of Human Genetics, Catholic University of Leuven, Leuven, Belgium

Objective. Diversity and frequency of recurrent rearrangements involving the short arm of chromosome 12 (12p) in a broad spectrum of haematological malignancies underline the crucial role of this region in leukaemogenesis. EV6 gene, located on 12p13, is frequently involved in balanced translocations. Using fluorescent in situ hybridisation (FISH), we studied the 12p region to identify new recurrent ETv6 partners, other breakpoint clusters regions outside ETv6 locus and to determine the smallest common region of 12p deletions in myeloid malignancies. Design and Methods. Patients with myeloid malignancies (acute myeloid leukemia (AML), myelodysplastic or myeloproliferative syndromes) and various 12p abnormalities were included in this study. FISH was performed on bone marrow cytogenetic spreads, using eight directly labeled probes located on 12p11 to 12p13, centromere 12 and 12p painting probes. Results. Seventy-four samples from 12 different centres were collected. Karyotypes were reviewed by 4GEF investigators according to FISH analysis criteria. Various chromosome aberrations were observed: balanced or unbalanced translocations (63%), insertions (2%), deletions (20%), additions 10%, duplications (5%). Complex karyotypes associated with a complete or partial deletion of chromosome 5 and/or 7 were observed in 36% of cases. Recurrent translocations were: t(3;12)(q26;p13)(3/4 cases), t(4;12)(q11-p13)(3 cases). Two other breakpoint regions, one centromeric to ETv6 (2 cases) and one telomeric to ETv6 (5 cases) were found and further investigated. In 35 cases, 12p deletion was observed, partial in 9 cases and associated with an unbalanced translocation in 5 cases. The smallest region of deletion was flanked by ETv6 (12p23) and GD204 (12p12) loci. No breakpoint cluster region was observed on 12p11 (17 cases). Conclusions. ETv6 gene remains the main breakpoint cluster region in myeloid malignancies, never associated with a deletion of the second allele. The recently cloned recurrent t(4;12), was identified in three cases, associated with AML-M0 phenotype, CD7-positive. The smallest deletion region always involved ETv6 and CDKN1B as previously described.

**SS-0519** Deletion of 6q16-21 in acute leukemias: a mapping and deletion-analysis study

Jackson A, Carrara P, Duve K, Papainnou M, Barnett T, Harrison CJ, Foroni L, Royal Free and University College School of Medicine, London, UK

Deletion of the long arm of chromosome 6 (6q) is a common chromosom al abnormality in human lymphoid malignancies, occurring in 4-13% of cases of acute lymphoblastic leukaemia (ALL) and 20-30% of non-Hodgkin’s lymphoma (NHL). Two distinct regions of minimal deletion (RMD) have been identified by loss of heterozygosity (LOH) studies at 6q25-27 (RMD1), predominantly in NHL, and RMD-2 at 6q21-23 in ALL, suggesting the presence of one or more tumour suppression genes. Although a recent study by fluorescence in situ hybridisation (FISH) has narrowed RMD-2 to a 2 Mb interval between the two markers D6S447 and D6S246, there is limited information on the region between these two markers. We have constructed a partial YAC STS contig map by initiating two yeast artificial chromosome (YAC) walks from the markers which flank RMD-2 region. The relative position of a number of markers was tested and new markers were identified. As a result of this study, we have extended the previously existing contig by approximately 600 kb between markers D6S447 and D6S246. This partial contig is composed of 17 overlapping YACs identified by 8 previously published STSs, 1 previously published EST, 9 novel YAC-end STSs and 1 novel EST. This contig has also allowed the accurate localisation of the brain-specific gene, HTLX (Genomics, 50, 34-43; 1998), that is thought to be involved in human brain development and the cell surface antigen CD24. Deletion of the YAC clones was investigated using dual colour FISH on a panel of 27 patients with acute leukaemia selected on the basis of a cytogenetically detectable 6q deletion. These studies have identified two distinct and non-overlapping deletions in 6q21-23 in ALL: (i) proximally (RMD-2A) around marker D6S246 and (ii) distally (RMD-2B) to marker CHLC.GGAT16C02.
SS-0521 RT-PCR detects TEL/AML1 but not MLL/AF4 expression in the cord blood of healthy new-borns

Trka J,* Zuna J,* Hrusák O, *Kalivova M,* Mužíková K,* Stávý J* for Paediatric Haematology Working Group in the Czech Republic

Division of Haematology, 2nd Department of Paediatrics; Institute of Immunology, 4th Medical School, Charles University, Prague, Czech Republic

TEL/AML1-positive acute lymphoblastic leukaemias (ALL) have been found to be of in utero origin in three cases of identical twins (1). Expression of the MLL/AF4 fusion gene, found typically in infant ALL, has recently been described in foetal haematopoietic tissues, the bone marrow (BM) of a healthy infant and BM of 13% of non-infant paediatric patients with t(6;11)-negative ALL (2). We applied a two-round reverse transcriptase-polymerase chain reaction (RT-PCR) approach for the detection of TEL/AML1 transcripts in cord blood (CB) samples of 235 healthy new-borns. Three of the analysed samples showed very low TEL/AML1 expression (less than 10-5 when compared to the limiting dilution series of TEL/AML1-positive leukaemic cells). However, we performed a parallel series of experiments which, although having comparable sensitivity to that of RT-PCR assay, could not prove the presence of MLL/AF4 transcripts in CB. Moreover, since June 1994 we have prospectively analysed BM samples from 235 newly diagnosed children with ALL for the presence of MLL/AF4 by two-round RT-PCR. We found 7 of 11 infant patient samples to be positive, contrasting with only 1 out of 224 (0.4%) from children aged 1-18 years. We therefore found no evidence for the hypothesis that expression of MLL/AF4 fusion gene may occur in the normal haematopoietic tissues of healthy individuals. Our findings concerning TEL/AML1 expression in CB of healthy new-borns need further elucidation. Nevertheless, together with the results of Ford et al. (1) these data are intriguing.

2. Uckun FM et al., 1998, Blood 92: 810-21

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SS-0522 Dissemination of gastric MALT lymphomas

Hofer G, Dragovic B,* Beham-Schmid Ch, Schauer S, Winter E, Lackinger E, Chott A
Institutes of Pathology, Universities of Graz and *Vienna, Austria

Objective. The goal of this study was to investigate the clonal relationship of gastric, concurrent or subsequent lymphomas occurring at sites other than the intestine. Design and Methods. Genomic DNA extracted from paraffin-embedded tissue was analyzed by PCR using primers amplifying the complementarily determining region (CDR) 3 and by cloning and sequencing of the PCR products. Results. As shown for concurrent gastric and intestinal lymphomas, the same clonal rearrangements of the immunoglobulin heavy chain gene were detected in three cases of gastric MALT lymphomas and in the concurrent lesions of the conjunctiva (two cases) and salivary gland (one case, respectively). Identical clonal rearrangements were also detected in a gastric MALT lymphoma and in a high grade B-cell lymphoma of a cervical lymph node which had developed after 18 months. In contrast, clones which differed in size and sequence of the CDR 3 were observed in a gastric MALT lymphoma and in a MALT lymphoma of the large intestine which had occurred seven years later. Conclusions. Our findings suggest that gastric lymphomas of MALT type may also disseminate to sites other than the intestine, such as ocular adnexae, salivary gland and non-regional lymph nodes. Subsequently developing high grade B-cell lymphomas of MALT, however, may also arise independently at gastric and intestinal sites.

SS-0523 Pseudohomozygosity for activated protein C resistance is a risk factor for venous thrombosis

Castaman G, Tosetto A, Ruggeri M, Rodighiero F
Department of Haematology and Hemophilia and Thrombosis Center, San Bartolo Hospital, Vicenza, Italy

Pseudohomozygosity for activated protein C resistance (APC r) is a rare condition due to the association of heterozygous FV Leiden mutation and partial type I FV deficiency. To assess the risk of venous thromboembolism in these subjects, seven families including 11 pseudohomozygotes and 45 relatives were examined. Sixteen of the relative members were heterogeneous for FV Leiden mutation, 9 had isolated partial FV deficiency and 20 showed no abnormalities. Four of the 11 (36.3%) pseudohomozygotes and 6/16 (37.4%) of the heterozygous FV Leiden patients suffered from deep vein thrombosis, whereas 1/9 (11%) of subjects with partial FV deficiency and 1/20 (5%) of normal subjects had a history of venous thrombosis. All the episodes and clinical significance of these events are documented. Pseudohomozygotes and heterozygotes for FV Leiden showed an increased risk of venous thrombosis in comparison to that in normal subjects (OR 8.8 and 5.7, respectively). However, the thrombotic risk of pseudohomozygotes was not significantly greater than that observed in heterozygotes for FV Leiden belonging to the same families (OR 1.6; 95% CI 0.43-5.7). By Kaplan-Meier thrombosis-free survival analysis within the seven affected families, at age 40 about 60% of pseudohomozygotes are expected to have suffered from thrombosis in comparison to 20% of heterozygotes for FV Leiden and 0% of normal subjects. However, no difference was observed in thrombosis-free survival between pseudohomozygotes and 45 consecutive outpatient FV Leiden carriers with thromboembolism identified in the same period. In conclusion, pseudohomozygosity for APC resistance carries a significantly greater risk for venous thromboembolism in comparison to that in normal subjects, but probably not in comparison to that associated with heterozygous FV Leiden mutation.

SS-0524 Prevalence of anti-prothrombin antibodies in patients with arterial and/or venous thrombosis without biological risk factors for thrombophilia

Reverter J, Muhóz FJ, Tascés D, Font J, Escolar G, Ingelmo M, Ordinas A
Hemotherapy and Haemostasis Dpt. and Systemic Autoimmune Unit; Hospital Clinic, Barcelona, Spain

Anti-prothrombin antibodies (aPT) have been recently identified in patients with the anti-phospholipid syndrome, and a possible association of aPT with pulmonary embolism has been suggested. However, the actual prevalence and clinical significance of these antibodies is still unknown. Objective. To evaluate the prevalence of aPT in patients with arterial or venous thrombosis without biological risk factors for thrombophilia. Methods. We studied 60 patients with arterial thrombosis [11 with myocardial infarction, 39 with ischemic stroke and 10 with peripheral arterial thrombosis] (33 male/27 female, median age 44 years, range 25-50 years) and 60 patients with venous thrombosis (28 male/32 female, median age 45 years, range 23-50 years) and 60 patients with venous thrombosis (28 male/32 female, median age 45 years, range 23-50 years) and 120 age and sex matched controls. Thrombotic patients did not show biological risk factors for thrombophilia defined as protein C, protein S, plasminogen or antithrombin III deficiencies, hyperhomocysteinemia, resistance to activated protein C associated to factor V Leiden mutation, presence of G20210-A mutation of the prothrombin gene, or antiphospholipid antibodies. aPT were determined by an EUSA using human purified prothrombin fixed to irradiated plates. Results. aPT were found in 4 controls (3.3%). In patients, aPT were found in 12 (20.0%) with arterial thrombosis and in 7 (11.7%) with venous thrombosis. Frequency of aPT in patients with arterial thrombosis was significantly higher than in controls (p<0.01). No differences in the prevalence of aPT were found in arterial thrombosis considering the different territories affected. Conclusions. aPT seem to be related to arterial thrombosis in patients without biological risk factors for thrombophilia.

SS-0525 Factor XIII Val34Leu is a genetic factor involved in the aetiology of venous thrombosis

Franco RF, Reitsma PH, Lourenço D, Maffei FH, Morelli V, Tavella MH, Araújo AG, Piccinato CE, Zago MA
Faculty of Medicine and Regional Centre of Ribeirão Preto, Faculty of Medicine of Botucatu and Medical School of the Federal University of S. Paulo, Brazil; Academic Medical Centre, Amsterdam, The Netherlands

Objective. A factor XIII mutation (FXIII Val34Leu) was recently reported to confer protection against myocardial infarction, but its relationship with venous thrombosis remains still unknown. Anti-prothrombin antibodies (aPT) were found in 12 (20.0%) with arterial thrombosis and in 7 (11.7%) with venous thrombosis. Frequency of aPT in patients with arterial thrombosis was significantly higher than in controls (p<0.01). No differences in the prevalence of aPT were found in arterial thrombosis considering the different territories affected. Conclusions. aPT seem to be related to arterial thrombosis in patients without biological risk factors for thrombophilia.
thrombosis is unknown. In addition, a mutation in the 5′-untranslated region of the FXI gene (46 C→T) was identified which is associated with low plasma levels of the protein. Its prevalence in patients with venous thrombosis is also unknown (see Methods and Results). We examined the frequency of the FXI Val34Leu and FXII 46 C→T mutations in 189 patients with deep venous thrombosis and in 187 age-, gender- and race-matched controls. Results. FXI Val34Leu was detected in 38.6% of the patients and in 41.2% of the controls. Interestingly, homozgyosity for the mutation was found in 1.6% of the patients and in 9.6% of the controls, yielding an odds ratio (OR) for venous thrombosis of 0.16 (95% CI: 0.03-0.5). The OR for heterozygotes was 1.1 (95% CI: 0.71-1.7). The FXII 46 C→T mutation was detected in 48.6% of the patients and in 48.6% of the controls. The OR for heterozygotes was 0.9 (95% CI: 0.6-1.4) and for homozgyozy the OR was 0.8 (95% CI: 0.3-1.9). Conclusions. Our data indicate that the FXII 46 C→T mutation is unlikely to be a major risk factor for venous thrombosis. In contrast, homozgyozy for FxII Val34Leu is a strong protective factor against venous thrombosis, which emerges as a novel genetic factor involved in the aetiology of thrombophilia.

SS-0528 Tissue factor independent procoagulant activity of leucocytes
Barrowcliffe TW,* Fabregas P,* Felez J*
*NIBSC, Potters Bar, UK; and *Institut de Recerca Oncologica, Barcelona, Spain

It is well established that leucocytes, especially monocytes, can develop procoagulant activity (PCA), primarily due to tissue factor (TF) in response to certain stimuli such as endotoxin or phytohaemagglutinin (PHA). However, previous studies from our laboratory have described PCA in some lymphoid cell lines which occurred despite the presence of very low levels of TF. The present study was designed to characterise further this PCA and to extend the range of cell lines studied. Jurkat & Molt 4 (T-lymphoid cells), Nalm 6 (B cells) & U-937 (monocytoid cells) were cultured under standard conditions, washed in RPMI and suspended at a concentration of 4 x 10^6 in Tris/NaCl pH 7.4. TF activity was measured by estimation of thrombin generation in plasma after addition of CaCl2 in the presence of cells or a standard phospholipid (PL) preparation. Tissue factor dependent activity was studied by substitution of freshly or deficient plasma for normal plasma. Some studies were also done on measurement of FXa generation with purified FXI, FVIII, & Fx, CaCl2 and cells. In normal plasma the amounts of thrombin generated by Jurkat, Molt 4, Nalm 6 and U-937 respectively were 80%, 78%, 30% & 81% of the PL standard. Activities were similar with or without PHA stimulation. In deficient plasma, similar high amounts of thrombin were generated, but in FIII deficient plasma the PCA was virtually abolished, indicating that most of the thrombin generation was stimulated by a tissue factor independent pathway. In the purified tissue system, FXa generation was also promoted by the cells, confirming that the activity was not due to tissue factor. These results show that a variety of cell types can exhibit PCA, and that this activity can be independent of tissue factor. The results suggest that the cells are providing procoagulant phospholipid for the reactions of the intrinsic system, and this activity could be important for enhancement of the generation of thrombin in situations where leucocyte PCA is triggered.
CIRIT/FIVPA
and an insertion which were distributed across the coding sequence of the ATM gene. Germline ATM mutations which indicate ATM carrier status, were found in 2 of the same 6 patients compared with a frequency within the general population of <0.5%. Although the precise function of the ATM protein is unknown, it is believed to have a role in programmed cell death, a deficiency of which would fit with the characteristic phenotype of prolonged cell survival seen in B-CLL tumour cells. Our results also suggest, for the first time, that carriers of ATM mutations may be at a particular risk for the development of B-CLL and this may partly explain the known genetic susceptibility to this disease. These preliminary results are now being extended and we are currently performing the frequency of ATM clones in patients with 11q deletions. In conclusion, abnormal expression of the ATM protein is a frequent finding in B cell chronic lymphocytic leukaemia.

SS-0530 11q sequence related breakage in CLL

Auer RL* James SP, Fegan CD*, Mullerbach R, James M, Milligan DW, Cotter FE*

+Molec. Haem Unit, ICH, London; °Birmingham Heartlands Hospital, UK

Objective. Trinucleotide repeats (TNR) have been associated with chromosome fragility and anticipation in some diseases, including CLL. Deletions of chromosome 11q occur in patients with CLL and are associated with a poor prognosis. We aimed to investigate the potential role of these TNRs. Results. and Locations. The results of several CCG-trinucleotide repeats on 11q were determined on a 40Mbp YAC contig spanning 11q22-qter. The YAC contig was used to analyse 11q deletions occurring in the malignant cells of 9 patients with CLL (mean age 58 years) to localise potential tumour suppressor genes. Whole chromosome 11 paints confirmed that in all cases the deletion was the sole chromosome 11 abnormality. Dual colour FISH with YACs from the contig, together with PAC and cosmid clones from 11q31-qter, defined a minimal region of deletion between 11q22.3-11q23.1. In four of the patients a common breakage point was identified within a CC-G repeat region. In three additional patients (2 without cytogenetic 11q deletions) haplotype analysis showed a smaller area of deletion which did not include ATM. However, ATM expression was reduced or absent. Within the minimal region of deletion a CCG-repeat was located, cloned and sequenced. The CCG repeat size was determined by a PCR assay. Normal controls (n=97) demonstrated that it is polymorphic, with n=4 or 12 copies of the CCG-trinucleotide representing the most common alleles. PCR analysis using DNA from the blood of 127 patients with CLL (13 with cytogenetically detected 11q deletion) revealed a significant increase in the presence of the larger CCG-repeat (n=12) within the CLL patients compared to within the normal group (p=0.025). In addition CLL patients with the larger repeat had a poorer outcome, suggesting this to be a poor prognostic marker. Conclusions. This data suggests trinucleotide repeats may play a significant role in the pathogenesis of chronic LPD and analysis of the polymorphic marker provides a marker for a poorer disease outcome.

SS-0531 Sequential analysis of p27KIP1 expression in B-CLL allows prediction of disease progression

Nataf J, Cotter FE*

+Molec. Haem Unit, ICH, London; °Birmingham Heartlands Hospital, UK

B-cell chronic lymphocytic leukaemia (B-CLL) is characterised by the accumulation of resting lymphocytes secondary to a defect in apoptosis. Conventional kinetic studies readily assess the late phases of the cell cycle and allow kinetic marker in B-CLL by providing instantaneous estimation of the disease doubling time. Therefore sequential analysis of p27 expression is likely to predict disease progression in B-CLL.

SS-0532 Heterogeneity of A-myb expression in CLL and Ig switching in B-CLL

Golay J, Facchinetti F, Bensacchi S, Barbi R, Rambaldi A, Isnard M, Istituto Ricerche Farmacologiche Mario Negri, Milan; Division of Haematology, Ospedali Riuniti, Bergamo, Italy

A-myb is a proto-oncogene of the family of myb transcription factors. It is expressed specifically in germinal center B cells and is involved in the regulation of mature B cell differentiation. Furthermore it is regulated during the cell cycle. We have previously shown that amongst neoplastic human B cells, A-myb is expressed specifically in the Burkitt’s lymphoma and B-ALL (L3) cells which show a germinal center phenotype. In addition, A-myb mRNA was found to be expressed at variable levels in about 40% of CLL cases. This was particularly surprising since CLL do not show a germinal center phenotype and are not proliferating. We have therefore studied 15 cases of CLL in an attempt to understand the basis for the heterogeneous expression of A-myb in this class of B cell tumors. A-myb expression did not correlate with surface phenotype (CD5, CD19, CD20, CD22, CD38, CD39, CD40 or CD71). Analysis of immunoglobulin transcripts in Northern blots and by PCR and of the state of Ig rearrangements in Southern blots suggests that A-myb is expressed only in CLL cells whose immunoglobulin genes have undergone isotype switching. Finally we show that the A-myb protein interacts with nucleolin and together with the latter is present in a protein complex that binds to switch region DNA. These data suggest that A-myb may directly regulate the switching process.

SS-0533 Ig-g-secreting lymphoplasmycidoid leukaemia: a B-cell disorder with extensively mutated Ig genes undergoing Ig isotype switching, frequently associated with trisomy 12

Garand B,* Sahota SS,* Avet-Löisieux H,* Talant P,* Robillard A,° Marceau A,* Guillard F,* Stevenson FK,* Bataille R*

*Laboratoire d'Hématologie; °Laboratoire d'Anatomie Pathologique, CHRU, Nantes, France; *Molecular Immunology Group, Tenuous Laboratory, University Hospital, Southampton, UK

Elevated serum monoclonal Ig is typically associated with multiple myeloma. However occasional cases have been reported with clinical and pathological features similar to Waldenstrom’s macroglobulinaemia. We have investigated 15 patients with a leukaemic B-cell lymphocytic disorder and high serum monoclonal Ig. Their clinical history was that of a non-aggressive disease, all but 3 patients being alive and well with a median follow-up (range: 14-205M). Spleenomegaly was found in all except one case, suggesting a splenic origin of disease. Circulating and bone marrow abnormal cells were predominantly small lymphocytes with variable percentages of lymphoplasmycidoid cells. Four patients’ disease was indistinguishable from chronic lymphocytic leukaemia (CLL), while histopathological features included a lymphoplasmacytic infiltrate in 8 cases. An AL amyloidosis nephropathy was observed in one case. In 11 cases flow cytometry showed an immunophenotype typical of lymphoplasmacytic lymphoma, i.e. CD19<CD5<CD23<4-14D<14C while 2 patients exhibited a CD19<CD5<CD23< phenotype of CLL. Comparison of serum and surface Ig revealed a coexistent monoclonal Ig secretion and/or expression with similar light chain to the IgAl paraprotein in 10 patients, whereas the others 5 cases only expressed IgG. Vh gene analysis was used to assess the clonal history of neoplastic cells. In 6/8 cases, Ig was also expressed or secreted by the leukaemic cells and Vh gene analysis indicated a clonal relationship between the IgM and IgG transcripts in 3 of 6 lymphomas and the same case being biclonal. In 2/8 cases where only IgG was expressed, a single IgG derived IgM transcript was identified. VH gene sequences were derived from Vh3 in 6/8 cases, and displayed a high level of somatic mutation with a pattern consistent with a role for antigen selection. Both IgM and IgG sequences displayed intrachromosomal homology. Cyto genetic studies showed a high incidence of trisomy 12 (56%) and 13q(14 deletion (33%). In conclusion, it would appear that the cell of origin is postfollicular, and neoplastic transformation has occurred at the point of isotype switch, with some cases arrested at both the IgM and IgG stage, and others as IgG+ve cells only.
Involvement of Fas ligand in the pathogenesis of neutropenia in large granular lymphocyte leukaemia

Lamy T, Liu JH, Wei S, Starkebaum G, Djeu JY, Loughran TP
From the Moffitt Cancer Center; Tampa, FL, USA, and the VA Hospital; Seattle, WA, USA

Large granular lymphocyte (LGL) leukaemia is a clonal lymphoproliferative disorder associated with chronic neutropenia. The mechanism of neutropenia is not known. Normal neutrophil survival is regulated by the Fas/Fas Ligand apoptotic interactions. We hypothesised that neutropenia in LGL leukaemia is mediated by dysregulated expression of Fas Ligand.

Methods. Forty-four patients suffering from LGL leukaemia entered this study. Serum samples were obtained at the time of diagnosis, before initiation of treatment for all patients and also in 11 of them while on treatment. Levels of Fas ligand in sera were measured using a Fas Ligand ELISA. The effects of LGL leukaemia sera on apoptosis of normal neutrophils (PMN) were determined by flow cytometry and morphologic measurement.

Results. High levels of soluble Fas Ligand (sFas.L) were detected in 41 of 44 sera from LGL leukaemia patients. sFas.L were undetectable in 10 normal sera. Patient sera caused Fas-dependent apoptosis on both normal PMN and PMN from LGL leukaemia patients. However, PMN from patients were more sensitive to Fas-mediated apoptosis than normal PMN. Eleven patients were treated with: methotrexate (n=10), 2CDA (n=1). Nine entered CR and 2 PR. Resolution of neutropenia was associated with disappearance or marked reduction in sFas.L in 10 of 11 treated patients. Recurrent neutropenia, observed in 2 patients after stopping treatment was associated with re-increased levels of sFas.L. Conclusions. These data support the hypothesis that Fas Ligand mediates neutropenia in LGL leukaemia. Methotrexate may be active by modulating Fas resistance and causing apoptosis of leukaemic LGL. Novel strategies targeting Fas Ligand (metalloproteinase inhibitor?) could be efficacious in LGL leukaemia.
Haematology-in-Focus Symposia

HIF7 Immunotherapy

HIF-0535 Adoptive immunotherapy of leukaemia with ex vivo expanded T cells

Leiden University Medical Center, The Netherlands

Allogeneic stem-cell transplantation (SCT) is the treatment of choice for a variety of haematological malignancies. The advantage of allogeneic SCT over autologous SCT is the fact that T lymphocytes derived from the stem cell donor may exhibit an immunological effect against the malignant cells. Unmodified T lymphocytes derived from the donor infused into the patient at the time of the transplantation, or preferably after engraftment of donor haematopoiesis in the patient, has been shown to be effective in suppressing the leukaemic cells in vivo. However, infusion of unmodified donor cells into the recipient has also frequently resulted in the development of Graft-versus-Host Disease (GVHD), therefore entailing a minimal myeloablative conditioning regimen, in patients with a high risk of GVHD. T cells recognising leukaemic precursor cells where found in high frequency in patients who responded after successful immunotherapy with donor lymphocytes for chronic phase chronic myeloid leukaemia (CML). We developed in an in vitro assay to select and expand T cells recognising leukaemic precursor cells mediating a minimal myeloablative conditioning regimen. By combining wells from a limiting dilution assay containing T cells that were capable of suppressing the outgrowth of leukaemic precursor cells, we generated T-cell lines capable of cell-mediated inhibition of leukaemic precursors as well as of circulating leukaemic cells as measured in a 3H-crystal release assay. Using these T cells, we treated a patient with refractory accelerated-phase CML following allogeneic SCT with three CML-reactive TCR-TCR T-cell lines at five-week intervals. Three weeks after the third infusion, a rapid disappearance of the leukaemic cells was observed. No acute GVHD developed. The patient entered a complete haematological and cytogenetic remission. After remission a second patient with a relapse of a lymphatic blast crisis of CML shortly after transplantation was treated with a combination of leukaemia-reactive T-cells and donor lymphocyte infusion. The combination of these treatments resulted in a complete remission. This patient however developed acute GVHD which was treated successfully with corticosteroids. In conclusion, the study shows the feasibility of the generation of leukaemia reactive T-lymphocyte lines under good manufacturing practice conditions and the potential benefit of treatment of patients with relapsed leukaemia after allogeneic SCT with these CTL lines.

HIF-0536 Donor lymphocyte transfusions for the treatment of haematological malignancies after transplantation


Hematopoietic Cell Transplantation, Medizinische Klinik III, University of Munich, GSF-National Research Center for Environment and Health, Munich, Germany

Allogeneic hematopoietic stem cell transplantation is an effective form of immunotherapy of leukaemia, but it carries the risk of severe graft-versus-host disease (GVHD). After allogeneic marrow transplantation of donor lymphocytes (DLT) have been used to treat relapse of leukaemia and other malignancies. In an EBM T study sustained remissions have been induced in more than 70% of patients with recurrent CML in chronic phase and cytogenetic relapse. GVHD was observed in 6% of patients. In patients with pre-existing GVHD the response rate was higher than in those without GVHD, but in 48% of patients without GVHD, GVHD responded to DLT too. Severe myelo-suppression developed in 20% of patients. Haematopoiesis could be rescued by the infusion of donor marrow. In patients with mild GVHD and in patients with low-grade lymphoma responses to DLT have been reported, the durability of which cannot yet be determined. DLT has been less successful in acute leukemias and advanced phase CML. In patients with AML treated with donor cell transfusions, remissions (CR) were observed in about 30% with lymphocytes only, and in 70% of patients given G-CSF mobilised peripheral blood cells (PBSC) and GM-CSF. Recurrent CML in accelerated phase can be treated with PBSC and GM-CSF with success in single patients without myeloablative conditioning. As a rule AML requires intensive chemotherapy prior to immunotherapy, but severe GVHD may be seen in these patients. Prolonged remissions are being observed in some patients with AML without intensive conditioning therapy. GM-CSF improves the response to DLT by better stimulation and antigen presentation. Alternatively PBSC may substitute antigen presenting cells.

HIF-0537 Immunotherapy using non-myeloablative regimens for allogeneic hematopoietic transplantation

Chapelin BM
University of Texas, MD Anderson Cancer Center, Houston, TX, USA

An immune mediated graft-vs-host-leukaemia effect is important to prevent relapse after allogeneic BMT for a range of haematologic malignancies. The most direct evidence of GVL is that most patients relapsing with CML post-BMT achieve durable remission by infusing additional donor lymphocytes. Response is associated with elimination of nonleukaemic host haematopoietic cells as well, suggesting that polymeric haematopoietic lineage related antigens could be involved. Similar data suggest an important graft-vs-host malignancy effect occurs with AML, as well as in indolent lymphoid malignancies and multiple myeloma. Given the efficacy of donor lymphocyte infusions to induce durable remissions in susceptible malignancies, an alternative strategy is to use a relatively nontoxic, nonablative preparative regimen to achieve engraftment, allowing subsequent infusion of additional donor lymphocytes. We have explored this at the MD Anderson Cancer Centre using fludarabine based nonablative chemotherapy in patients with a range of malignancies. We have utilized “standard dose” combination regimens producing myelo-suppression but sufficiently immunosuppressive to allow engraftment of an allogeneic blood stem cell or bone marrow transplant. Preliminary results indicate that engraftment of patients treated with fludarabine with initial mixed chimaerism generally follows by progression to complete chimaerism within 3 months. If engraftment is achieved complete remissions have been achieved in the majority of patients with CML, CLL, low grade lymphoma and myeloma. Durable remissions have occurred in chemotherapy sensitive, but not chemoresistant AML. Pilot studies in breast cancer suggest a graft-vs-tumour effect may also occur against solid tumors; several patients have achieved CR simultaneously with developing acute GVHD or after donor lymphocyte infusion. This general strategy holds promise of reducing the morbidity and mortality associated with high dose chemotherapy and potentially allowing effective transplantation in older or infirm patients who are not presently candidates for BMT.

HIF-0538 Phase I study of vaccination therapy using dendritic cells pulsed with her-2/neu or muc-1 derived peptides

Department of Haematology, Oncology, and Immunology, and Institute for Cell Biology, Tübingen, Germany

Vaccination of cancer patients using antigen-pulsed dendritic cells (DC) was shown to be effective for B cell lymphoma and malignant melanoma. We recently demonstrated that DC derived from peripheral blood monocytes are potent stimulators of Her-2/neu specific CTL in vitro when stimulated with TNF-α and peptides derived from the Her-2/neu protein. The use of Her-2/neu epitopes, however, is restricted by its limited expression. In contrast, MUC-1 is a glycosylated transmembrane protein that is overexpressed on the cell surface of many human epithelial malignancies, making MUC-1 an attractive target for broadly applicable immunotherapeutic strategies. Using a computer analysis of the amino acid sequence of the MUC1 protein, we identified 2 HLA-A2 restricted T-cell epitopes presented by various tumour cells naturally expressing MUC-1. Based on these preclinical results, we started a phase I study for treatment of patients with progressive metastatic breast and ovarian cancer refractory to chemotherapy using monocyte-derived DC generated in serum free medium using GM-CSF, IL-4 and TNF-α, and pulsed with Her-2/neu or MUC-1 derived synthetic peptides. So far, 10 patients have been included in this ongoing trial. Each patient received 3–10 D (range 3–8–10) subcutaneously per vaccination. The administration of DC was well tolerated with no side effects. One patient with metastatic breast cancer developed partial remission of the cutaneous lesions and another one has stable disease after having progressive disease before vaccination. Both patients were vaccinated with MUC-1 pulsed DC. Using FACS analysis for intracellular IFN-γ staining, Her-2/neu specific CD8+-T cells could be detected in one patient. Data from ongoing clinical evaluations and immunological monitoring using ELISPOT assays, intracellular IFN-γ staining, 3H-cr-elase assays and cytokine production will be presented and discussed.
HIF-0539  Bcr-abl breakpoint derived oncoregene fusion peptide vaccines in patients with chronic myelogenous leukaemia: a phase I trial


We have shown that four short peptides and one longer peptide derived from amino acid (aa) sequences crossing the b3a2 breakpoint in the bcr-abl generated P210 oncogene elicit class I restricted cytotoxic T lymphocytes in vitro and class II responses respectively. This provided the rationale for a peptide based vaccine phase I dose escalation trial to evaluate the safety and immunogenicity of bcr-abl breakpoint peptides when administered to patients with CML. Fourteen adult patients (29-73 years old) with chronic phase CML characterised by the b3a2 junction have been vaccinated with a preparation of four peptides of 9-10 aa in length, and a 25 aa peptide. Cohorts of 3 patients each received either 50 µg, 150 µg, 500 µg or 1500 µg total peptide mixed with 100 µg of QS-21 as adjuvant in 5 subcutaneous injections over a ten week period. Delayed type hypersensitivity (OTR) and up-regulated ex vivo autologous proliferative (H-thymidine) and cytotoxicity (Cr release) responses were measured. Three patients at each dose level are currently evaluable. All vaccinations were well tolerated without significant adverse effects. Three of six patients treated at the two highest dose levels of vaccine generated peptide specific T cell proliferative responses, DTH responses or both. One patient maintained a proliferative response for 5+ months after all vaccinations were completed. None of the 14 patients developed disease progression. In conclusion, a bcr-abl derived peptide vaccine can be safely administered to patients with chronic phase CML and can elicit a bcr-abl peptide specific immune response. Phase II trials to evaluate immunity and therapeutic benefit further have been initiated.

HIF-0541 Flow cytometric detection of minimal residual disease in acute leukaemia

Campsana D., Costam-Smith E., Neale GAM, Pui C-H (Department of Hematology-Oncology, St. Jude Children’s Research Hospital, and University of Tennessee, Memphis, Tennessee, USA)

We developed flow cytometric techniques which allow the detection of a leukemic cell in 10^-9 bone marrow mononuclear cells. In a previous study, we prospectively applied these methods to monitor minimal residual disease (MRD) in 158 uniformly treated children with acute lymphoblastic leukaemia (ALL) in clinical remission [1]. The proportion of patients with MRD was 23% at remission induction and 17% at week 14 of continuation therapy, decreasing to 5% and 4% at weeks 52 and 56, respectively. By contrast, for the 9 patients who relapsed, the actuarial probability of relapse and survival was 100% and 0% respectively. For this risk group, sensitivities of at least 10^-4 are sufficient for stratification of treatment. This is in contrast to the results of the large multicenter MRD study of the International St. Jude ALL study group [2]. The above described MDR study of the I-BFM-SG showed that, preferentially, quantitative MDR information should be provided and for reasons of potential Ig/TCR target instability at least two targets per patient should preferably be used. The first is possible with the TaqMan technology (Leukemia 1998; 12:2006-14) and the latter can be achieved in >80% of the patients. The choice of the various types of Ig/TCR targets is dependent on the occurrence, sensitivity, and predicted stability (e.g. absence of oligoclonality at diagnosis). This should be determined for each patient. Clinical studies with MBD-based stratification or adaptation of treatment need reliable strategies for the MRD analyses, we use the two methodologies that allow standardisation of all logistical and technical aspects.

HIF-0542 A prospective study on residual disease monitoring of the ALL 1/ A4F transcript in patients with t(4;11) translocation acute lymphoblastic leukaemia

Cimino G. on behalf of the GIMEMA Cooperative Study Group (Dipartimento di Biotechnologie Cellulare ed Ematologia, Università “La Sapienza”, Rome and Dipartimento di Scienze Biomediche ed Oncologia Umana, University of Torino, Italy)

Twenty-two patients (20 adults and 2 infants) with ALL/A4F-positive acute lymphoblastic leukaemia (ALL) were prospectively monitored by RT-PCR between January 1992 and December 1998. At presentation, a rearranged configuration of the ALL1 gene was detectable by Southern blot analysis in all cases, whereas a t(4;11) translocation was karyotypically evident in 15 patients. Following high-dose intensive induction and consolidation chemotherapy without bone marrow transplantation, all patients achieved a complete haematological remission. By nested RT-PCR (sensitivity 10^-3), conversion to PCR negativity was observed in 9 cases (41%). All 13 patients who did not achieve a molecular remission relapsed at a median time of 4 months (range 1-20). Of the 9 patients who became PCR-negative, 5 returned PCR-reve within 1 to 14 months. Four of these 5 patients progressed to haematological relapse after 2, 3, 4 and 7 months, from the reappearance of the ALL1 A4F transcript. For patients with acute lymphoblastic leukaemia (ALL) with t(4;11) translocation, the actuarial probability of relapse and survival was 100% and 0% at 14 and 24 months, respectively. For the 9 patients who reached molecular remission, the relapse rate was 9% at 46% and 53% at 84 and 100 months, respectively. The difference in the actuarial survival rate between the two groups was statistically significant (p < 0.005). This study represents the first prospective analysis of residual disease monitoring carried out in a substantial group of patients (n=11) ALL patients. Our results emphasise the clinical relevance of RT-PCR monitoring of minimal residual disease in this acute leukemia subset.
Acute myeloid leukaemia (AML) with the t(8;21) karyotype highlights a group of patients with good prognosis disease leading to modification of treatment for such patients in the most recent MRC trial. Detection of the underlying AML1-ETO translocation can be performed with a high degree of sensitivity by RT-PCR although there is little agreement as to the relevance of its detection in terms of long-term survival. Objective. To assess the relationship between PCR status post chemotherapy and outcome in patients with t(8;21) +ve AML. Design and Methods. A prospective study was undertaken on 22 (14 male, 8 female) patients with t(8;21) +ve AML treated in the MRC AML XII trial between 1995-98. All received induction chemotherapy and 3 or 4 cycles of consolidation chemotherapy as per protocol but were not transplanted in first complete remission (CR). RNA was extracted from peripheral blood or bone marrow mononuclear cells at diagnosis and following each successive cycle of chemotherapy. A nested RT-PCR five-step acquisition procedure through a SSC/CD33+/ive gate (live gate) was done. Results. Seventeen patients became PCR negative by the end of their 3rd course of chemotherapy, remained so during and after treatment and are in CR 9-36 months post diagnosis. Five patients were PCR positive at this stage and of these, 4 have subsequently relapsed. The fifth became PCR negative following a further course of chemotherapy and remains in CR. Conclusions. Assessment of PCR status following 3 cycles of chemotherapy is a good predictor of prognosis in t(8;21) +ve AML and may help identify patients at higher risk of relapse.

HIF-0544 Minimal residual disease monitoring in multiple myeloma patients following high-dose chemotherapy using a high sensitive immunophenotypic approach


Monitoring of minimal residual disease (MRD) in multiple myeloma (MM) patients is usually performed by PCR techniques. Immunophenotyping is an attractive alternative that has been proven to be a great utility for MRD investigation in acute leukemias, based on the presence of antigenic aberrations in blasts that can be used as ‘tumor markers’ for discrimination from normal cells in following samples. Using the same sensitive immunophenotypic approach (based on triple antigen combinations and a two-step acquisition procedure through a SSC/CD33+/ive gate) we analyzed plasma cells (PC) from 61 MM patients. Overall, 87% of MM patients displayed an aberrant phenotype at diagnosis that allows the discrimination between normal ad malignant PC with a sensitivity limit of 10-1. Based on this information we investigated MRD in 25 MM patients who underwent autologous Tnx and had entered into morphological complete remission (<5% CP) following induction treatment (4 cycles of VBCMP/VBAD). Before Tnx, the mean number of myelomatous PC identified by immunophenotypic analysis was 3.4±4.5 while this number to 0.26±0.63 and 0.09±0.16 in the BM samples obtained three and twelve months after Tnx, respectively. Therefore the percentage of PC decreased one and two logarithmic orders, respectively. Since our method allows discrimination between normal and myelomatous PC, we also analyzed the evolution of normal PC during follow-up. At diagnosis, only ±2.7% of the total PC were considered to be phenotypically normal (normal residual PC); following induction treatment, the proportion of normal PC slightly increased (18%±28%) and, interestingly, at three and twelve months after Tnx the proportion of normal PC rose to 70±33% and 18±19 respectively. Therefore, following Tnx, most PC present in the BM are normal and not myelomatous. We conclude that the use of immunophenotyping could be a valuable tool for monitoring residual disease following high-dose chemotherapy in MM patients.

HIF-0546 Hereditary disorders of platelet receptors for adhesive proteins: collagen and von Willebrand factor

Clemetson KJ, Clemenson JM, Theodor Kocher Institute, University of Berne, Berne, Switzerland

The most common platelet receptor disorder is Glanzmann’s thrombasthenia where platelet GPIa,b, is deficient or defective. This is followed in frequency by disorders of the GP Ib–IX–CX (CD42) complex, in particular, the Bernard–Soulier syndrome (BSS) where the receptor complex is completely or partially absent or dysfunctional due to a mutation or deletion in one of three of the four genes of the complex, GP Ibα, GP I bβ1, and GP IIbβ3. However, the absence of GP Ibα in BSS is due to the lack of expression of the rest of the complex. Many mutations have been identified in the other three genes leading to the partial or complete absence of the complex. Some of these are deletions or point mutations leading to frameshifts and premature termination causing lack of the membrane binding domain. In these cases there may be an increase in soluble protein in plasma. More interesting mutations affect the leucine-rich domains or cystine-bridges causing folding problems or affecting complex formation. In populations of N. European origin a very common mutation (perhaps up to 50% of BSS cases) is the Asn45-Ser mutation in GP Ibα. Mutations in the larger loop of the double loop domain of GP Ibα give increased binding of vWF to platelets causing the rare platelet-type vWD disease. Disorders in expression of collagen receptors are known affecting α2β1, GPⅥ and CD36 receptors. Deficiencies of α2β1 or GPⅥ are very rare and lead to mild bleeding disorders. CD36 is absent in 4–7% of East Asian and Sub-Saharan African populations but do not affect haemostasis or thrombotic tendencies in these groups. Polymorphisms in GPⅥbα and α2β1 have been reported to affect thrombotic tendencies.
**HIF-0547** **Autosomal dominant type 1 von Willebrand disease due to C1130F mutation: description of five families**

Carmanan G, Eikenboom JC, Misigia E, Rodeghiero F
Department of Hematology and Hemophilia and Thrombosis Center, San Bartolo Hospital, Vicenza; Italy; Haemostasis and Thrombosis Research Center, Leiden University Medical Center, The Netherlands

The molecular bases of classic autosomal dominant type 1 von Willebrand disease (vWD) have been elucidated in a very few cases so far. Recently, we reported two missense mutations in exon 26 of the von Willebrand factor (vWF) gene in 1 family with autosomal dominant type 1 vWD of Dutch and two unrelated patients of Italian origin (Blood 88, 2433, 1996). Both the mutations (C1149R in Dutch and C1130F in Italian subjects, formerly referred to as C368R and C367F) cause the loss of a cysteine in the D3 domain which is involved in multimerisation. The C1149R mutation has been expressed in vitro and a dominant-negative effect on the synthesis of the normal allele has been demonstrated. Since both the mutations abolish a restriction site in exon 26 of the vWF gene detectable by Alu I or Fnu4HI digestion, we screened for both the mutations 24 additional apparently unrelated Italian patients with similar phenotypes and a clear autosomal pattern of inheritance of bleeding symptoms and laboratory phenotype. While all the patients resulted normal for the C1149R mutation, three patients and four affected relatives were heterozygous for the C1130F mutation, confirmed by Taq 1 digestion. When all five families were examined, the mutation appeared to segregate with vWD phenotype. The patients had normal platelet vWF content and plasma multimeric pattern and responded well to desmopressin infusion. This suggests that the C1130F mutation too could have a dominant-negative effect on the secretion of the normal protein and that desmopressin is able to overcome this effect, thus being clinically effective.

**HIF-0548** **Molecular characterisation of 22 families with factor VII deficiency**

Peviani F, Mannucci PM, Jenkins PV, Perry DJ
Hemophilia Centre and Haemostasis Unit, Royal Free Hospital and School of Medicine, London, UK; *Angelo Bianchi Bonomi Hamophilia and Thrombosis Centre, IRCCS Maggiore Hospital, Milan, Italy

Factor VII (FVII) deficiency is a rare inherited coagulation disorder with an autosomal recessive pattern of inheritance and a variable clinical phenotype. Forty-three mutations in the FVII gene have previously been identified and analysis of these has proved invaluable in subsequent structure-function studies. We have studied 22 families with FVII deficiency from 6 geographically separate countries. Nineteen mutations have been identified of which 13 are novel and 6 have been previously reported. Of the 13 novel mutations, 9 are missense mutations, 3 are splice-site mutations, 1 is an insertion-type mutation. Arg304Gln was found in a homozygous form in 2 unrelated Iranian kindreds; Cys310Phe in a homozygous form in 2 unrelated Iranian kindreds and in a heterozygous form in 1 Italian kindred and responded well to desmopressin infusion. This suggests that the C1130F mutation too could have a dominant-negative effect on the secretion of the normal protein and that desmopressin is able to overcome this effect, thus being clinically effective.

**HIF-0549** **Identification of 5 novel factor XI mutations in French basques**

Zuelin A, Bauduer F, Kornbrot N, Ducoulot L, Rosenberg N, Seligson U, Sheba Medical Center, Tel-Haasomer, Israel; and Centre Hospitalier de la Côte Basque, Bayonne, France

Hereditary factor XI deficiency is rarely observed in most populations except for Jews in whom 2 mutators, a stop mutation (type II) and a missense mutation (type III) predominate. Recently, a cluster of 39 patients with factor XI deficiency was identified among French Basques (Bauduer et al. Br J Haematol 1998; 102:137a). In this study we searched for nucleotide sequence alterations by SSCP analysis of exons and exon-intron boundaries and identified mutations by direct sequencing and ASRA. Five novel missense mutations were detected among 11 subjects belonging to 8 unrelated families as follows:

<table>
<thead>
<tr>
<th>Mutation Exon</th>
<th>Nucleotide</th>
<th>Amino Acid</th>
<th>Genotype</th>
<th>ASRA</th>
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</thead>
<tbody>
<tr>
<td>1 3</td>
<td>T155C</td>
<td>Val 20 Ala</td>
<td>Heterozygote</td>
<td>Mbo III (+)</td>
</tr>
<tr>
<td>2 3</td>
<td>T209C</td>
<td>Cys 38 Arg</td>
<td>Heterozygote</td>
<td>TaI (+)</td>
</tr>
<tr>
<td>3 7</td>
<td>C659T</td>
<td>Pro 188 Ser</td>
<td>Compound</td>
<td>BstI (-)</td>
</tr>
<tr>
<td>4 8</td>
<td>G807A</td>
<td>Cys 237 Tyr</td>
<td>Compound heterozygote with type II</td>
<td>Alw26I(-)</td>
</tr>
<tr>
<td>5 13</td>
<td>G1057T</td>
<td>Cys321 Phe</td>
<td>Compound heterozygote with type II</td>
<td>Pst I (+)</td>
</tr>
</tbody>
</table>

*Artificially created restriction site.

Each of two siblings bore 3 different mutations, two (no. 4 and 5) occurred on the same allele, and the type 11 mutation, common in Ashkenazi Jews. Indeed, this family is of mixed Basque and Jewish origin. The subject bearing mutation #3 is a compound heterozygote with one mutation yet to be detected. All novel mutations predict changes in the heavy chain of factor XI. Three of them involve substitutions of cysteine residues which are probably important for the folding of the so called apple domains (Cys 38 Arg; Cys 237 Tyr) and dimersisation of factor XI (Cys 321 Phe).

**HIF-0550** **Bcr/abl fusion genes**

Saglio G, *; Pane F, *; Rege Cambrin G, *; Guerrasio A, *; Martrelli G, *; *Dept. of Clinical and Biological Sciences, University of Turin; "Dept. of Biochemistry, University "Federico II", Naples; "Institute of Hematology "L & A Seragno"; University of Bologna, Italy

The different leukaemia phenotypes found associated with the presence of a Ph-chromosome can, at least in part, be ascribed to the presence of different types of BCR/ABL rearrangements. The constant molecular consequence of the Ph-chromosome translocation, whereas the breakpoints on chromosome 9 are always 5' to ABL exon 2 or, sporadically, to ABL exon 3, the breakpoints on chromosome 22 may differ more widely within the BCR gene, giving origin to 5 different types of BCR/ABL junction. In CML, two different types of BCR/ABL junction (c13a2 and e1a2) are generally present. Both lead to the presence of a protein of 210 Kd (p210). A third type of BCR/ABL junction (e1a2) which results in a BCR/ABL protein of 190 Kd (p190) has been identified almost exclusively in Ph-ALL. However, cases of CML expressing exclusively p190 have been described and their incidence in a large cooperative Italian study is approximately 0.5%. Although these cases may show increased monokaryosis, their clinicalbehavior is similar to that of p210 CML. More recently, a rare breakpoint located at the 3' portion of the BCR gene, which produces an e1a2z junction and predicts a 230 Kd protein, has been identified in patients frequently showing a milder form of CML, denominated Ph-positive CML. In addition to these "major" types of BCR/ABL fusions, sporadic patients bearing unique types of BCR/ABL transcripts have also been described. A careful observation of the haematological and clinical features of these cases, true, naturally occurring "deletion mutants", may help to understand the mechanisms by which the BCR/ABL hybrids contribute to the hematopoietic.
HIF-0551 Therapeutic decisions in CML
Goldman J
Department of Haematology, Hammersmith Hospital/ICSM, London, UK

It is today conventional to start treatment soon after CML is diagnosed. Treatment options include cytotoxic drugs, interferon-α (IFN-α), allografting and autografting. In some cases there is general agreement as to optimal therapy - eg allografting for a young patient with an HLA-identical sibling or chemotherapy for a 75-year-old with ischaemic heart disease. In other cases the choice may be highly controversial. Interferon-α is widely accepted that IFN-α prolongs life in comparison with hydroxyurea but uncertainty remains as to whether this benefit applies mainly or exclusively to those who achieve a cytogenetic response. The addition of other drugs, especially cytarabine, may confer additional benefit. Whether prior treatment with IFN-α increases the risk of mortality following an allograft is not yet clear. Allografting, particularly in patients in the chronic phase, has a high probability of being cured. The risk of mortality associated with the transplant is appreciable and unfavourable risk factors include patient age, advanced phase disease, duration of prior chronic phase, HLA mismatching and accelerated phase disease. Using these and other criteria a risk profile can be calculated for individual patients. Autografting. The evidence that autografting with 'unmanipulated' myeloid stem cells prolongs survival is suggestive but not conclusive. The evidence that the patient is autografted with predominantly Ph-negative stem cells, such as the technique of in vivo mobilisation developed in Genoa, appear promising but have not yet been shown conclusively to prolong life. Decision making. There are various possible approaches to decision making for the newly diagnosed patient: (1) decide immediately whether the patient could be a candidate for allografting. If yes, search for the best available donor and proceed to transplant. If no, choose between IFN-α or an initial autograft; or (2) give an initial trial of IFN-α. If the patient responds, continue; if the patient does not respond, proceed to allografting; or (3) enter the patient as a prospective study, such as the current CML-Philadelphia chromosome study. The above decisions are performed in the same reaction vessel and ii) the complete PCR quantification of BCR-ABL fusion transcripts using the LightCycler™ technology. This device combines rapid thermocycling with fluorescence online quantification of BCR-ABL fusion transcripts using the LightCycler™ technology. This device combines rapid thermocycling with fluorescence online detection of PCR product formation. The analysis is based on fluorescence resonance energy transfer (FRET). The use of two adjacent hybridisation probes carrying donor and acceptor fluorophores complementary to ABL exon 3 enables detection of all known BCR-ABL variants and ABL introns as internal controls. We quantified BCR-ABL and total ABL transcripts in 126 samples. Peripheral blood, 19 samples were from 55 patients with CML in chronic phase treated with IFN-α. Nineteen patients expressed b2a2, 29 b3a2 and 7 b2a2 & b3a2 BCR-ABL transcripts. BCR-ABL transcripts were detected in 125/126 samples. We could amplify down to 10 transcripts per reaction. The ratios BCR-ABL/ABL were compared with the results of cytogenetics, Southern blot and competitive PCR: A) For 63 samples contemporaneous cytogenetic results were available (complete response n=31, partial response n=21, no response, n=17), which correlated with real time PCR results (r=0.87, p<0.0001). B) In 83 samples contemporaneous Southern blot for M-bcr rearrangements was performed. Real time PCR results correlated with the ratio of rearranged BCR bands/total BCR bands (r=0.77, p=0.008). C) In 100 CD34 samples the ratio BCR-ABUABL was determined by nested competitive PCR which compared well with the same ratio determined by real time PCR (r=0.89, p<0.0001). We conclude that real time PCR with hybridisation probes is a fast, reliable, and sensitive method to follow CML patients treated with IFN-α. Major advantages of the method are i) PCR and PCR product analysis are performed in the same reaction vessel and ii) the complete PCR analysis takes less than 60 minutes.

HIF-0552 Quantification of residual disease in CML patients treated with interferon-α using a novel real time RT-PCR approach with fluorescent hybridisation probes
III Med. Klinik, Klinikum Mannheim, Universität Heidelberg, Roche Molecular Diagnostics, Penzberg, Germany; Imperial College School of Medicine, Leukaemia Research Unit London, UK

Interferon-α (IFN-α) induces cytogenetic and molecular responses of variable degree in patients with chronic myelogenous leukaemia (CML). We have developed a rapid and reliable RT-PCR approach for detection and quantification of BCR-ABL fusion transcripts using the LightCycler™ technology. This device combines rapid thermocycling with fluorescence online detection of PCR product formation. The analysis is based on fluorescence resonance energy transfer (FRET). The use of two adjacent hybridisation probes carrying donor and acceptor fluorophores complementary to ABL exon 3 enables detection of all known BCR-ABL variants and ABL introns as internal controls. We quantified BCR-ABL and total ABL transcripts in 126 samples. Peripheral blood, 19 samples were from 55 patients with CML in chronic phase treated with IFN-α. Nineteen patients expressed b2a2, 29 b3a2 and 7 b2a2 & b3a2 BCR-ABL transcripts. BCR-ABL transcripts were detected in 125/126 samples. We could amplify down to 10 transcripts per reaction. The ratios BCR-ABL/ABL were compared with the results of cytogenetics, Southern blot and competitive PCR: A) For 63 samples contemporaneous cytogenetic results were available (complete response n=31, partial response n=21, no response, n=17), which correlated with real time PCR results (r=0.87, p<0.0001). B) In 83 samples contemporaneous Southern blot for M-bcr rearrangements was performed. Real time PCR results correlated with the ratio of rearranged BCR bands/total BCR bands (r=0.77, p=0.008). C) In 100 CD34 samples the ratio BCR-ABUABL was determined by nested competitive PCR which compared well with the same ratio determined by real time PCR (r=0.89, p<0.0001). We conclude that real time PCR with hybridisation probes is a fast, reliable, and sensitive method to follow CML patients treated with IFN-α. Major advantages of the method are i) PCR and PCR product analysis are performed in the same reaction vessel and ii) the complete PCR analysis takes less than 60 minutes.

HIF-0553 CGP57148B can eradicate bcr/abl+ leukaemic cells in nude and SCID mice, depending on the initial tumour load
Gambacorti-Passerini C, Corneo GM, Poitetti P, Cleris L, Marchesi E, Formelli F, Fogliani EM, le Coutre P
Istituto Nazionale Tumori, Milano, Italy and Section of Haematology, S. Gerardo Hospital, Monza, Italy

CGP57148B is a selective inhibitor of ABL kinase and BCR/ABL oncogenic kinase and has been shown to inhibit the proliferation of BCR/ABL+ cell lines as well as fresh cells obtained from CML patients. We recently showed that CGP57148B, when administered p.o. at 160 mg/kg three times per day for 11 days, caused continuous in vivo block of BCR/ABL kinase activity and eradicates the growth of 5x10^6 human BCR/ABL+ leukaemic cells (KU812) injected into the flank of nude mice. When the treatment was initiated 24 hours after leukaemic cell injection, the complete eradication of leukaemic growth was obtained in all treated animals (18/18). When animals were treated 8 days after injection, with an estimated tumour load of 3x10^6-10^7 cells, nodules disappeared in all animals within 10 days; however in 4/12 animals nodules reappeared 12 to 15 days after treatment discontinuation. This difference is statistically significant (p=0.0018). Treatment at day 15 (tumour load of 10^8 cells) resulted in regression of nodules in most of the animals. Methods to encourage autologous treatment of relapsed animals did not obtain permanent eradication of leukaemic growth, and in vivo CGP57148B-mediated inhibition of the BCR/ABL kinase activity was reduced or absent. Prolongation of treatment duration from 11 days to 18 days also failed to decrease the risk of relapse. BCR/ABL+ leukaemic cells obtained from relapsed animals were restested in vitro for sensitivity to CGP57148B by HTDSCR uptake and in general showed the same sensitivity as parental cells. The main variable able to predict the efficacy of CGP57148B in eradicating leukaemic growth in this nude mouse model is represented by the initial tumour load; leukaemic cells obtained from relapsed mice do not appear intrinsically resistant to CGP57148B. These data show that the number of leukaemic cells present at the beginning of treatment is an important variable, even when considering a specific anti-leukaemic treatment. Additional data obtained in SCID mice injected with fresh CML cells from patients in chronic phase support the above mentioned results, and will also be presented.

HIF-0554 The t(8;13) myeloproliferative syndrome
Cross NCP
Department of Haematology, Imperial College School of Medicine, Hammersmith Hospital, London, UK

The t(8;13) myeloproliferative syndrome is a rare, aggressive condition that shows similarities to CML at clinicopathological and molecular levels. At presentation, the peripheral blood and bone marrow have the appearance of a chronic MPD with prominent eosinophilia. In addition, there is a high incidence of non-Hodgkin's lymphoma that is of either B or, more commonly, T-cell phenotype. Following a relatively stioil chronic phase the disease transforms into an acute leukaemia. The chronic phase appears to be non-eradicable by conventional chemotherapy, although haematological remission may be induced by IFN-α and some patients have apparently been cured by allogeneic BMT. The syndrome is usually associated with the reciprocal translocation t(8;13)(p11;q21), but similar clinical features are seen in patients with two other translocations, the t(8;9)(p11;q34) and the t(6;8)(q27,p11). These three translocations have been cloned and shown to fuse three unrelated genes 2Nf1918 at 13q12, FAN at 3q24 and FOP at 6q27) to the fibroblast growth factor receptor-1 (FGFR1) at 8p11. All three chimaeric genes retain the FGFR1 tyrosine kinase domain. To determine the transforming properties of 2Nf198-FGFR1 we transfected constructs encoding this chimaeric gene into the IL-3 dependentcell line Ba/F3. Growth factor independent subclones were obtained in which 2Nf198-FGFR1, STAT1 and STAT5 were constitutively tyrosine phosphorylated. Self-association of 2Nf198-FGFR1 was demonstrated by coimmunoprecipitation with an epitope-tagged derivative. Transfection of COS7 cells followed by immunostaining with anti-FGFR1 demonstrated that 2Nf198-FGFR1 is localised in the cytoplasm, as is the case for BCR/ABL. The identification of substrates that are common to ZNF198-FGFR1 and BCR/ABL is an important area of research, particularly with regards to understanding the mechanisms by which these two chimaeric proteins induce such a wide range of biological consequences.

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HIF11 - New trends in haemoglobinopathies

HIF-0555 Modulators of fetal haemoglobin synthesis: an update
Loukopoulos D.

Increasing the proportion of fetal haemoglobin (HbF) in patients with thalassaemia major and sickle cell disease is expected to significantly improve their quality of life through (a) efficient neutralisation of the excess a-chains in the polymer and (b) inhibition of polymerisation in the latter condition. Of the various agents so far proposed, hydroxyurea (HU) and butyric acid (and derivatives) attract most interest; HU may (a) "recruit" the erythroid precursors, which maintain the γ-chain synthesis programme but remain "dormant" in the marrow of the normal adult, unless called up by an acute erythropoietic stress and (b) selectively promote the transcription of genes which are expressed at a very low level in the adult. As the F-cells have a longer survival, the apparent increase of HbF is higher than its rate of synthesis. At the clinical level, HU proves precisely for the prevention of crises in sickle cell disease; in addition, it may also reduce the haemolytic process. HU may increase HbF also in thalassaemia, but as usefulness is still controversial. As of this date, prolonged administration of HU has not been associated with any major side effects; however, the potential of carcinogenicity is always present and imposes searches for alternative drugs. Butyric acid and derivatives may play this role. Following the initial controversial clinical impressions, recent, better controlled studies showed that administration of Arginine Butyrate resulted in a significant increase of HbF (11/15 patients with sickle cell syndromes), which could be documented by γ-mRNA hybridisation. Butyrate studies promise to offer new approaches to the management of thalassaemia and sickle cell anemia. Major effects of hydroxyurea in red cell membranes are potentially ascribable to this oxidative pathophysiology. Sev- eral clinical trials are planned to investigate the efficacy of hydroxyurea and its derivatives in thalassaemia and sickle cell disease.}

HIF-0556 Thrombophila in β-thalassaemia major
*Tel-Aviv Sourasky Medical Center, The Schleifer Faculty of Medicine, Tel-Aviv University; "Hadasah University Hospital and *Shaare Zedek Medical Center, Jerusalem, Israel; INSERM unit 3484, Hôpital Lariboisière, Paris and *Hôpital Beaujon, Clichy, France

A higher than normal incidence of thromboembolic events has been observed in patients with β-thalassaemia major (TM). Several haemostatic abnormalities have been found in these patients which suggest the existence of a chronic hypercoaguable state (Blood 1991; 77:1748). Thalassaemic red blood cells (RBC) were demonstrated to facilitate thrombosis formation due to altered asymmetry of the membrane phospholipids (Thromb Haemostas 1996; 76:322). The enhanced exposure of phosphatidylserine on the RBC membranes, characterised by annexin V binding, correlated significantly with the expression of p-selectin on TM platelets (Brit J Haematol 1997; 98:51). We have now studied markers of thrombophilia in 26 children (aged 2-9 years) and 36 adults with TM who were in steady state without any evidence or signs of an active thrombotic disease. The concentrations of protein C (PC) and protein S (PS) were significantly decreased in all TM patients investigated, regardless of age, while the PS binding protein (C4BP) and AT-III levels were normal. PC antigen was 51±1% (mean±SD, N=31), PC activity 52±1%, and free PS antigen (p<0.01) compared to healthy individuals). The frequency of factor V Leiden (FV Leiden), MTHFR C677T and prothrombin G20210A mutations or anti- phospholipid antibodies was not increased in TM patients. ALL TM patients had a subclinical chronic hypercoaguable state, manifested by highly elevated urinary levels of 11 dehydro- thromboxane B2 and elevated plasma levels of thrombin antithrombin III (TAT) complexes and these anomalies were found in all the TM children and adults. The levels of factor II were decreased (p<0.001) in TM, V, W and S who have had thrombotic events. In accordance with previous studies of patients with chronic iron overload, we found a negative correlation between the values of S and factor V (r=-0.43, p<0.01). To conclude, thrombophilia leading to a chronic hypercoaguable state exists in children and adults who suffer from β-thalassaemia major. The significant haemostatic anomalies detected already in early childhood may contribute to the cardiac and pulmonary anomalies and the thrombotic events which occur later in life, as TM patients live much longer than previously.

HIF-0557 The potential role of antioxidants in the treatment of sickle cell anemia and thalassemia
Hebbel RP
Department of Medicine/Hematology; University of Minnesota, Minneapolis, Minnesota, USA

In vitro studies have identified the probable components of an oxidation pathobiology affecting the red cell membrane in thalassaemia and sickle cell anemia. This oxidative scenario involves: (a) excessive oxidation of haemoglobin, as a source of oxidant generation (superoxide, peroxide, hydroxyl radical); (b) compartmentalisation of cellular iron, so that abnormal deposits of iron (free heme, haemoglobin and hemechromate, and molecular iron) reside at the membrane/cytoplasm interface; (c) ability of abnormal iron deposits to reduce oxysterols, so as to catalyze conversion of peroxide to hydroxyl radical; (d) therefore, the targeting of oxidative damage to membrane constituents. Many effects of haemoglobinopathic red cell membranes are potentially ascribable to this oxidant pathobiology. Several are highly likely to be explained by this scenario: stimulation of K+ co-transport activity; abnormal membrane microstructure, membrane vesiculation tendency, Band 3 clustering and immunoglobulin accumulation, enhanced membrane leakiness to deformation. In two studies of murine thalassaemia the support the notion that membrane iron deposits contribute to red cell pathobiology in thalassaemia. These studies show that removal of membrane molecule iron is accompanied by improvements in red cell cation content, thrombolysis, deformability, and survival. Thus, insopr as oxidative processes contribute to the pathobiology of thalassaemia and sickle cell anemia, intervention with antioxidants may be of value. As above, the precedent is established for an ameliorating effect of removal of catalytic iron. There is no known way to inactivate (harmlessly) heme iron on the membrane. Therefore, interventions with antioxidant substances should be considered. These might include membrane protectants (e.g., vitamin E, scavengers (e.g., n-acetylcysteine), or reducing agents (e.g., ascorbate). The ability of some of these substances to participate in iron redox activity must be, however, taken into account. Therefore detailed studies are necessary to verify the hypothesised beneficial effects of "antioxidant therapy" in these disorders.

HIF-0558 Increased expression of red cell glycoproteins in sickle cell disease
Kristman R., Brown KA.,* Pearson TC.,* Macey M.,* Spring FA.,* Parsons SF.
Departments of Immunology and *Haematology, The Guy’s, King’s College and St Thomas’ Hospitals Medical and Dental School, London;
**Department of Haematology, The Royal London Hospital, London; the *Bristol Institute for Transfusion Sciences, Bristol, UK

Objective. This study addressed the hypothesis that the binding of sickle red blood cells (RBCs) to endothelial cells induces microvascular occlusions and leads to the painful crises of sickle cell disease (SCD). The purpose of the study was to determine whether RBC glycoproteins, considered to have adhesion-promoting properties, were highly expressed on RBCs from patients with SCD. The percentage of RBCs expressing Lutheran (Lw), King’s, Indian, Lutheran, LW, Rh and Wr(b) glycoproteins were abnormally expressed on RBCs from patients with SCD. The percentage of RBCs expressing Lutheran during disease crisis (mean 60%) was increased (p<0.01) when compared to the percentage of control cells (mean 51%), and were elevated in patients out of crisis (mean 72%; p<0.001). LW glycoprotein was present on 64% control RBCs, 83% RBCs from patients in crisis (p<0.001) and 93% RBCs from patients out of crisis (p<0.001). The other RBC glycoproteins were normally expressed on patients. The percentage of RBCs expressing Lutheran during disease crisis (mean 60%) was increased (p<0.01) when compared to the percentage of control cells (mean 51%), and were elevated in patients out of crisis (mean 72%; p<0.001). LW glycoprotein was present on 64% control RBCs, 83% RBCs from patients in crisis (p<0.001) and 93% RBCs from patients out of crisis (p<0.001). The other RBC glycoproteins were normally expressed on patients. The percentage of RBCs expressing Lutheran during disease crisis (mean 60%) was increased (p<0.01) when compared to the percentage of control cells (mean 51%), and were elevated in patients out of crisis (mean 72%; p<0.001). LW glycoprotein was present on 64% control RBCs, 83% RBCs from patients in crisis (p<0.001) and 93% RBCs from patients out of crisis (p<0.001). The other RBC glycoproteins were normally expressed on patients.
Graft versus host disease (GVHD), graft rejection, disease recurrence and long term toxicity remain significant obstacles to successful allogeneic bone marrow transplantation (BMT) in children with β-thalassaemia major and genetic diseases. In an attempt to improve results, we used a preparative regimen consisting of three alkylating agents, busulfan (BU), thiotepa (TTP), and cyclophosphamide (CY), for T-cell depleted allogeneic bone marrow transplantation instead of the conventional BU-CY protocol. The effect of this intensified regimen was investigated in 26 consecutive cases of children with β-thalassaemia major and other genetic diseases who underwent T-cell depleted BMT from HLA identical siblings. The diseases included β-thalassaemia major (n=14) osteopetrosis, severe combined immunodeficiency, Wiskott-Aldrich syndrome, familial agranulocytosis, congenital idiopathic haemolytic anaemia, Gaucher’s disease, Niemann-Pick disease, Hurler’s syndrome, and adrenoleukodystrophy. The conditioning regimen consisted of BU 4 mg/kg × 4 days (-8 to -5), TTP 5 mg/kg × 2 days (-4 and -3), and CY 60 mg/kg × 2 days (-2 and -1). Engraftment was normal, with WBC >1.0 × 10^9/L at day +19 (10-33), ANC >0.5 × 10^9/L at day +22 (10-56) and platelets >25 × 10^9/L at day +32 (18-131). Overall survival and disease free survival (DFS) at 60 months’ follow-up were both 77%. Our results with the BU-TTP-CY regimen followed by T-cell depleted BMT in β-thalassaemia major and other genetic diseases may provide a basis for prospective comparison with the standard conditioning regimen of BU-CY in the management of children suffering from these conditions.

HIF-0561 Severe chronic neutropenias: clinical course, response and safety of long term treatment with G-CSF

for the Severe Chronic Neutropenia Registry, Medical School, Hannover, Germany; University of Washington, Seattle, USA

Severe chronic neutropenia (SCN) is a heterogenous group of haematological disorders characterised by severe neutropenia with absolute neutrophil counts of less than 500 µL⁻¹, including congenital neutropenias (CN), cyclic neutropenia (CNy) and idiopathic neutropenia (IN). Since 1987 murine/hu-G-CSF (Filgrastim, G-CSF) has been used for the treatment of these patients leading to a benefit for more than ninety percent of SCN patients due to an increase in absolute neutrophil counts. By the end of 1998, the Severe Chronic Neutropenia International Registry (SCNIR) had collected data on clinical course and long term safety of G-CSF treatment in 646 SCN patients (CN 310, CNy 131, IN 205) with a maximum follow up of more than 10 years. Data document a different clinical course and treatment response depending on the SCN subtype: 1. Filgrastim dosing varies by diagnosis (median dose in µg/kg/d is CN 6.9, CyN 2.14, and IN 1.2 with a range of 0.01-240). 2. Long-term Filgrastim does not cause bone marrow exhaustion. 3. There is a reduced risk of leukaemia to the SCN subtype of congenital neutropenias. 4. Cases with chronic granulocytopenia of less than 100 µL⁻¹ have been limited to patients with CN. 4. No cases of MDS/AML have occurred among patients with cyclic or idiopathic neutropenia. 5. Osteopenia/osteoporosis has been reported both at baseline and during G-CSF treatment and the majority of patients reported within the SCNIR (CN 23.3%, CyN 5.6%, IN 7.4%), suggesting that it is a concomitant symptom in CN, but not CNy or IN. These data led to further investigation of the underlying pathomechanism of SCN. For example, leukaemogenesis mutations of the G-CSF receptor seem to play an important role.

HIF-0559 The role of thiopeta in allogeneic bone marrow transplantation for β-thalassaemia major and other genetic diseases

Nadler A, Varadi G, Or R, Naphasted E, Aker M, Slavin S
Bone Marrow Transplantation Department, Hadassah University Hospital, Jerusalem, Israel

The study of minimal residual disease (MRD) has drawn great attention in clinical oncology for the potential of tailoring, treatment and for the possibility of gaining insights into the nature of cure. Several methods have been proposed to detect MRD in leukemias and extensively reviewed. In all rearrangements in which Ig and TCR genes result in unique recombinations of variable (V), diversity (D) and joining (J) gene segments, the junctional regions between these gene segments can be regarded as fingerprint-like sequences due to the deletion and random insertion of nucleotides during the rearrangement process. PCR-based MRD detection via clone-specific junctional regions generally reaches sensitivity of 10⁻⁴ to 10⁻⁵. Several retrovires indicated that the detection of MRD in childhood ALL is prognostic value, although the results of these studies are not fully concordant. In the context of the International BFM study group (I-BFM-SG), we have previously demonstrated in the largest series of ALL patients prospectively evaluated that molecular detection of MRD during the first three months of treatment gives relevant prognostic information about the in vivo effectiveness of treatment. Even in the subset of B-cell precursor ALL, in which relapse cannot predicted by using current clinical and biological features at diagnosis, a case-controlled study showed the predictivity of MRD detection in the identification of patients at higher risk of relapse. Although technically feasible several questions are still open and will be further discussed:
1. How can we use MRD data for future clinical studies in childhood ALL?
2. Which patients could potentially benefit? 3. Do we have clinical options: i.e. intensification of treatment in patients with MRD transplants was extremely poor with a survival of 4%.

HIF-0562 Fanconi anaemia: progress in genetics and therapy

Gluckman E, Guardiola P, Socie G
Hôpital Saint-Louis, Department of Haematology/Oncology Paris, France

Fanconia anaemia (FA) is an autosomal recessive disorder belonging to the group of chromosomal instability syndromes. Diagnosis is usually performed by cytogenetic analysis which shows increased chromosomal breaks following exposure to DNA cross-linking agents. In order to diminish this risk, since 1997 our patients no longer received irradiation, but a conditioning regimen including cyclophosphamide predisposition and chronic GVHD were associated with an increased risk of cancer. In order to diminish this risk, since 1997 our patients no longer received irradiation, but a conditioning regimen including cyclophosphamide (total dose 40 mg/kg) and thiotepa (total CN 6 mg/kg), 4. For this regimen has been well tolerated with good engraftment, minimal GVHD and severe GVHD. From the database of the European Blood and Marrow Transplant Group (EBMT), we have analyzed the outcome of 92 patients transplanted from 1979 to June 1998 either with unrelated (N=75) or matched unrelated (N=17) donors. Overall survival at 54 months was 74.4% and 58.5% at 100 months. Acute graft versus host disease (GVHD) developed in 50% of the patients and chronic GVHD in 70% of 43 patients surviving for more than 90 days. Adverse factors for survival were a high number of transfusions prior to transplantation and the occurrence of GVHD. Patients without chronic GVHD had a 100% long-term survival. The most important complication was the development of cancer with an 8-year projected incidence of 24%. Genetic predisposition and chronic GVHD were associated with an increased risk of cancer. In order to diminish this risk, since 1997 our patients no longer received irradiation, but a conditioning regimen including cyclophosphamide (total dose 40 mg/kg) and thiotepa (total CN 6 mg/kg), 4. For this regimen has been well tolerated with good engraftment, minimal GVHD and severe GVHD. From the database of the European Blood and Marrow Transplant Group (EBMT), we have analyzed the outcome of 92 patients transplanted from 1979 to June 1998 either with unrelated (N=75) or HLA mismatched related donors (N=17). The overall 3 year probability of survival was 26.5%. With a 3-year overall survival of 42%, patients who received T cell depleted unrelated transplants had the best outcome. A severe constitutional phenotype, positive recipient CMV serology, elevated liver transaminases, severe cytopenia, conditioning without irradiation and use of female donors were associated with poor outcome. The outcome of mismatched related transplants was extremely poor with a survival of 4%. The day 30 probability of engraftment was 76%. The probability of developing grade II-IV GVHD was 46±5%. Our current conditioning regimen
includes cyclophosphamide 40 mg/kg, single dose 4.5 Gy total body irra-
diation and 5 doses antithymocyte globulin followed by T cell depleted
bone marrow infusion. High resolution typing for class I and Class II HLA
antigens should improve donor recipient selection and therefore further
improve current results.

HIF-0563 The severe juvenile form of hemochromatosis maps
to chromosome 1q
Camaschella C,* Roetto A,* Cazzola M, Bosio S,* Ciciliano M, *
D’Ascola G,* Totaro A,*, Carella M,*, Kelly AL, Cox TM,* Gasparini P,*
*Dipartimento di Scienze Cliniche e Biologiche, Università di Torino;
*IRCCS CSS San Giovanni Rotondo; IDipartimento di Medicina Interna e
Oncologia, IRCCS Policlinico San Matteo, Università di Pavia; *Centro
Microcitemie, Ospedale di Reggio Calabria, Italy; *Department of Medi-
cine, University of Cambridge, UK

Juvenile haemochromatosis (JH) is an autosomal recessive disorder which
leads to severe iron loading early in life. The disease affects equally both
sexes. Prevalent clinical symptoms are hypogonadism and cardiac dis-
ease, which if untreated can be fatal. In contrast to hereditary haemochro-
matisosis (HH), JH patients do not have mutations in the HFE gene, the gene
responsible for hemochromatosis, and do not show linkage to chromo-
some 6p. We have performed a genome-wide search (GWS) using 375
markers of the ABI PRISM Linkage Mapping Set (Perkin Elmer, USA) at
approximately 10 cM distance in 9 JH families, 6 with consanguineous par-
ents and 3 with multiple affected patients. PCR was performed using flu-
orescent primers and aliquots of PCR reaction were run in an ABI PRISM
373 or 377 DNA sequencer. The results were processed by the GENESCAN
software and allele assignation was carried out using the Genotype™ soft-
ware. Using this approach the JH locus was mapped to chromosome 1q.

The results were processed by the GENESCAN software and allele assignation was carried out using the Genotype™ soft-
ware. Using this approach the JH locus was mapped to chromosome 1q. A maximum lod score of 5.75 at a recombination fraction of 0 was detect-
ed with marker D1S498. Homozygosity mapping in consanguineous fami-
lies defined the limits of the candidate region in an approximately 4 cM
interval between markers D1S442 and D1S2347. Analysis of genes mapped
in this interval excluded obvious candidates. These results repre-
sent the first step towards the cloning of the gene and offer a tool for
the early recognition of patients and carriers within the affected families. Since
the JH locus does not correspond to the chromosomal localisation of any
known iron gene its cloning will provide new insights in iron metabolism.

HIF-0564 Methotrexate resistance in vitro can be overcome with
long-term continuous exposure in childhood acute myeloid
leukaemia
Rots MG, Kappers GJL, Pieters R, van Zantwijk CH, Creutzig U,
Noordhuis P, Peters GJ, Veerman AJP, Jansen G
Univ. Hospital Vrije Universiteit, Dept of Pediatric Hematology/Oncology
and Medical Oncology, Amsterdam, The Netherlands

Objective. AML is presumed to be resistant to methotrexate (MTX), based
on studies in few patients using low doses and mainly i.v bolus or short-
term infusions. We compared MTX metabolism and sensitivity in B-cell pre-
cursor (BCP-) ALL and AML and explored the possibility of overcoming
MTX resistance in AML in vitro. Design and Methods. We adapted a MTX
sensitivity assay based upon the MTXinduced inhibition of the thymidylate
synthase (TS) catalysed conversion of 3HdUMP to dTMP and 3H₂O. Blasts
from children with BCP-ALL and AML were also analysed for 3H-MTX accu-
mulation and -polyglutamylation after a 24 hour exposure to 1 µM 3H-MTX.
Finally, the blasts were analysed for levels of folylpolyglutamate synthetase
(FPGS, catalyses formation of polyglutamates) and folylpolyglutamate
hydrolase (FPGH, breaks down polyglutamates). Results. AML cells (n=22)
were 6-fold more resistant to MTX than BCP-ALL cells (n=43) after a 3-hour
drug exposure followed by an 18 hour drug-free period (IC50 values 2.20
and 0.38 µM respectively, p<0.001). This was accompanied by a 4-fold
higher accumulation of the pharmacologically more important and intra-
cellularly better retained long-chain MTX polyglutamates (MTX-Glu4-6) in
BCP-ALL (n=40) than in AML (n=14, 906 and 225 pmol/10⁹ cells respec-
tively, p<0.001). This poor polyglutamylation in AML could be explained by
both a 2-fold decreased activity of FPGS (6.9 vs 14.6 pmol of MTX-[3H]Glu2
formed/hr/10⁶ cells respectively, n=19 vs 39, p=0.004) and a 3-fold
increased activity of FPGH (1.15 vs 0. 36 nmol MTX-Glu 2 hydro-
lised/hr/10⁶ cells respectively, n=33 vs 94, p<0.001). The MTX resis-
tance upon short-term drug exposure was overcome with continuous 21-
hour exposure to MTX: AML cells were as sensitive to MTX as the BCP-ALL
samples in the TS inhibition assay under these circumstances. Conclu-
sions. In vitro resistance to MTX in childhood AML, at least partly related
to poor MTX-polyglutamylation caused by unfavorable FPGS and FPGH
activities, was overcome by long-term continuous exposure to MTX Sup-
pported by the Dutch Cancer Society (grant WJ 94-679).
SS0565 Expression of Ikaros isoform IK5 causes a reduction in γδ intrathymic lymphocytes and NK1.1+ T-cells

Tucker SN, Jessep HK, Wison CB
University of Washington Department of Immunology, Seattle, WA, USA

To elucidate the role the zinc finger protein Ikaros plays in cells committed to lymphoid development independent of its effects in hematopoietic stem cells, a naturally occurring, dominant negative Ikaros isoform (IKS) was overexpressed in the lymphoid progenitors of transgenic mice (bi-5 mice). The promoter used to express IKS was a combination of the Iκκ proximal promoter and the immunoglobulin heavy chain enhancer. The bi-5 mice exhibited a reduction in the T-cell lineages with extrathymic origins but have relatively normal numbers of conventional T and B cells. Comparisons between bi-5 mice and littermate controls demonstrated a substantial decrease in the percentage and numbers of γδ-intestinal intrathymic lymphocytes (IEL) in the bi-5 mice. Bi-5 γδ-δ-IEL averaged 8.3±2% s. d. of 3.3% whereas littermates averaged 23.5±6% s. d. of 8.6% (N=6 pairs). There was also a decrease in the percentage and numbers of NK1.1+ CD8α+ gated thymocyte population versus 11%±s. dev. of 4.4% of bi-5 mice (N=6 pairs, age 8-14 weeks). This phenotype is remarkably similar to the development phenotype observed from disruption of the IL-15 signaling pathway (Suzuki, et al.)

SS0566 Igl light chain expression is determined by ordered gene rearrangement and allelic exclusion processes

van der Bum M, Tümkaya T, Boerma M, de Bruin-Versteeg S, Langerak AW, van Dongen JM
Department of Immunology, Erasmus University Rotterdam, The Netherlands

In normal and malignant B-cells functional expression of immunoglobulin kappa (IGK) genes occurs more frequently than functional expression of immunoglobulin λ (IGL) genes, resulting in an Igκ+/IgL- distribution of approximatly 1:4. Two models have been proposed to explain this relative ‘over usage’ of IGK genes: the ordered and the stochastic model. The first model argues that IGK genes rearrange prior to IGL. The stochastic model argues that both Ig light chain genes rearrange totally independently, but that other factors handicap IGL gene rearrangements, such as inefficient recombination signal sequences and the complex structure of the human IGL. To determine which model is applicable to the human Ig light chain gene rearrangements, the configuration of both IGK and IGL alleles was studied by Southern blotting, PCR and sequencing, in a series of 53 Igκ+ and 59 IGL+ cases. Igκ+ cases were used as a ‘single-cell’ model system. Half of the Igκ+ CBL had one rearranged IGK allele with IGL genes in germine configuration, while the other half had biallelic IGK gene rearrangements or one deleted and one rearranged IGK allele. Six percent (3/53) of the Igκ+ CBL also had IGL rearrangements. In the group of Igκ+ CBL, all cases had at least one deleted IGK allele and approximately 80% (47/59) of them had biallelic Igκ gene rearrangements. Twenty-five percent (15/59) of the Igκ+ CBL had biallelic IGL rearrangements. Ten percent showed one rearranged and one deleted IGK allele. The data indicate that the Igκ+ light chain gene rearrangements can best be explained by an ordered model from germine (G) via rearrangement (R) to deletion (D): one rearranged IGK allele (R/G) → both IGK genes rearranged (R/R) → one deleted IGK allele (R/D) and occasionally one rearranged IGL allele (R/G) → both IGL alleles deleted (D/D) and one or two IGL gene rearrangements (G/R or R/R). Several Igκ+ CBL and Igκ+ CBL have in-frame IGL and in-frame IGK genes, respectively. Therefore, dual Ig light chain expression can occur if no allelic/isoform exclusion mechanism is present. However, dual Ig light chain expression is a rare event, implying that exclusion of two functional Ig light chain genes might be regulated at the transcription, translation or post-translation level.
SS8 – Acute myeloid leukaemia

Dendritic cells (DC) represent the most powerful professional antigen-presenting cells (APC). In spite of the large information accumulated in recent years about DC, the results reported about their phenotypic and functional characteristics are not always uniform. This could be due to the use of different methodological approaches both for the enrichment and analysis of DC. The aim of the present study was to analyse the immunophenotypic profile of DC from normal peripheral blood (PB) and their ability to produce cytokines. A total of 67 erythrocyte-lysed whole PB samples from heas in a significant percentage of patients with acute leukaemia, there is a need for mild growth factors. These promising results encouraged us to test Fc epsilon RI redirected BsAb against CD11b, CD17 and HLA- II in whole blood assays against mature B-cell line ARH-77, [FcεRI x HLA-II] and [FcεRI x CD20] BsAb mediated significant lysis, whereas [FcεRI x CD13] showed only marginal cytolytic activity and [FcεRI x CD37] was completely ineffective. Subsequently, we substantiated our promising results with [FcεRI x CD20] with a wide range of malignant B-cell lines. In conclusion, [FcεRI x CD20] bispecific antibodies are an interesting approach to improve effector cell recruitment for CD20-directed immunotherapy of lymphoma.

SS8 – Acute myeloid leukaemia

SS8 – Acute myeloid leukaemia

SS571 Core binding factor leukaemias are a discrete group: experience from MRC AML 10, 11 and 12 trials

Burnett AK, Goldstone AH, Wheway K on behalf of MRC Adult Leukaemia Working Party, UK

Between 1988-1998 AML10, 11 and 12 Trials of primary treatment of AML recruited 4653 patients of whom 3757 (81%) were cytogenetically abnormal. The best remission rate was achieved with CMML and the worst with AML with t(8;21) and t(15;17). We have previously shown that additional cases may be detected by RTPCR for bcr-abl

SS572 SHC adaptor proteins and ERK/JNK map kinases in acute myelogenous leukaemia


Institute of Medical Pathology and Chair of Hematology, University of Parma; Chair of Haematology, University of Perugia, European Institute of Oncology, Milano, Italy

It has been reported that the capacity of autonomous in vitro proliferation of leukaemic blasts is associated with highly clinical aggressivity of acute myelogenous leukaemia (AML), but the mechanisms involved are not yet completely understood. It is mandatory to understand whether or not different signalling proteins are activated in AML blasts and whether the activation is higher than in normal haemopoietic precursors.In this paper, we report the results of the analysis of signal transduction pathways in twenty-five cases of AML. All the cases were examined at diagnosis by studying samples presenting more than 90% leukaemic cells. FAB classification, immunological, cytogenetic and DNA staining on monocytes/macrophages, neutrophils and eosinophils. As the numbers of these effector cells and their functional activity can be enhanced by application of G-CSF or GM-CSF, lysis via [FcεRI x CD20] BsAb was significantly enhanced with whole blood from patients during therapy with multimodal growth factors. These promising results encouraged us to test Fc epsilon RI redirected antibodies against more B-cell related antigens. Comparing Fc epsilon RI redirected BsAb against CD19, CD17 and HLA- II in whole blood assays against mature B-cell line ARH-77, [FcεRI x HLA-II] and [FcεRI x CD20] BsAb mediated significant lysis, whereas [FcεRI x CD13] showed only marginal cytolytic activity and [FcεRI x CD37] was completely ineffective. Subsequently, we substantiated our promising results with [FcεRI x CD20] with a wide range of malignant B-cell lines. In conclusion, [FcεRI x CD20] bispecific antibodies are an interesting approach to improve effector cell recruitment for CD20-directed immunotherapy of lymphoma.
SS-0573 Secondary cytogenetic changes in acute promyelocytic leukaemia. Prognostic importance in patients treated with an ATRA plus anthracycline-based protocol


Acute promyelocytic leukaemia (APL) is characterised by the translocation t(15;17)(q22;q11-12), leading to the formation of PML/RARα and RARγ/PML fusion products. Usually, this translocation is the sole cytogenetic abnormality. Other PML/RARα cases also show other structural chromosome aberrations, especially del(5) in addition to t(15;17). The influence of these secondary cytogenetic changes on the clinical outcome of APL cases remains uncertain. The purpose of this study was to analyse the relationship between additional cytogenetic abnormalities and other clinical and biological characteristics, as well as their prognostic importance, in patients with newly diagnosed APL. Patients treated with an anthracycline-based protocol (idarubicin 12 mg/m² only one day (course 1), idarubicin mg/m² daily for 5 days (course 2), and IDR 12 mg/m² only one day (course 3). As maintenance therapy, all patients were administered mercaptopurine (22.9-75) than the p-gp negative samples (mean RMFI 96.5, range 77.5-110) after overnight culture, p<0.01. These results indicate that p-glycoprotein plays a drug-efflux-independent role in augmenting cell survival in acute myeloblastic leukaemia (AML) and MDR activity in adult de novo acute myeloid leukaemia.

SS-0574 Angiogenesis in myelodysplastic syndromes and myeloid malignancies


Acute promyelocytic leukaemia (APL) is characterised by the translocation t(15;17)(q22;q11-12), leading to the formation of PML/RARα and RARγ/PML fusion products. Usually, this translocation is the sole cytogenetic abnormality. Other PML/RARα cases also show other structural chromosome aberrations, especially del(5) in addition to t(15;17). The influence of these secondary cytogenetic changes on the clinical outcome of APL cases remains uncertain. The purpose of this study was to analyse the relationship between additional cytogenetic abnormalities and other clinical and biological characteristics, as well as their prognostic importance, in patients with newly diagnosed APL. Patients treated with an anthracycline-based protocol (idarubicin 12 mg/m² only one day (course 1), idarubicin mg/m² daily for 5 days (course 2), and IDR 12 mg/m² only one day (course 3). As maintenance therapy, all patients were administered mercaptopurine (90 mg/m²/day p.o.), methotrexate 15 mg/m² week IM, and intermittent ATRA 45 mg/m² daily for 15 days every 3 months. Secondary cytogenetic abnormalities were observed in 19 (23%), Trisomy 8, the most frequently found secondary change, was detected in 11 out of 19 patients (58%). Other different structural rearrangements in addition to t(15;17) were detected in nine patients. There were no correlations between secondary cytogenetic changes and presenting features (including clinical, cytophysiological, immunophenotypic and molecular characteristics). There were no differences in CR, molecular response and relapse rates between cytogenetic subgroups. This study indicates that there is no rationale for administering more intensive treatment in those APL patients with secondary cytogenetic abnormalities receiving ATRA plus anthracycline-based chemotherapy.

SS-0575 P-glycoprotein plays a drug-efflux-independent role in augmenting cell survival in acute myeloid leukaemia

Pallis M, Russell NH

Acute promyelocytic leukaemia (APL) is characterised by the translocation t(15;17)(q22;q11-12), leading to the formation of PML/RARα and RARγ/PML fusion products. Usually, this translocation is the sole cytogenetic abnormality. Other PML/RARα cases also show other structural chromosome aberrations, especially del(5) in addition to t(15;17). The influence of these secondary cytogenetic changes on the clinical outcome of APL cases remains uncertain. The purpose of this study was to analyse the relationship between additional cytogenetic abnormalities and other clinical and biological characteristics, as well as their prognostic importance, in patients with newly diagnosed APL. Patients treated with an anthracycline-based protocol (idarubicin 12 mg/m² only one day (course 1), idarubicin mg/m² daily for 5 days (course 2), and IDR 12 mg/m² only one day (course 3). As maintenance therapy, all patients were administered mercaptopurine (90 mg/m²/day p.o.), methotrexate 15 mg/m² week IM, and intermittent ATRA 45 mg/m² daily for 15 days every 3 months. Secondary cytogenetic abnormalities were observed in 19 (23%), Trisomy 8, the most frequently found secondary change, was detected in 11 out of 19 patients (58%). Other different structural rearrangements in addition to t(15;17) were detected in nine patients. There were no correlations between secondary cytogenetic changes and presenting features (including clinical, cytophysiological, immunophenotypic and molecular characteristics). There were no differences in CR, molecular response and relapse rates between cytogenetic subgroups. This study indicates that there is no rationale for administering more intensive treatment in those APL patients with secondary cytogenetic abnormalities receiving ATRA plus anthracycline-based chemotherapy.
SS-0577 Interferon alpha (IFN-a) protects Philadelphia-negative haemopoiesis in chronic myeloid leukaemia (CML) patients with cytogenetic response


In previous studies we documented a rapid decline of normal LTC-IC with time in CML patients (pts). Such exhaustion could reflect a suppressive effect of the Ph-positive clone and/or could be induced by treatment with IFN-alpha. We have studied 30 normal donors, 24 newly diagnosed CML pts. (group A), and 21 CML pts. beyond one year from diagnosis (12-84 months): of these 15 showed no cytogenetic response to IFN-alpha (group B), and 21 CML pts. beyond one year from diagnosis (12-84 months) had a significantly lower number of Ph-LTC-IC than the pts. at diagnosis (p<0.01) and C-reactive protein (C-reactive protein) (p<0.02). Furthermore, cy of bone marrow steady state (BM) Ph-neg. LTC-IC/10^6 MNC in normal donors was 6.7 (1.2-30), in CML pts. at diagnosis 0.8 (0-30), in non responders (NCyR) to IFN-alpha (group B) had a significantly lower number of Ph-LTC-IC than the pts. at diagnosis (p<0.01) and C-reactive protein (C-reactive protein) (p<0.02). Furthermore, we have compared the peripheral blood collection yield of pts. of group A and B mobilised with chemotherapy (ICE or mini-ICE) and G-CSF with the yield of normal donors and pts. of group C mobilised with a short course of G-CSF only, Ph-negative LTCIC/10^6 kg yield was 29 (0-952), 0.4 (0-32, 45 (6-207), 108.8 (5.9-259.5) for pts. of group A, B, normal donors and group C respectively. Patients of group B had statistically significantly lower amounts of Ph-neg. progenitors than patients of group A and C (p<0.01). Interestingly, 5 pts. of group B harbored Ph-neg progenitors and were able to mobilize a reasonably adequate numbers of Ph-neg progenitors. This may suggest that there is a proportion of pts. in whom IFN-alpha may be able to protect Ph-neg progenitors while cytogenetic conversion in the maturing compartment, is not reached. Altogether, these findings indicate that normal hematopoietic reservoir is consistently preserved in pts. given IFN-alpha early after diagnosis and who achieve a stable cytogenetic response. It should be noted that IFN-alpha treatment had been protocoted for a median of 3.5 years (range 1.5 to 5). These data also suggest that the decline of normal hematopoietic progenitors, currently observed in the majority of CML pts, is not induced by IFN-alpha treatment, but to be is likely due to the duration and persistence of the expansion of the leukemic clone. It appears that any tool which limits the expansion of Ph-positive clone and enables normal haemopoiesis to function can also preserve it from exhaustion.

SS-0578 Combination therapy with interferon-alpha (IFN-a) and Ara-c in chronic myeloid leukaemia (CML) may suppress residual normal haemopoiesis

Marley SB, Davidson RJ, Goldman JM, Gordon MY

Department of Haematology, Imperial College School of Medicine, London, UK

IFN-alpha selectively reduces the self-replication of CML CFU-GM in vitro (J Clin Invest 102:710-5). Self-replication of CFU-GM is measured by picking and replating individual primary CFU-GM then scoring the number of secondary colonies produced. The degree of self-replication is expressed as Area-Under-The-Curve (AUC) of the distribution of secondary colonies. At physiological concentrations (100 U/mL), IFN-alpha in vitro has no significant effect on primary CFU-GM survival in NBM or CML. NBM AUC is also unaffected by IFN-alpha (91±16% (mean±s.e.m.) of controls, n=2, p=0.53) but CML AUC is significantly reduced (58±9.6% (mean±s.e.m.) of controls, n=24, p=0.0001). Ara-C has a differential effect on CML CFU-GM, with a significant difference in the treatment of CML. Dose response experiments in vitro showed that CML CFU-GM were significantly more sensitive to Ara-C than NBM CFU-GM both in terms of their primary proliferation (inhibition above 5 ng/mL vs 500 ng/mL, p=0.01, n=9 and p=0.0001, n=4, respectively) and their replating ability (inhibition above 5 ng/mL vs 50 ng/mL, p=0.04, n=5 and p=0.0001, n=6, respectively). Addition of Ara-C to IFN-alpha did not further reduce the AUC for CML CFU-GM but significantly reduced the AUC for the normal CFU-GM. This reduced the differential effect on CML progenitors. The therapeutic action of IFN-alpha on CML in vivo may be at least in part through the selective reduction of leukemic progenitors, as measured by this assay. The results presented here suggest that combination therapy with Ara-C may act to negate the selective advantage of normal haemopoiesis seen in the presence of IFN-alpha alone.

SS-0579 Autografting with mobilised hematopoietic progenitor cells in chronic myelogenous leukaemia

Carolina AM, Lerma E, Corsetti MT, Bastida P, Dejana A

N.O.A Haematology and ABMT, Ospedale S. Matino, Genoa, Italy

Seventy-four patients with chronic myelogenous leukaemia (CML) in early or late chronic phase underwent autografting. Thirty-three pts were in early chronic phase (ECP) and had not been previously treated with IFN-alpha (group A), 12 pts were in ECP pretreated with IFN-alpha (group B), 14 pts were in LCP (>24 months from Dx) pretreated with IFN-alpha (group C). All pts were in LCP (>24 months from Dx) pretreated with IFN-alpha (group D). All pts of groups B, C and D were cytogenetically refractory to IFN-alpha. The pts were treated with ICE or mini-ICE protocols and hematopoietic progenitor cells (HPC) were mobilised when wbc were >0.8×10^9/L and, in the last cohort of patients, also when CD34+ cells was >10/L in the peripheral blood. High-dose therapy consisted of a TBI-containing regimen (single dose TBI, idarubicin, etoposide) in 19 pts or high-dose busulfan (16 mg/kg in four days) in 55 pts. All pts received i.v. prophylaxis with Amphotericin B and antibiotics when N<1×10^9/L. Twenty-four of the 33 patients in group A, six of 13 pts in group B, nine of 14 pts in group C and three of 14 pts in group D, were rescued with entirely Ph-negative collections. The combination IFN-alpha (3 MU/d) ± low-dose IL-2 (2 MU/d × 5 days every 8 weeks) was given to the pts soon after HPC engraftment. Thirty-one (94%) pts in group A are alive at a median of 22 mo. (range, 2 to 76) after autografting and nineteen of them are Ph-negative or MCyR. Twenty-one (64%) pts in group B are alive at a median of 33 mo. (range, 17 to 83) after autografting and 3 pts are Ph-negative. Seven (50%) pts in group C are alive at a median of 30 mo. (range, 4 to 70) after autografting and three are Ph-negative or MCyR. Nine (64%) pts in group D are alive at a median of 23 mo. (range, 4-49) and three are Ph-negative or MCyR. Febrile episodes, mucositis and G1 toxicities were rare in group A and more frequent in pts of groups B, C and D. To date, a significant statistical difference in terms of actual survival from autograft has been found in favor of patients of group A vs group B (p=0.0047), group C (p=0.0125) and group D (p=0.0162). In conclusion, these results show encouraging trends in terms of survival in patients autografted early in the first few months after diagnosis. Our procedure (mobilisation of Ph-negative or predominantly Ph-negative HPC followed by IFN-alpha and Ara-C) is now being investigated in randomised trials.

SS-0580 Hydroxyurea versus busulphan for chronic myeloid leukaemia: a meta-analysis of the randomised trials


Chronic Myeloid Leukaemia Trials’ Collaborative Group, Clinical Trial Service Unit, Oxford, UK

Although interferon-alpha (IFN-alpha) has been shown to prolong survival in chronic myeloid leukaemia (CML), it is not the treatment of choice for a large number of patients. Therefore it is valuable to know the relative effects of hydroxyurea and busulphan. In order to establish which treatment gives better survival, data was collected for each patient in the three trials which randomised between hydroxyurea and busulphan. Intention-to-treat analyses were performed within trial and the results combined by standard methods. Analyses were also done for predefined subgroups ( Sokal stage, age, gender). In Philadelphia chromosome (Ph) positive CML, hydroxyurea was significantly better than busulphan (p=0.03). Survival at 5 years was 30% with busulphan and 40% with hydroxyurea, an absolute benefit of...
SS10 - Infections

SS0583 Incidence of HSV infection during therapy induced mucositis (TIM) in oncology, haematology and stem cell transplantation (BMT): a prospective multicentre study

Miplidy N*, Cordieroin C**, Domenge C*, Landais P†, Morand P. ‡
*de Laboretty C for the Giaou Welcomme France HSV Study Group, **Hematology, Nantes, France; †Clinical Hematology, Créteil, France; §Cancerology, Villejuif, France; ¶Biostatistics, Paris, France; ¶Virology, Grenoble, France; ¶Giaou Welcomme, France

According to several reports the incidence of HSV infection associated with TIM is 50-90% after BMT, 50% after chemotherapy for AL and 20-30% after chemotherapy or chemoradiotherapy for solid tumours. However, the accuracy of these numbers is questionable since no precise epidemiological study was performed. Between October 97 and July 98 viral cultures were performed on mouth swabs harvested on the first day of WHO grade ≥ 2 TIM in 330 patients without antiviral prophylaxis in 37 centres: 183 patients were treated for solid tumours (onc) 121 patients underwent a BMT (88 auto, 32 allo) and 106 patients were treated for haematologic malignancies (haemat). According to a predefined score of mucosal toxicity for the 10 most chemotherapeutic agents and for BMT, allo BMT and haemat patients respectively received a high mucotoxic regimen and 48%, 3% and 43% an intermediate mucotoxic regimen. Of the 94 patients serologically tested, 72% were HSV seropositive. At time of sampling the median duration of TIM was 2 d (0-13), the WHO grade was 2 in half of the patients and 4 in 10-15%. The incidence of HSV infection was 14%, 39%, 28% and 27% in oncology, auto BMT, allo BMT and haemat respectively (p<0.001). The TIM herpesvirus seropositive patients has documented HSV infection. The incidence of HSV infection in seropositive patients was 38%, 50% and 33% in auto BMT, allo BMT and haemat patients respectively. In each group of patients the incidence of HSV infection was associated with any of the following factors: age, grade or duration of TIM, neutropenia, fever, history of HSV, expected mucosal toxicity of the chemotherapy. The incidence of HSV infection associated with TIM, as shown in this prospective study on a large number of patients is lower than expected, even in HSV seropositive patients. Such results could be a basis for a reasoned use of antiviral drugs in these settings.

SS0584 Combined foscarnet and ganciclovir at half doses for the pre-emptive treatment of CMV infection: a pilot study

Royal Free & University College Medical School, London, U.K

Ganciclovir provides effective pre-emptive antiviral treatment for CMV infection but treatment-related neutropenia and infection are problematic. Foscarnet can be nephrotoxic. We hypothesised that pre-emptive treatment of CMV viraemia with ganciclovir plus foscarnet at 50% of standard dose would reduce toxicity and preserve efficacy. Patients undergoing allogeneic BMT were monitored twice weekly by PCR for CMV DNA and therapy initiated if 50% positive consecutive PCRs were detected. CMV viraemia with ganciclovir plus foscarnet at 50% of standard dose appears effective in patients receiving concomitant nephrotoxic agents and one had cyclosporin-related thrombotic microangiopathy. Combination therapy with ganciclovir and foscarnet at 30% standard doses remains effective for CMV viraemia and prevention of CMV disease post allogeneic BMT and may reduce the myelotoxicity of treatment. Two patients with HHV-6 infection

SS0581 IFN-α or IFN-α and ARA-C combination treatment chronic myeloid leukaemia: a single centre experience

Liberti AM, Bettì AR, Venducci N, Al Sharjaby N, Schiappa M, Degli Angeli PS, Basseti A, Doni E, Mettivier V
Sect. Intern. Med. Oncol. Sci., Univ. of Perugia, Italy

Aims. The aims of this study were to confirm or not: 1) the therapeutic usefulness of IFN-α therapy and 2) the response to the combination of IFN-α and low-dose (25-40 mg/die) ARA-C used at diagnosis or as second line therapy (after 12 mths) in a cohort of chronic myeloid leukaemia (CML) pts treated in a single center. Patients: Sixty-eight CML pts (50-38 F/M), 64 Ph+ (4-74%) and 4 Ph-, but all bcr-abl rearranged were observed from 1979 to 1998. Three pts were allotransplanted at diagnosis and 4 were treated with hydroxyurea treatment. The other 9 pts were treated within the first month following diagnosis with the combination of IFN-α and ARA-C. This combination was also administered to 36 pts (four had been off IFN-α for more than 1 year) in order to induce a complete or major cytogenetic response. These patients are heterogeneous as shown by the durability of the cytogenetic response and the level of residual myeloblasts while in CCR and one of transformation to ALL. In 3 of the 20 patients who obtained a complete or major CCR, 63.5 mth (13-213) for the 18 pts (5 still alive) who obtained a complete or major KR, 63.5 mth (13-213) for the 18 pts (5 still alive) with no KR.

SS0582 Characteristics of complete cytogenetic responders to IFN therapy in CML. Importance of durability of response

Allan NC, Shepherd P, Richards S, on behalf of the MRC CML Working Group
Western General Hospital, Edinburgh, Scotland

Interferon-α therapy in CML offers the chance of extended and possible longterm survival in CML. The best survival is seen in patients who achieve a complete cytogenetic response. These patients are heterogeneous as shown by the durability of the cytogenetic response and the level of residual BCR/ABL transcripts. In the MRC CML Intl Trial 20 out of 215 Ph+ patients evaluated for both haematological and cytogenetic response achieved a complete cytogenetic remission (CCR). Nineteen out of 20 had good haematologic control of the white count (<10 x 10^9/L) at six months. Most showed a white cell count of <5 x 10^9/L at six months. Of the 20 patients who achieved a Ph-CR and ARA-C combination treatment 19 pts (95%) had no plateau; for patients treated in a single center. Patients: Sixty-eight CML pts (50-38 F/M), 64 Ph+ (4-74%) and 4 Ph-, but all bcr-abl rearranged were observed from 1979 to 1998. Three pts were allotransplanted at diagnosis and 4 were treated with hydroxyurea treatment. The other 9 pts were treated within the first month following diagnosis with the combination of IFN-α and ARA-C. This combination was also administered to 36 pts (four had been off IFN-α for more than 1 year) in order to induce a complete or major cytogenetic response. These patients are heterogeneous as shown by the durability of the cytogenetic response and the level of residual myeloblasts while in CCR and one of transformation to ALL. In 3 of the 20 patients who obtained a complete or major CCR, 63.5 mth (13-213) for the 18 pts (5 still alive) who obtained a complete or major KR, 63.5 mth (13-213) for the 18 pts (5 still alive) with no KR.

10% (95% confidence interval=0.4% to 19.2%). There was no subgroup for which the treatment difference was statistically significantly different from the average. The survival of patients not treated with interferon is better with hydroxyurea treatment than with busulphan.

Patients: Sixty-eight CML pts (50-38 F/M), 64 Ph+ (4-74%) and 4 Ph-, but all bcr-abl rearranged were observed from 1979 to 1998. Three pts were allotransplanted at diagnosis and 4 were treated with hydroxyurea treatment. The other 9 pts were treated within the first month following diagnosis with the combination of IFN-α and ARA-C. This combination was also administered to 36 pts (four had been off IFN-α for more than 1 year) in order to induce a complete or major cytogenetic response. These patients are heterogeneous as shown by the durability of the cytogenetic response and the level of residu
SS-0586 Response to vaccination against influenza, genetic delivery systems to enhance the immune response to vaccination.

M. K. Gandhi, W. T. Inman, J. C. Grose, M. Reaves
Departments of Haematology, Addenbrooke's, Cambridge and Immunology, Northern General Hospital, Sheffield, UK

Background. After autologous and allogeneic stem cell transplant there is significant risk of morbidity and mortality from influenza. Without restoration of adequate humoral immunity, it is unlikely that these patients will mount a protective antibody response to influenza vaccination. Information regarding the efficacy of influenza vaccination in this setting is minimal, and there is to our knowledge no published data on the humoral response to this vaccine following autologous EBMT. EBMT guidelines recommend influenza vaccination to all survivors in the first 2 years following transplantation. Objective. To assess antibody response to a single-dose trivial influenza vaccine (H3N2, H1N1, B) in 37 non-T-cell-depleted transplant recipients (22 autologous, 7 allogeneic and 8 alloBMT). Patients. Twelve were 15F, 22M; median age at transplant: 47 yrs (range 15-63); diagnosis: 13 NHL, myeloma, 6 AML, 5 HD, 4 CML, 1 ALL. Median vaccination was 12 mo. post-transplant (range 4-28). Haemagglutination-inhibition (HI) antibody titres were determined prior to and at 1 and 6 mo. post-vaccination. Susceptibility was compared to an unvaccinated control population of 26 healthy volunteers and 9 transplant recipients. One out of 26 healthy; 0/9 transplanted controls and 0/37 vaccinated patients had protective titres against all 3 strains at baseline. Humoral response (i.e. a degree of response that reflects both a protective antibody level and a response to the current vaccine) was defined as an HI titre >40 to the components of the vaccine and a ≥4 fold rise in HI titre after vaccination to any of the 3 strains used. Results. Overall, 12/27 (57.4%) had a humoral response to all 3 strains, and 5/37 (13.5%) responded to one or two serotypes. Antibody responses were maintained at 6 mo. post-vaccination. No responses were seen after alloBMT. There was no significant difference in immunogenicity between vaccine strains. Conclusions. Influenza vaccination is almost completely ineffective after allogeneic stem cell transplantation. Many different groups compete for a limited supply of vaccine each year, and routine use after stem cell transplant cannot be recommended. We are currently exploring novel antibody delivery systems to enhance the immune response to vaccination.

SS-0587 Advantages of adding tazobactam to the piperacillin/gentamicine regime as initial empirical antibiotics in neutropenic fever due to Hickman line sepsis

Lee ES, Chu P
Department of Haematology, Royal Liverpool University Hospital, Liverpool, UK

Neutropenic fever has been an important cause of morbidity after high dose chemotherapy for haematological malignancies. Infections account for most of neutropenic fevers, and with the widespread use of indwelling Hickman lines and oral prophylactic antibiotics against Gram-negative bacteria, Gram-positive infections, particularly the coagulase negative staphylococci (CNS) have become an important cause of neutropenic fever. However, many of the antibiotic combinations used in this situation (e.g. piperacillin and gentamicin (PG)) are often ineffective against CNS and additional antibiotics, e.g. glycopeptides, are often required. As tazobactam, a beta-lactamase inhibitor, has been shown to extend the spectrum of piperacillin, we have evaluated the clinical benefits of adding tazobactam to the PG regime, with particular regard to glycopeptide and other antibiotic usage, in neutropenic fever. A group of 79 neutropenic patients with a Hickman catheter and neutropenic fever were randomised into receiving piperacillin/tazobactam (PTG) or PG as initial empirical treatment. The initial therapeutic regimens were as follows: PTG (10/13.5 mg/kg/d) or PG (22 autoPBSCT, 7 autoBMT and 8 alloBMT). There was no significant difference in immunogenicity between vaccine strains. Conclusions. Influenza vaccination is almost completely ineffective after allogeneic stem cell transplantation. Many different groups compete for a limited supply of vaccine each year, and routine use after stem cell transplant cannot be recommended. We are currently exploring novel antibody delivery systems to enhance the immune response to vaccination.

Christie Hospital NHS Trust, Manchester; PHLS, Whittington, Manchester; PHLS, Colindale, London, UK

Vaccination against influenza and Streptococcus pneumoniae is recommended for elderly and immunocompromised individuals. However there is little information concerning the efficacy of vaccination in specific groups of patients. The aim of this study was to document the degree of susceptibility of patients with multiple myeloma to infection by influenza, S. pneumoniae and H. influenzae type b (Hib) and ascertain their serological response to vaccination against these three organisms. Fifty-two patients were offered vaccination as they attended a weekly out patient clinic. The vaccines used were: Fluvirin (Evans) containing inactivated A/Singapore/6/86 (H3N1), A/Wuhan/359/95 (H3N2) and B/Beijing/184/93, Pneumovax II (Pasteur-Merieux) and ACT-HIB (Familon). Serum was analysed prior to vaccination and between 4-6 weeks afterwards. Antibody titres against S. pneumoniae and Hib were compared with reference values corresponding to the geometric mean titres of a healthy UK population. The vaccines used were: Fluvirin (Evans) containing inactivated A/Singapore/6/86 (H3N1), A/Wuhan/359/95 (H3N2) and B/Beijing/184/93, Pneumovax II (Pasteur-Merieux) and ACT-HIB (Familon). Serum was analysed prior to vaccination and between 4-6 weeks afterwards. Antibody titres against S. pneumoniae and Hib were compared with reference values corresponding to the geometric mean titres of a healthy UK population. The vaccines used were: Fluvirin (Evans) containing inactivated A/Singapore/6/86 (H3N1), A/Wuhan/359/95 (H3N2) and B/Beijing/184/93, Pneumovax II (Pasteur-Merieux) and ACT-HIB (Familon). Serum was analysed prior to vaccination and between 4-6 weeks afterwards. Antibody titres against S. pneumoniae and Hib were compared with reference values corresponding to the geometric mean titres of a healthy UK population. The vaccines used were: Fluvirin (Evans) containing inactivated A/Singapore/6/86 (H3N1), A/Wuhan/359/95 (H3N2) and B/Beijing/184/93, Pneumovax II (Pasteur-Merieux) and ACT-HIB (Familon). Serum was analysed prior to vaccination and between 4-6 weeks afterwards. Antibody titres against S. pneumoniae and Hib were compared with reference values corresponding to the geometric mean titres of a healthy UK population.
SS11 - New trends in stem cell transplant

SS-0589 Radioimmunotherapy with 111In-relabelled anti-CD164b monoclonal antibody: results of a phase I/II study

*Depts of Internal Medicine III, *Transfusion Medicine and Nuclear Medicine, Uln University Hospital, Ulm, Germany

In order to reduce the risk of relapse after a stem cell transplant in patients with high-risk leukemias we have employed radioimmunonjugates. We have used an anti-CD164b monoclonal antibody (NCA 95) which binds to early granulopoiesis. The antibody was labelled with 111In as an almost pure ß-emitter. Dosimetry was performed with the 111In-labelled antibody. Twenty-two patients have so far been included in the pilot study: AML >1, CR 11 pts, AML 1 CR (high-risk) 2 pts, CML >1.c.p. 3 pts, ALL 2nd CR 1 pt, ALL 1 CR (Ph+). 2 pts, CLL 1 pt. All 20 pts showed favourable dosimetry defined as a red marrow dose higher than that of the dose limiting organ. The marrow doses ranged from 2.7 Gy to 19.2 Gy with a median of 13 Gy. All 20 pts received additional conditioning with TBI 12 Gy or Bu 16 mg/kg plus CTX 120 mg/m2. Eight pts were given T-cell depleted allogeneic cell grafts (1 BMT, 17 PBSCT) from family (n=12) or matched unrelated donors (n=6). 2 pts received unpurged autologous PBSCT. MILD nausea and vomiting were the only adverse effects associated with the i.v. application of the antibody. No excess early mortality was observed, one patient died of late VOD, 1 pt of CMV-IP and 1 pt of septicaemia. All patients showed rapid engraftment, which was durable in 19. Mild aGVHD was observed in 4 pts. Sixteen pts are alive and in remission, 1 pt has relapsed. These preliminary data suggest that we have a simple, safe and effective method of selectively intensifying the conditioning regimen for high-risk patients.

SS-0590 Donor lymphocyte infusions (DLI) for patients who relapse after allogeneic bone marrow transplantation (BMT) for chronic myeloid leukaemia (CML): the use of RT-PCR for bcr-abl transcripts to predict durability of response and survival

Dazzi F, Sydlov RM, Craddock C, Olavarría E, Kaeda I, Cross NCP, Deane M,* Lawler M, Koh M,* Wilson A°
Dept of Haematology, Imperial College School of Medicine, Hamner smith Hospital, London, UK

We analysed response to treatment with DLI in 64 consecutive patients who relapsed after primary treatment by allogeneic BMT for BCR-ABL-positive CML. The transplant donor was an HLA-identical sibling (n=34) or a ‘matched’ unrelated volunteer (n=30). Fifty-six patients were transplantant in chronic phase (n=45, 71% in accelerated phase and one in second CP). The recognition of relapse was based on molecular, cytogenetic or haematologdistic criteria. The median interval from transplant to relapse was 12 months (range 4-77 months). The probability of survival for patients was significantly better for patients who achieved a molecular remission lasting 4 months or longer than for those who failed to achieve molecular remission (97% vs 55% at 3 yr post-DLI). We conclude that serial RT-PCR studis are valuable for monitoring patients treated by DLI and help to define a population likely to experience long-term leukaemia-free survival.

SS-0591 Fludarabine based conditioning ("mini transplant") for second allograft in acute leukaemia

*Royal Free Hospital & University College Medical School, and †Trinity College, Dublin, Ireland

Fludarabine-containing non-myeloablative regimens have been shown to be effective as conditioning for allogeneic progenitor cell (PC) transplantation. We report on 6 patients in whom the combination of fludarabine, high dose ara-C and G-CSF with or without idarubicin (FLAGIda) was used as conditioning for PB or BM PC transplantation for treatment of relapse post-BMT. Three patients had AML and 3 and were in CR1 at 1 BMT in 4 cases and CR2 in 2 cases. The transplants were from fully matched related donors using Cy/TBI conditioning. GvH prophylaxis was partial T-cell depletion in 2, cyclosporin A (CyA) in 4 and CyA plus MTX in 3. None developed GvHD. One hundred percent donor chimaerism was attained in 3 cases and mixed chimaerism (90-98%) in the other 3. Remis- sions after first BMTs lasted 3-66 (median 10) months. One patient with Ph1+ ALL had 3 relapses (BM, iE[m]oslin} 2018 and CyA and Ph1+ ALL in 3. Haematological reconstitution was rapid NTS ( > 0.5 x 10^9/L) in 16.5 days and PTS (> 5 x 10^9/L) in 21 days. Five patients became full donor chimeras (1 result awaited). Three patients developed acute GvHD grade I-III and 3 chronic (2 limited, 1 extensive). Two patients suffered a BM relapse and 2 TBI myeloablation and 1 PBPC. The others survive in remission 1, 2, 11, 16 and 33 months post second transplant. Remission duration after second transplant exceeded that after first BMT in 3 patients. FLAGIda plus donor PB or marrow PCs is effective treatment for relapse following BMT. The treatment is safe but associated with a high incidence of GvHD. This approach may be more effective in DU in relapsed acute leukaemia (cfr. CML).

SS-0592 Induction of chimaerism using minimal conditioning and related or unrelated stem cell donors in patients with leukaemia up to the age of 70 years

Div. Hematology/Oncology, Dept. of Medicine II, Dept. of Radiology and Blood Bank, University of Leipzig, Germany; Fred Hutchinson Cancer Research Center, University of Washington, USA

Allogeneic stem cell transplantation is associated with unacceptable morbi- dity and mortality in older patients. Therefore, we conducted a graft-versus-leukaemia effect, we investigated the possibility of inducing a chimaerism with minimal conditioning regimen and peripheral blood stem cells (PBSCT) as published previously by Storb et al. Blood 99: 3204, 1997. Five patients, one with AML in PR, three with CML (1 chronic phase, 2 acc. phase), one with AML in 3rd relapse received PBSCT from HLA-identical siblings (n=4) or from an BLA-matched unrelated donor (n=1). Conditioning was performed with 20/60mg/m2 CyA. More than 90% donor genomes were given as postgrafting immunosuppression. Chimerism, as shown by amplification of short tandem repeat loci and, where appropriate, FISH for sex chromosomal differences was induced in all patients. Complete chimaerism (CC) has developed in the patient with ALL in the patient with CML in chronic phase and in the patient with AML, who had steroid responsive acute GvHD grade II of the skin and liver and is now 160 days posys-plant in CR. All these 3 patients are in CR from their leukaemia. Mixed chimaerism (MC) is present in the other 2 patients, who were treated with donor lymphocyte infusions. We conclude that induction of CC or MC is feasible in high risk patients using related or unrelated PBSCT donors with min- imal conditioning regimen and without severe toxicity.

SS-0593 Monitoring minor histocompatibility antigens (mHags)-specific CTLs after BMT using tetramers of HLA-class I-mHag peptide complexes

*Dept of Immunohaematology and Blood Bank; †Dept of Haematology, Leiden University Medical Center, Leiden, the Netherlands; †John Rad-cliffe Hospital, Oxford and *Dept of Haematology, University Hospital, Birmingham, UK

Graft versus host disease (GvHD) remains a major complication of allogeneic bone marrow transplantation (BMT). In recipients of HLA-identical BMT, GvHD may be caused by cytotoxic T cells (CTLS) directed at minor histocompatibility (mHags) antigens in the host. We previously identified mHags that are recognised by CTLS in association with HLA-A2 and -B7.

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We have now generated tetramers of HLA-A2 and -B7 molecules complexed by mHAgs HA-1 and H-Y to visualise antigen-specific cytotoxic T cells in patients who received BMT mismatched for HA-1 and H-Y. In a retrospectively designed study, we studied thirteen recipients of H-Y and four recipients of HA-1 mismatched BMT. Patients’ PBMC, isolated before and at different times after BMT, as well as donors’ PBMC were surface stained with CD8 antibodies and tetramers of HLA-A1/L1-H-Y complexes. Tetramer positive T cells were selected and analysed for antigen specificity, HA-1 and H-Y specific T cells were detected in PBMC of all recipients after unmodified BMT and at low level in some marrow donors PBMC. HA-1 and H-Y tetramer positive cytotoxic T cells were detected as early as 14 days after BMT. The frequency of tetramer staining positive CD8 cells increased significantly during GVHD, and declined following successful GVHD treatment. Tetramer positive CD8 cells showed the minor histocompatibility antigen specificity in vitro. In conclusion monitoring mHAg specific CTLs with tetrameric HLA-HA-1/H-Y complexes may facilitate early GVHD diagnosis and provide information on the GVHD treatment efficacy.

SS-0594 Mismatch at the TNFα microsatellite locus correlates with CTLp frequency in unrelated donor-recipient pairs

Wang XN, Middleton PG, Cavanagh G,* Dickinson AM
Department of Haematology, University, School of Clinical laboratory Sciences and *National Blood Service, Newcastle, UK

Objective/Methods. The use of unrelated donors for bone marrow transplantation is associated with increased morbidity and mortality compared with matched related donors. We studied fifteen donor-recipient pairs matched for HLA-A, B, C, DR and DQ on which CTLp frequency and D6STNF a microsatellite locus D6STNF a alleles were determined, and confirmed the association of CTLp reactivity in 26 cases examined. Results/Conclusions. In the present report we studied 43 donor-recipient pairs matched for HLA-A, B, C, DR and DQ on which CTLp frequency and D6STNF a genotype were determined, and confirmed the association between the two assays for patient-recipient matching. The mean value of alloreactive CTLp frequency was significantly lower in the D6STNF a matched group (1.83,341; p=0.025). The results suggest a further relevance of the D6STNF a locus in MUD selection for BMT.

SS-0595 [t(14;18)] positive follicular lymphoma: front-line treatment with CHOP and anti-CD20 (Rituximab) monoclonal antibody

Bergamo, Aviano, Bologna, Firenze, Milano, Pavia, Roma, Siena, Udine, Torino, Italy

This multicenter study was designed to assess the efficacy of the sequential administration of CHOP (6 cycles) and the humanised anti-CD20 monoclonal antibody Rituximab in untreated follicular non-Hodgkin’s lymphoma patients (stage II or more) with a molecularly proven t(14;18). The main endpoint of this study was to evaluate the PCR negativity and that an effective in vivo purging of residual disease after CHOP may be obtained by 4 weekly infusions of Rituximab. The sequential PCR monitoring of t(14;18) on bone marrow (BM) and peripheral blood (PB) lymphocytes. Patients who at the end of the last cycle of CHOP proved PCR positive on two determinations (baseline), were eligible for antiCD20 administration (375 mg/m² weekly for 4 weeks). Patients were recruited in 10 centers, while the molecular analyses were centralised in 2 laboratories (Bergamo, Torino). So far, 77 patients carrying a t(14;18) have been assigned to receive CHOP chemotherapy after 6 cycles of CHOP. Of the 45 (33%) evaluable patients proved PCR negative on two determinations both on BM and PB lymphocytes. At this time, 24 patients have completed Rituximab treatment and 20 have been evaluated for minimal residual disease on BM and PB samples collected as planned after 12, 28 and 44 weeks from baseline as summarised below:

<table>
<thead>
<tr>
<th>PCR status</th>
<th>12 weeks</th>
<th>28 weeks</th>
<th>44 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM+/PB-</td>
<td>10</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>BM+/PB+</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
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</table>

Nine of the 15 patients molecularly negative after CHOP chemotherapy who did not receive Rituximab were also molecularly re-evaluated after 12 weeks from the base line and 3 of them became PCR positive in the BM. Taken together, this preliminary analysis indicates that in a proportion of t(14;18) positive follicular lymphomas, CHOP alone may lead to a PCR negativity and that an effective in vivo purging of residual disease after CHOP may be obtained by 4 weekly infusions of Rituximab. Whether obtainment of PCR negativity bears clinical-prognostic implications remains to be determined by a sufficiently prolonged follow-up.

SS-0596 Rituximab for salvage treatment of lymphoma relapse post transplant: an EBMT survey

Second Department of Medicine, University of Kiel, Germany

To evaluate the possible role of the chimaeric anti-CD20 antibody rituximab in the management of lymphoma recurring post transplant a survey was performed within the European Blood and Marrow Transplant Group (EBMT) asking for experience with rituximab in this situation. Eighteen centres from 6 countries reported salvage therapy for post transplant lymphoma relapse in 38 recipients of autographs (n=37) or allografts (n=1). Diagnoses were follicular lymphoma in 19 cases and other B cell lymphomas in 9 cases, whereas in 10 cases the lymphoma subtype was not indicated. Fifty percent of the patients presented with an unfavourable (high intermediate or high) International Prognostic Index (elevated LDH in 72%). High-dose regimens consisted in TBI/cyclophosphamide combinations (n=13), BEAM (n=15) or other chemotherapy regimens. Rituximab treatment was commenced 26 (2-113) months post transplant. Rituximab was administered as salvage chemotherapy regimen for nodal and/or BM relapse. In 1.0 cases, rituximab was combined with chemotherapy. Grade 3-4 toxicity was reported in 14% of the cases (cytokine release syndrome or cytopenia). Response to rituximab occurred in 26 (72%) patients (CR 31%; PR 42%). A normal LDH was highly predictive for response (p=0.013). Thirty-eight percent of responders have relapsed with a median follow-up of 4 (0-6) months. Responders had a significantly longer estimated overall survival than non-responders (10 vs. 7 months; p=0.018). We conclude that rituximab is an active and well tolerated salvage regimen for lymphoma relapse post transplant.
SS-0597 A multicenter, randomised trial of fludarabine (FLU) versus fludarabine and idarubicin (FLU-ID) as first-line treatment in patients with low-grade non-Hodgkin's lymphoma (LG-NHL)

Zinzani PL on behalf of an Italian Cooperative Study Group on Lymphomas. Institute of Hematology and Medical Oncology "Seraponi", University of Bologna, Italy

Background. FLU has been shown to be an effective agent in the treatment of indolent lymphoma, either used alone or in combination with other drugs. Design and Methods. From 9/95, 185 patients aged 25-76 (median 55) yrs with newly diagnosed stage I-II lymphoma were enrolled in this multicenter, 1:1-randomised study. Of the 173 patients currently evaluable, 88 were allocated to the FLU arm to receive 6 monthly cycles of FLU 25 mg/m²/day on day 1 to 5, while 88 were allocated to the FLU-ID arm to receive 6 monthly cycles of FLU 25 mg/m²/day on day 1 to 3 and ID 12 mg/m² on day 1. The two groups were comparable in terms of histological type, stage, extranodal site involvement, age and sex distribution. Clinical response to treatment was evaluated immediately after completion of the chemotherapy schema and defined according to 3 categories: CR, PR and failure. Results. Accordingly, 37 of 88 (42%) patients in the FLU and 38 of 88 (43%) in the FLU-ID arm achieved a CR, while in both arms 36 patients (41% and 42%, respectively) achieved a PR. Moreover, an in-depth analysis of the clinical response with respect to histological type showed that FLU treatment appeared to be more effective than FLU-ID against follicular lymphoma (54% vs. 38% CR). No differences were observed for the remaining histological types. Furthermore, no striking differences were observed for any histotype between the 2 protocols in terms of overall response (ORR), and toxicity, which was generally mild. However, with a median follow-up of 12 (range: 5-31) mos, only 21 (57%) patients who received FLU alone have maintained their first CR, as compared to 25 (89%) of those who received FLU-ID therapy. Conclusions. We conclude that although the FLU-ID regimen did not significantly improve the induction of CR in most LG-NHL patients, our preliminary data do suggest that with respect to FLU alone it may be capable of conferring a longer-lasting, and thus better-quality, CR.

SS-0598 Autologous stem cell transplantation (ASCT) improves survival after first progression for patients with a follicular lymphoma (GELF68 protocols)


A study from the GELA, Hôpital Saint Louis, Paris, France

Purpose. All patients with follicular lymphomas (FL) included in the GELF68 trials (1986-1995) who progressed/relapsed after initial treatment were retrospectively analyzed to determine prognostic factors after relapse and the influence of high dose therapy (HDT) on ASCT survival. Design. Of the 556 patients with confirmed FL registered in the GELF68 trials, 376 progressed/relapsed. After first relapse, patients were treated with radiotherapy (9%), single agent chemotherapy (28%) or polychemotherapy (63%) followed by HDT (32%) with allogeneic SCT (n=8) or ASCT (n=68). Before HDT, 76% had chemosensitive relapse, 64% received a conditioning regimen with total body irradiation. Overall survival (OS) was calculated from the date of diagnosis. Results. The median time to first relapse was 24 mo (33% on therapy). Histological transformation was confirmed in 24% of 217 who had a second biopsy. Sixty-four percent of patients responded to salvage treatment. The OS at 5 years was 48%. The 5 yo OS was 67% for patients who received HDT at first relapse vs 44% for patients treated with standard treatment (p=0.0001). Adverse prognostic factors at relapse (Cox's analysis) were NO HDT at first relapse, age > 50 y, initial high tumour burden, early relapse before 3 years after diagnosis and histological transformation. In the multivariate analysis the relative risk of death with HDT is 0.6. Conclusions. Results of this study suggest that HDT with ASCT is the optimal treatment for patients with FL and high tumour burden at diagnosis who progress or relapse after first line treatment.

SS-0599 HIV-related lymphoma: a randomised study of dose intensive risk adjustment treatment


In May 1993 the European Intergroup Study NHL-HIV started a randomised study with a stratification of lymphoma patients into three groups according to the prognostic factors, prior AIDS, CD4+ ≤ 100/mm³, performance status > 1. In the low risk group (i.e. absence of the three prognostic factors) patients were randomised between the intensive regimen ACVB (Am J Med 1993; 95:188-96) and CHOP with GC/FSF support. One hundred and fifty-nine patients have been included, 80 in the ACVB and 79 in the CHOP arm. Forty-one percent had diffuse large cell (DLCL) lymphoma, while 60% had an International Index prognostic (IPI) score ≥ 2. Median CD4 count was 200 (μl). Complete response (CR) was achieved in 66% and 60% for respectively ACVB and CHOP. For the ACVB arm survival and EFS were 51% and 46% respectively and did not differ from those in the CHOP arm (50% and 42%). Toxic deaths were observed in 5% and 3% of patients in the ACVB and CHOP arm respectively. Grade 4 hematotoxicity was observed in 3% and 4%, respectively. During follow-up 13% of the 75 patients died in CR. In the intermediate risk group (with the presence of only one prognostic factor) patients were randomised between CHOP and CHOP reduced to 50%. Of the 110 patients, 59 were in the CHOP arm and 51 in the 50% reduced CHOP; 43% had DLCL and 63% had an IPI score ≥ 2. Median CD4 count was 60 (μl). The 63% CR rate in the CHOP arm was superior to the 39% in the reduced CHOP (p=0.01). We did not translated into a significantly better OS (25% vs 30%) and EFS (25% vs 26%) between the 2 arms despite a better CR rate in the conventional arm. However, 24% of 75 pts died while in CR. Outcome of NHL-HIV patients remains poor and is mainly related to severity of immunodeficiency.

Supported by ISS, Sidaction and Roche laboratory.

SS-0600 Primary central nervous system lymphomas: long-term results of the GOELAMS LCP 88 trial with a focus on neurological complications among 152 patients


Maladies du Sang, Hôpital Sud, CHU Amiens, France

GOELAMS LCP 88 trial combined 3 courses of MVBP (Ma 3 g per m² dL & d15, Teniposide 100 mg d2 & d3, BCNU 100 mg d4, and methyl-prednisolone 60 mg d1 to d5), 6 LP and a 40 Gy in toto cerebral irradiation. We treated 152 HIV-negative patients (pts); sex-ratio was 0.97 and median age 61 years (from 16 to 75). An obvious CR was noted in 31 pts (20%) after the first MVBP, in 69 pts (45%) after the 3 courses and in 99 pts (65%) after irradiation. In January 1999 we noted 29 relapses (from 6 to 94 months - median 16), 6 non-related deaths and 85·specific·deaths (13 early deaths, 39 failures, 22 relapses, 10 leucencephalopathies and 1 AML). The 3-, 5- and 7-year·specific·survival·rates were respectively 49, 36 and 29% for all the pts and 75, 62 and 45% for the 99 pts in CR. Analysis found no prognostic value for many factors (sex, clinical data, location and number of lesions, mode of diagnosis, CSF, lymphopenia...) while 3 independent factors have a strong pejorative value: age ≥ 60 years, PS ≥ 2 and LDH ≥ N:

<table>
<thead>
<tr>
<th>Factor</th>
<th>Yes/No</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>85/67</td>
<td>18/73 mo</td>
</tr>
<tr>
<td>PS</td>
<td>85/62</td>
<td>15/58 mo</td>
</tr>
<tr>
<td>LDH</td>
<td>50/95</td>
<td>13/56 mo</td>
</tr>
</tbody>
</table>

We also studied the risk of occurrence of a severe neurological complications, e.g., leucencephalopathy, recurring vascular strokes, severe extra-pyramidal syndrome. As shown above the 5-year risk is 36±7% for all the 152 pts. Age is an important factor while all other ones (PS, LDR initial site, extension, CSF...) are not significant: risk is 48±11% among pts aged 60 years or more vs 25±6% (p=0.03) and this risk is very low among the 13 pts less than 40 years (9% [p>0.8]). Neuropsychologic data will be reportied by M. Gardembas in another abstract. However, when adding risks due to lymphoma and to neurological complications, the prognostic of a primary central nervous system lymphoma in pts aged less than 60 years is not bad: the 7.2-year event free survival rate is 41±8%.

Haematologica vol. 84 (EHA4 Abstract Book); June 1999
Acute myeloblastic leukaemia II

PO-0603 Risk-adapted induction and consolidation therapy including autologous peripheral blood stem cell transplantation (PBSCT) in de novo AML patients aged <60 years

Dept. Haematology/Oncology Univ. of Hannover; Ulm, Frankfurt, Hamburg, Freiburg, Gart, Augsburg, Berlin, Auenbl, Germany

Treatment of 218 de novo AML patients (median age 43 years, range 16 - 60 years) was stratified according to their risk profile as defined by karyotype and chemosensitivity of the blasts. Patients with a t(8;21) or inv(16) or a normal karyotype and a good response to the first induction course (absence of blasts in the peripheral blood and ≤5% blasts in the day 15 bone marrow) were allocated to a standard risk (SR) group. All others were regarded as high risk (HR) cases. Patients with a t(15;17) were excluded.

Good responders to induction I (idarubicin, Ara-C, etoposide) received a second induction course with the same drug combination (IVA II) starting on day 21 ("double induction"). For patients with >5% bone marrow blasts induction II consisted of Ara-C (1g/m², 8 doses)/m-AMS A (30 mg/m², 3 doses). Early consolidation included Ara-C (1g/m², 8 doses) daunorubicin or M-AMSA. For SR patients late consolidation consisted of 1 cycle of high-dose Ara-C (3 g/m², 12 doses) and daunorubicin (45 mg/m², 3 doses); HD-Ara-C (OND), while patients with a normal karyotype and an HLA matched sibling were to be allotransplanted. HR patients were to be allotransplanted or undergo autologous PBSCT. PBSCT were mobilised after early consolidation by fligrastim (10 µg/kg, s.c.). The overall CR rate was 75% (90% in the SR and 59% in the HR group) and 8% died during induction. Median duration of neutropenia (25,000/µl) was 18 days (16 days) during induction I, 15 days (9 days) during induction II, 10 days (9 days) during early and 24 days (24 days) during late consolidation. In contrast, median duration of neutropenia (thrombocytopenia) after autologous PBSCT was 9 days (14 days).

A median follow-up of 17 months 20/103 SR patients relapsed, 8 patients died during HD-Ara-C (OND) therapy and another 4/16 patients after allotransplantation. In the HR group 23/61 patients relapsed, including 6/14 autotransplanted patients and another 7/16 patients died during alloSCT. The probability of relapse-free survival (CCR) at 40 months was 5% for the SR group while the outcome of patients with a normal karyotype did not differ from those with a t(8;21) or inv(16). In contrast the probability for CCR for the HR patients was 34% (median=325 days) only.

We retrospectively evaluated the impact of karyotype on the outcome of AML patients who received first line induction therapy, (standard or fludarabine combinations FLAG, FLANG, FLAG-Ida) and allogeneic or autologous bone marrow transplantation, when possible. t(8;21), inv(16) and del(5q)/del(20q) were considered favourable (f) karyotypes; -5q/-7q, -7q/17q12, t(11q23) were defined unfavorable (uf); 46,xx/xy and all other abnormalities were of intermediate (int) prognostic value.

<table>
<thead>
<tr>
<th>Standard therapy</th>
<th>Fludarabine combinations</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>46,xx/xy</td>
</tr>
<tr>
<td>Karyotype</td>
<td></td>
</tr>
<tr>
<td>N. pts</td>
<td>8</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>49 (33-63)</td>
</tr>
<tr>
<td>N. CR</td>
<td>6/8</td>
</tr>
<tr>
<td>%CR</td>
<td>75</td>
</tr>
<tr>
<td>%Surv</td>
<td>44</td>
</tr>
<tr>
<td>NDFS</td>
<td>50</td>
</tr>
</tbody>
</table>

(3 year projection)

In 38 secondary AML patients receiving fludarabine combinations karyotype had a major influence on CR rate: 11/19 (56%) and 4/19 (21%) CRs in patients with intermediate or unfavorable karyotype respectively. Survival and DFS were poor in both groups. Our data confirm the strong prognos-
tic value of karyotype in de novo and secondary AML patients. Fludarabine combinations achieved promising results in patients with intermediate prognosis karyotype. Unfavorable karyotype stands as an unresolved problem and requires innovative therapies.

**PO-0605 Outpatient management of acute myeloid leukaemia after consolidation chemotherapy**


Dipartimento di Biotecnologie Cellulari ed Ematologia, Università "La Sapienza", Rome, Italy

The feasibility and safety of outpatient management of AML during the aplastic phase after intensive consolidation chemotherapy, the incidence and types of complications requiring readmission to hospital, and the number of hospital days spared by this policy have been prospectively evaluated. After chemotherapy administration, patients were evaluated on an outpatients basis. In the event of any complication they were referred to the Emergency Unit (EU) of our Department dedicated to outpatients with haematologic diseases. Ninety-eight patients with AML observed over a four year period were eligible for intensive chemotherapy. After the achievement of complete remission they received a total of 137 consolidation courses and in 125 instances they were followed on an outpatients basis during the aplastic phase. There were 70 cases (56%) of hospitalisation, 69 for fever and only one for severe anemia. Only one patient died due to a brain haemorrhage. Coagulase-negative staphylococci and Streptococcus viridans were the organisms most frequently isolated from blood. Empirical once-a-day antibacterial therapy with ceftriaxone and amikacin was effective in 87% of the cases and it made it easy discharge possible in 30% of the cases who continued the antibiotic therapy in an outpatient setting. Patients were managed out of the hospital for 70% of the post-consolidation neutropenia period. Thanks to the availability of an EU specifically dedicated to outpatients with haematologic diseases, community management of AML patients after consolidation therapy, appears to be safe, well accepted, potentially cost-saving, and could contribute to avoiding the risk of developing severe nosocomial infections.

**PO-0606 Detection of higher levels of glutathione S-transferase deletions in AML patients born after 1960**

Tothal K, Lambert DS, Kadkhodaei-Biaderani M, Sheikhiha MH, Macheta M, Liu Yin JA

University Department of Haematology, Manchester Royal Infirmary, Manchester, UK

The reduced ability of individuals to detoxify environmental and occupational mutagens and carcinogens has been associated with increased risk of developing cancers. The glutathione S-transferase (GST) family of enzymes plays an important role in metabolising and detoxifying mutagens and carcinogens. It has been reported that individuals with deletion (null genotype) of the GST theta 1 (GSTT1) have an increased risk of developing cancers and is increased in acute myeloid leukaemia (AML). The GST theta 1 (GSTT1) have an increased risk of developing severe myelodysplastic syndromes (MDS). We investigated the level of GSTT1 and the GSTM1 null genotype in 253 normal subjects and 250 AML patients and normal individuals born in any decade before 1960. Howev-

**PO-0607 Expression of inducible nitric oxide synthase (iNOS) is increased in acute myeloid leukaemia**

Brandao MM, Soares E, Salles TSI, Saad ST

Department of Pharmacology, Haematology - Hemotherapy Center State University of Campinas - UNICAW, Campinas - SP, Brazil

Nitric oxide (NO) is capable of activating RAS by binding itself to p21, increasing the concentration of the GTP-bound form. NO is one of the most important intracellular signalling molecules present in all mammalian cells. Recently, NO was discovered as a cell division trigger, beyond the interaction with p53 and other oncogenes. In this study we analyse iNOS expression in 18 patients (15 with AML, 2 with CLL and 1 with Lymphoma) and 7 normal controls. We also studied the expression of iNOS in lipopolysaccharide (LPS)-stimulated human and noninduced leukaemic cell lines (HL60, U937, and K562). Positive staining for iNOS was obtained in all patients. iNOS was expressed in cytosol, close to the inner cellular membrane. By flow cytometry and immunocytochemistry we verified that patients with AML express high iNOS compared to patients with CLL and Controls. We also found high iNOS expression in U937 and HL60 cell lines compared to K562. There was no correlation between the iNOS expression and the expression of p53 and K, H, and N-RAS mutation and expression, suggesting that the high expression of iNOS could be an effect of lower scavenger action caused by lower levels of haemoglobin in AML or by the action of transcription factors expressed in AML.

**PO-0608 Different leukaemia-free survivals in acute myeloid leukaemia (AML) with t(8;21) and inv(16).**


CETILAM Group, Spain.

Objective. We evaluated the results of a protocol including high-dose Ara-C (HDAC) or autologous stem cell transplantation (auto-SCT) in adult AML patients (pts) with favorable cytogenetics such as t(8;21) or inv(16). These pts were treated as a part of a prospective multicenter trial between May '94 and November '98. Design and Methods. Twenty-three pts ≤60 years (yrs), mean age ±SD: 38±10 yrs, m/f: 13/10, with t(8;21) (n=10) or inv(16) (n=19) were included. Induction chemotherapy consisted ofidarubicin, Ara-C standard dose and VP16. First intensification included mitoxantrone (MTX) and intermediate-dose Ara-C (IDAC). Following this treatment the intention was to administer 2 courses of HDAC 3gr/m²/12h, days 1-3, or to perform an auto-SCT after cyclophosphamide and total body irradiation. Pts who relapsed were treated with allogeneic SCT if an HLA-identical sibling was available or with auto-SCT provided that the initial treatment was HDAC. Results. CR in 19 (83%) pts (8(8;21), 11 inv(16)), death during induction in 3 (13%), and refractoriness in 1(4%). The 19 CR pts, one with inv(16) died during the MTX and IDAC course and another relapsed early after this treatment. Three pts did not receive further therapy: two with inv(16) relapsed and the patient with t(8;21) is in continuous CR. Twelve of the remaining 14 pts, 6 (t(8;21) and 6 inv(16), received 2 courses of HDAC; all pts with t(8;21) remain alive and leukaemia-free in contrast to 2 pts with inv(16) who relapsed and another who died after the 2nd HDAC course. Of the two remaining pts, 1 (t(8;21) and 1 inv(16), were autografted in first CR and both relapsed after the procedure. Five of the 6 pts with inv(16) who relapsed were subsequently transplanted: two received an auto-SCT and are in CR and 3 were allografted, with one-transplant related death and 2 pts remaining in remission. In summary, CR was achieved in the same proportion of pts with t(8;21) and inv(16). The subsequent outcome was different with only one relapse in the t(8;21) group in contrast to 2 deaths in CR and 6 relapses in the inv(16) group. Conclusions. These results support the opinion that pts with AML t(8;21) should not be transplanted in first CR because of the outstanding results of HDAC. The best approach in inv(16) remains controversial; LFS in this series was poor but most pts could be rescued with SCT after relapse.

**Acute myeloblastic leukaemia II**

Haematologica vol. 84 (EHA-4 Abstract Book); June 1999
PO-0609 Influence of HLA-matched sibling donor availability on treatment outcome for patients (pts) with newly diagnosed acute myeloid leukaemia (AML)

Thomas Le QH, Belhabri A, Chelgoum Y, Charrin C, Michallet M, Fabre D
Service d’Hématologie, Hôpital Edouard Herriot, Lyon, France

The optimal post-remission therapy for patients with AML in first complete remission (CR) remains controversial. In order to evaluate the place of HLA-identical allogeneic bone marrow transplantation (alloBMT) in first CR, we compared it to other post-induction therapies in adults with previously untreated AML. Between 1985 and 1998, 152 consecutive AML pts aged between 10 and 68 years, seen in our institution, were treated according to 3 different successive protocols (LYLAM85, LAM90, AML10). Overall, 114 (152 pts) (75%) achieved a CR. One hundred and forty-one of the 152 pts entered a prospective study in which they were registered at the time of diagnosis for presence or absence of HLA-identical donor and analysed an intention-to-treat basis, every pt in CR with an HLA-identical sibling being scheduled to receive alloBMT. Fifty one pts (36%) had a family donor (group 1) and were offered alloBMT in case of CR achievement. The 90 pts without a donor were allocated to group 2 and were assigned to receive chemotherapy or autologous transplantation as post-remission treatment according to the protocol they were initially included in. Pts from both groups had similar disease characteristics at diagnosis. From group 1, 34 pts (67%) were actually transplanted in first CR. The presence of a family donor was of good prognosis for CR achievement and CR duration (respectively p<0.001 and p=0.01). Karnofsky at diagnosis was defined as low risk (88-90) or intermediate (11.14) or high risk (other abnormalities). In univariate and multivariate analysis, karyotypic status was the main prognostic factor for CR achievement (p<0.001), CR duration (p<0.01), and overall survival (p=0.001). The overall survival from diagnosis for pts who received alloBMT was also different to that of pts without a donor (median overall survival respectively at 16 months and 26 months with estimated survival at 5 years respectively at 33% and 35%). There was also no statistical difference between the 2 groups in terms of relapse rates. Pts receiving alloBMT effectively did not have a better survival than patients who did not. Overall survival for all assigned pts correlated with the prognostic grouping of the karyotypes. However, there was no relation between karyotype groupings and the long term results of the 2 post-remission therapies. We conclude that the availability of an HLA-identical sibling donor did not provide a better prognosis than other post-remission therapies in young adults with AML in first CR.

PO-0610 Analysis of treatment outcome of patients with hypoplastic acute myeloid leukaemia

Colovic MD, Jankovic GM, Bogdanovic AD, Donfrid MD, Bogunovic M, Suvajdic ND, Cermencic VM, Jovanovic V, Tomasevic R, Bogdanovic G, Nedeljkovic N
Institute of Haematology, Clinical Centre of Serbia, and Blood Bank, Belgrade, Yugoslavia

Objective. Hypoplastic acute myeloid leukaemia (AML) is a rare type of leukaemia, in which FAB and MIC classifications are difficult to apply. In our group, we achieved therapeutic response (complete or partial remission) in 7 patients, lasting 4.5 months. Two patients with previous MDS reverted to MDS but after a short remission died in florid leukaemic relapse. Four patients died during therapy and there was no difference in death rate between the two treatment regimens (2 vs 2).

In the past 12 years we treated 18 patients with hypoplastic AML (HypAML); 16 pts had de novo HypAML, while 2 had a history of previous MDS. None had elements to suggest secondary, therapy-related AML. All patients had hypocellular bone marrow for the age with overt leukaemia on bone marrow trephines. In this group there were 3 pts with AML M, 2 pts M2, 2 pts M3, and 1 Pt M4. Others were not classifiable according to the FAB classification. However, there was no statistical difference between the 2 groups in terms of relapse rates. Pts receiving alloBMT effectively did not have a better survival than patients who did not. Overall survival for all assigned pts correlated with the prognostic grouping of the karyotypes. However, there was no relation between karyotype groupings and the long term results of the 2 post-remission therapies. We conclude that the availability of an HLA-identical sibling donor did not provide a better prognosis than other post-remission therapies in young adults with AML in first CR.

PO-0611 Do elderly patients with acute myeloid leukaemia benefit from standard intensive induction chemotherapy?

Bogdanovic AD, Jankovic GM, Donfrid MD, Bogunovic M, Suvajdic ND, Rajic Z, Vidovic A, Cermencic VM, Tomasevic R, Bogdanovic G, Nedeljkovic N
Institute of Haematology, Clinical Centre of Serbia, and Blood Bank, Belgrade, Yugoslavia

Objective. We tried to evaluate benefits of standard, intensive induction treatment (SIT) (3+7 or ADE/MAE schedule) in patients with AML over 60 yrs of age. We analysed on an intention-to-treat basis, every pt in CR with an HLA-identical sibling being scheduled to receive alloBMT. Fifty one pts (36%) had a family donor (group 1) and were offered alloBMT in case of CR achievement. The 90 pts without a donor were allocated to group 2 and were assigned to receive chemotherapy or autologous transplantation as post-remission treatment according to the protocol they were initially included in. From both groups had similar disease characteristics at diagnosis. From group 1, 34 pts (67%) were actually transplanted in first CR. The presence of a family donor was of good prognosis for CR achievement and CR duration (respectively p<0.001 and p=0.01). Karnofsky at diagnosis was defined as low risk (88-90) or intermediate (11.14) or high risk (other abnormalities). In univariate and multivariate analysis, karyotypic status was the main prognostic factor for CR achievement (p<0.001), CR duration (p<0.01), and overall survival (p=0.001). The overall survival from diagnosis for pts who received alloBMT was also different to that of pts without a donor (median overall survival respectively at 16 months and 26 months with estimated survival at 5 years respectively at 33% and 35%). There was also no statistical difference between the 2 groups in terms of relapse rates. Pts receiving alloBMT effectively did not have a better survival than patients who did not. Overall survival for all assigned pts correlated with the prognostic grouping of the karyotypes. However, there was no relation between karyotype groupings and the long term results of the 2 post-remission therapies. We conclude that the availability of an HLA-identical sibling donor did not provide a better prognosis than other post-remission therapies in young adults with AML in first CR.

PO-0612 RNA and DNA analysis of HPRR/TIF1α gene in AML


The HPRR/TIF1α gene belongs to a family of nuclear proteins characterised by the presence of the RBCC motif and with structural similarity to PML gene of Acute Promyelocytic Leukaemia (APL). HPRR/TIF1α appears to be a ligand and/or co-activator of the AR-2 activity of a variety of nuclear receptors, including RARα, RXRα, vitamin D3 receptor (VDR), and estrogen receptor (ER) (1.2). RARα is a crucial element of the RA-dependent myeloid differentiation pathway, and its disruption is obtained by the formation of the fusion protein PML-RARα in APL. The ability of HPRR/TIF1α to mediate the activity of RARα, and the structural similarity with PML, prompted us to evaluate the possible role of this gene in the pathogenesis of Acute Myeloid Leukemias. A set of probes was assembled in 2138 pts and sequenced by the Sanger DNA coding sequence of the gene (3) were generated, and the fragments obtained utilised as hybridisation probes in Northern and Southern blot experiments. RNAs from 23 cases belonging to different subtypes of AML, according to the FAB Classification, were analysed by Northern blot analysis and the level of the expression of the gene compared with the expression of two different housekeeping genes, such as β3GPDH and β-actin. Southern Blot analysis of HPRR/TIF1α DNA was performed in the same group of patients in order to...
to study the HPRR/TIF\(_1\) genomics organisation. In 22 of the 23 cases HPRR/TIF\(_1\) RNA was found expressed but at variable levels, with highest values in the M1 and M2 subtypes. In the HPRR/TIF\(_1\) DNA of the 23 cases so far studied, no abnormal fragments were found. Therefore, these preliminary results suggest that HPRR/TIF\(_1\) appears not to be rearranged in the Acute Myeloid Leukemias, but its variable level of expression suggests a possible role in the gene in the physiopathology of the myeloid differentiation pathway. 1. Gandini D et al., Blood 1994; 84, 439a.

Le Douarin B et al., EMBO J 1995; 14, 2020-2033.

PO-0613 A novel translocation t(1;7)(p36;q34) in three patients with acute myeloid leukemia
Specchio G, 1 Curotto A, 2 Lisio V, 1 Contino R, 1 Gentile E, 1 Pastore D, 1 Rocchi M, 2 Castoldi GL
1Department of Haematology, University of Bari, Italy, 2Institute of Haematology, University of Ferrara, Italy, 3Genetic Institute, University of Bari, Italy

Objective. Studies of large numbers of patients have enabled the identifi-
cation of a number of common chromosomal changes, such as inv(3)(q21q26), t(6;9)(p23;q34), t(8;16)(p11;p11), whose clinical biological significance is gradually becoming clearer. Translocations involving chromo-
somes 1 and 7 are relatively rare in myeloid neoplasias, being found in far fewer cases, mostly in the form of an unbalanced translo-
cation (t(1;7)(p11;p11)), resulting in complete loss of 7q, associated with therapy-related or environmentally-induced high-risk myelodysplasia. Descri-
ing the translocation we recently observed in three patients with AML with a pre-
viously unreported translocated balanced translocation t(1;7)(p36;q34). To character-
ize the translocation better we performed FISH experiments using two PCPs specific for the long arm of chromosome 7 (PCP#57) and for 7q31.32-
7qter (PCP#52). Results. G-banding analysis of the karyotype in our cas-
es indicated the presence of a balanced translocation involving the telom-
entric regions of chromosomes 1 and 7. In all three patients a t(1;7)(p36;q34) was found. Case 1 (FAB AML-M0) showed a normal karyotype with 46 chromosomes, Case 2 (FAB AML-M4) exhibited t(1;7)(p36;q34) and t(6;9)(p23;q34) in all metaphases. Case 3 (FAB AML-M2) did not reveal additional chromosome abnormalities. The YAC 750G5 was found to be centromeric with respect to the breakpoint on 1p. The results suggest that the breakpoints were localised at 1p36 and 7q34. Conclusions. The response to chemotherapy observed in our cases suggests that variable clinical features might be present in the broad cytogenetic category usu-
ally referred to as “7q abnormalities” and contributes to an interesting pre-
vious observation of prolonged disease-free survival in a subset of AMLs with 7q- as the isolated chromosome change.

PO-0614 Marrow leukemic index (MLI), age and CD34 in the evaluation of clinical outcome in elderly AML patients treated with standard chemotherapy
Haematology, University of Bari, Italy

Objective. Acute myeloid leukemia (AML) is mainly a disease of the elder-
ly, who account for more than 50% of the cases among the general pop-
ulation. We analysed a cohort of 120 cases of de novo AML, in order to identify clinical and laboratory parameters with prognostic significance. Design and Methods. The patients' study group comprised 120 patients with de novo AML. The median age was 68 years (range 60-86; 64 M 56 F). Distribution according to FAB was as follows: M0 (8 cases), M1 (3 cases), M2 (17 cases), M4 (118 cases), M5 (12 cases), M6, 20 M7 (1 case), unclassifiable (1 case). Sixty-nine (58%) patients were defined as eligi-
bale for standard chemotherapy and were treated with induction therapy includ-
ing Mitoxantrone 7 mg/m\(^2\) on days 1, 3 and 5, Etoposide 100 mg/m\(^2\) on days 1, 3, and 5, and idarubicin 12mg/m\(^2\) on days 1, 2, and 3, followed by L-Asparaginase 15,000 IU/m\(^2\) on days 1, 2, and 3. The treatment began despite the severe haemorrhagic syndrome that occurred in one patient with AML. In order to study the HPRR/TIF\(_1\) genomic organisation. In 22 of the 23 cases HPRR/TIF\(_1\) RNA was found expressed but at variable levels, with highest values in the M1 and M2 subtypes. In the HPRR/TIF\(_1\) DNA of the 23 cases so far studied, no abnormal fragments were found. Therefore, these preliminary results suggest that HPRR/TIF\(_1\) appears not to be rearranged in the Acute Myeloid Leukemias, but its variable level of expression suggests a possible role in the gene in the physiopathology of the myeloid differ-

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Objective. Studies of large numbers of patients have enabled the identifi-
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Le Douarin B et al., EMBO J 1995; 14, 2020-2033.
High levels of LRP mRNA in leukaemic cells from patients with AML are associated with poorer response to chemotherapy and prior treatment with mitoxantrone.

**PO-0617**

Gruber A, Xu D, Arestrom I, Pisa P, Peterson C.*

Department of Oncology, Karolinska Hospital Stockholm, and Clinical Pharmacology, Faculty of Health Sciences, Linköping University, Sweden

Expression of the multidrug resistance-associated protein, and Lrp (Lung resistance associated protein) genes is associated with transport related MDR. We quantified their mRNA levels of those genes by competitive reverse transcriptase polymerase chain reaction in 128 samples of leukaemic cells from 92 patients with acute myelogenous leukaemia (AML). There was a wide variation between the samples in mRNA levels of all the genes. The mean mdr1 mRNA level was 1.3 transcripts per cell (range: undetectable-15.8), the mean mdr2 mRNA level was 7.9 (range: 0.1-36.2), and mean Lrp mRNA level (range: 0.1-29). There was a weak positive correlation between mdr and Lrp mRNA levels in samples taken both at diagnosis and at relapse or resistant disease. No correlation was found between mdr1, mdr2 or Lrp mRNA levels, and WBC, FAB type or CD34 expression. However, complete remission rates were significantly lower in MDR1(+), mdr2(+) and Lrp(+) patients (5/14 vs 13/41 in all patients). A high degree of multiresistance (D>0.15) was found in 4/14 patients with AML. The diagnosis of AML was made according to the French-American-British classification and that of AML/MDS according to the proposal of the World Health Organization.

**PO-0618**

Staneva M, Guenova M, Hadjiev I.

National Centre of Haematology and Transfusiology, Sofia, Bulgaria

Objective. Multidrug resistance is responsible for the decrease in sensitivty of leukaemic cells to normally occurring anti-cancer drugs thus being a major factor limiting successful treatment of AML. This resistance is correlated with the expression and activity of a membrane protein - P-Glycoprotein (P170) which is encoded by the MDR1 gene and functions as a drug extruding pump. However, its clinical significance is still controversial.

**Design and Methods.** The level of MDR1 expression was studied in 25 cases of newly diagnosed adult AML using the monoclonal antibody MRK16 that binds to an epitope of P170. Indirect immunof luorescent labeling was performed and samples were analysed by flow cytometry. K-Mogeneity test was used to estimate MRK16 staining. A D>0.15 for labeling of gated leukaemic blasts as compared to that of the isotypic control was defined positive and compared to clinical data. All patients were treated with standard induction protocols. The mean follow-up was 11.5 months. Results. P170 expression as determined by MRK16 positivity (>0.15) was found in 14/25 patients (56%). A high degree of variability in intensity of staining was observed. No correlation was found with regard to age, sex, WBC, FAB type or CD34 expression. However, complete remission rates were significantly lower in MRK16 (+) patients (5/14.36%) than in MRK16 (-) cases (8/11.73%) (p<0.05). CR duration was also shorter in MRK16 (+) cases but the difference was not statistically significant.

Conclusions. Flow cytometric analysis of MDR using MRK16 monoclonal antibody may be a helpful tool for identification of patients with poor prognosis in terms of CR induction.

**PO-0619**

Cytogenetic analysis of de novo acute leukaemia with trilineage myelodysplasia

Suvacic M, Mirasjelic D, Pantic M, Djordjevic V, Novak A, Bogdanovic A, Jankovic G, Bozikovic D and Colovic M.

Institute of Haematology, Clinical Centre of Serbia, Belgrade, Yugoslavia

Characteristics of karyotypes were analysed in 60 de novo acute myeloid leukaemia (AML) cases with trilineage myelodysplasia (AML-TMDs). There were 28 males and 32 females ranging from 17 to 78 years with 43% patients over 60 years. The diagnosis of AML was made according to the FAB classification and that of AML/MDS according to the proposal of the British-Baebulite et al. (1957). There were 11 M5, 17 M4, 16 M2, 5 M1, 3 M3. FAB subtypes and 8 unclassified cases. In 43/60 patients there was an abnormal karyotype (22 single and 16 complex). The most frequent aberrations were 5/5q- in 5 pts.; -7/7q in 4 pts.; -9/9q- in 5 pts.; -12/12p- in 6 pts. Clonal evolution or cytogenetically related clones (subclones) were found in 11/60 patients. An unbalanced karyotype was recorded in 32/43 patients. Balanced karyotype included cases displaying any balanced aberration regardless of coexisting unbalanced aberrations and unbalanced included cases with only unbalanced aberrations (Misawa et al. 1998). Loss of a sex chromosome was observed only once in a 68-year-old male despite the relatively advanced age of the patients. According to these results, it would appear that the nature and incidence of the most frequent chromosome abnormalities do not differ much between AML/TMDs and myelodysplastic syndrome, a conclusion which supports the view that AML/TMDs passes through several preleukemic stages at diagnosis, as has been well documented for myelodysplastic syndromes.

**PO-0620**

Novel T-cell receptor d gene rearrangement involving a recombining element located 2.6 kb 3' from the VD2 segment

Przybylski G, Oettle H, Siepert W, Schmidt CA.

*Z* Berlin, UXVR, Abt. Haematologie, Berlin Germany; **Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland

In this study we describe a novel T-cell receptor d (TCRd) gene rearrangement observed in acute myeloid leukaemia with coexpression of T-lymphoid antigens (Ly-AML) and in peripheral blood leucocytes (PBL) from 2 out of 10 healthy donors. The rearrangement was identified by Southern blot analysis using a joining region (Jd1) specific probe and amplified by polymerase chain reaction (PCR) with a variable region (Vd2) and Jd1 specific primers. The nucleotide sequence analysis of an abpical 3 000 bp PCR product allowed localisation of the breakpoint within the TCRd gene locus, 2.6 kb 3' from the Vd2 gene segment. A regular Dd2-Dd6-j1 joining was found at the 3' end of the breakpoint, indicating that the rearrangement was mediated by the Vd2 recombinase, but no TCRd gene segment was detected at the 5' end. Analysis of the germline sequence 3' from the breakpoint revealed an isolated recombination signal sequence (RSS) capable of initiating a rearrangement. The RSS motif described by us is the second TCRd recombinating element (Rec2). The oRec2 (Dd6)1 recombinatation is a rather rare event and can be found in acute leukaemia and in PBL from healthy individuals. Most likely the nonfunctional oRec2 (Dd6)1 rearrangement is a transient stage during Vd2 recombinase. It may potentially lead to deletion of the oRec2 (Dd6)1 complex and either to direct join of a Vd6 region to one of the downstream Jd6 regions or to a rearrange-ment of the TCRd gene.

Poster Discussions: Non-Hodgkin's lymphoma II

**PO-0621**

Cost-effectiveness of epoetin to avoid allogeneic blood transfusions for coronary by-pass surgery

Marchetti M, Barosi G

Laboratory of Medical Informatics, RCCS Policlinico S Matteo, Pavia, Italy

Objective. Perioperative allogeneic blood transfusions carry a risk of both infectious and non-infectious complications; moreover they are expensive and carry substantial intangible costs, since patients prefer not to receive them. Recent guidelines recommended that patients undergoing elective interventions may be offered a preoperative autologous blood donation (PADD) with or without epoetin (EPO) as a means to expand red blood cell mass. Nevertheless, the cost-effectiveness of the intervention has not been evaluated. In this study we aimed to quantifying the impact of preoperative EPO on the cost-effectiveness of PADD for CABG (3). The high cost of the drug, although, probably the reason why preoperative EPO is not used in Italy.

In this study we aimed to quantifying the impact of preoperative EPO on the cost-effectiveness of PADD for CABG (3). The high cost of the drug, although, probably the reason why preoperative EPO is not used in Italy.
cost incurred to avoid the transfusion of one unit of allogeneic blood was $730. However, given its improving safety, each unit of allogeneic blood averted produced a very small benefit, of 0.00008 QALYs. This caused cost-effectiveness of EPO therapy, either alone or with PAB, to be higher than 6 million dollars/QALY. Conclusions. The analysis calculated that EPO was not cost-effective as a means of escaping health effects of perioperative blood transfusions. Therefore we discourage EPO for elective cardiac surgery. These results are in accordance with those by other authors who investigated this issue in the setting of orthopedic surgery. A common reason for the very high cost-effectiveness ratios is the very low impact of allogeneic blood transfusions on life expectancy.


**PO-0622** High-dose cyclophosphamide followed by autografting can improve the outcome of relapsed or resistant non-Hodgkin's lymphomas with involved or hypocellular bone marrow

Santrini G, De Souza C, Congiu AM, Marino G, Nall S, Darmas E
Department of Haematology, S. Martino Hospital, Genova, Italy

We report our experience of high-dose cyclophosphamide (HD Cy) followed by high-dose therapy (HDT) and peripheral blood progenitor cell (PBPC) autografting in patients with aggressive non-Hodgkin's lymphomas who have failed to benefit from conventional treatment. From 1991 to 1996, 54 consecutive patients treated with a median of two chemotherapy lines entered the study. Eighty patients (33%) were in sensitive relapse, and 20 patients (37%) were in PR after chemotherapy (CT). Sixteen patients (30%) were considered primary resistant or resistant at relapse. Thirty-nine patients had bone marrow involved by disease and fifteen had hypocellular marrow following conventional treatment. Patients received HD Cy (7 g/m^2) and G-CSF or GM-CSF in order to collect PBPC. Median number of CD34+ cells collected was 12.3 ± 10^9/kg (range 0.7-197). After HDT (BEAM or Melphalan + TBI) 50 patients underwent PBPC autografting. According to the intention to treat, 44 (81%) out of 54 patients achieved complete remission (CR) (50% after HDT and 31% after HD Cy).

<table>
<thead>
<tr>
<th>Status</th>
<th>Pts(n)</th>
<th>CR after HDT</th>
<th>CR after HD Cy</th>
<th>Final CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td>20</td>
<td>11 (55%)</td>
<td>4 (20%)</td>
<td>15/20 (75%)</td>
</tr>
<tr>
<td>Sen/Rel</td>
<td>18</td>
<td>13 (72%)</td>
<td>4 (22%)</td>
<td>17/18 (94%)</td>
</tr>
<tr>
<td>Rel/Rel</td>
<td>12</td>
<td>2 (16.5%)</td>
<td>7 (58.3%)</td>
<td>9/12 (75%)</td>
</tr>
</tbody>
</table>

Procedure related death occurred in 6 patients (11%), one after HDT Cy and 5 after autografting. Twenty-nine (66%) out of 44 patients are still in CR after 7 to 63 months (median 27 months) after the procedure. Three-year probabilities of survival, disease-free survival and progression-free survival were 63%, 64% and 52% respectively. In conclusion, HD Cy is an effective procedure not only in mobilising PBPC, but also in reducing tumour burden. HDT with PBPC support may further improve the outcome in this category of high-risk non-Hodgkin's lymphomas.

**PO-0623** The first experience of liposomal daunorubicin in treatment of haematological malignancies in adults

Medvedev PV, Stroyakovsky DL, Moiseeva TN, Skorohod AA, Pivnik AV
Haematological Research Centre of Russia, Moscow, Russia

Use of liposomal anthracyclines is highly perspective in terms of decreasing cardiac toxicity and the possibility of increasing the dose. Since 1996 we have used liposomal daunorubicin - DaunoXome (Dx) in the treatment of 21 patients with different haematological malignancies, including 14 cases of non-Hodgkin's lymphoma, 3 cases of relapsed Hodgkin's disease, 2 cases of relapsed acute promyelocytic leukaemia, one case of primary brain lymphoma and one case of transformed CLL. In primary aggressive lymphoma 5 cases were treated with the COP-DX regimen (CHOP containing Dx). The study included 8 males and 6 females with intermediate grade lymphoma. Median age was 56 yrs (range 17-70). The patients received 2 - 4 cycles at a very small interval 1 week interval. Dose of Dx was 80 mg/m^2, with escalation in 2 cases to 100 mg/m^2 and in 1 case to 130 mg/m^2. Six pts achieved complete remission (43%), 5 pts partial response (36%), progression was noted in 2 patients, and no response in one patient. Median follow-up is 18.6 months from the progression of the disease (28.5%). Dx was used in 3 cases of relapsed Hodgkin's disease as salvage therapy in different regimens. From 3 pts one achieved complete remission, 1 pt had partial response and 1 pt died from the progression of the disease. Dx was successfully used in 1 patient with primary brain lymphoma. After surgical removal of tumour the patient received COP-DX regimen of chemotherapy and achieved complete remission. In one patient with transformed CLL Dx (60 mg/m^2) was used in course M2. The patient achieved partial response. Two patients with relapsed acute promyelocytic leukaemia were treated with ATRA and 7 + 3, containing Dx. The dose of Dx was 60 mg/m^2/day and in the third course 90 mg/m^2/day. In both patients complete remission was achieved with follow-up of 7 and 5 months. Toxicty was predominantly haematological. Non-hematological toxicity was mild. Occasional extravasation of Dx in 4 cases was unremarkable. Even in patients in whom high doses of Dx were used (total dose 1050 mg) no deterioration in cardiac function estimated by EKG and cardiac ultrasound measurements was noted. We conclude that Dx is a well tolerated drug with acceptable toxicity. Preliminary results show that its activity in haematological malignancies is not higher than that of standard forms of anthracyclines.

**PO-0624** Hepatitis B and C virus infection in patients with B-cell non-Hodgkin's lymphoma

Ciucuianu A, Patru M, Duma M, Basarab C, Bojan A, Vaslacie A and Petrov I
Cancer Institute Cluj, Haematology Dept., Cluj, Romania, University of Medicine and Pharmacy, Haematology Dept., Cluj, Romania

We studied the incidence of hepatitis B (HBV) and hepatitis C (HCV) virus infection in a cohort of 52 consecutive B-cell non-Hodgkin's lymphoma (NHL) patients diagnosed and treated in our institution during the December 1997 - December 1999 period. The patients were 31 men and 21 women, aged 27-75 years (mean 53). Twenty one cases were diagnosed as low grade NHL and 31 patients as aggressive NHL (8 high grade and 23 intermediate grade according to the Working Formulation). In 27 patients (51.9%) we found evidence of either HBV or HCV infection. Anti-HCV antibodies were found in 16 patients (30.7%) and HBV surface antigen (HBs) was also found in 16 of our patients. In 5 patients, both HBs and anti-HCV were present. Anti-HCV antibodies were present in 9 out of 21 low grade NHL cases (42.8%) and only in 7 out of 31 aggressive NHL cases (22.5%). HBs was found in 7 of 21 low grade NHL cases (33.3%) and in 9 of 31 aggressive NHL cases (29%). Evidence of liver disease reflected by elevated transaminases or alteration at liver biopsy (performed only in 2 cases) was present in 7 patients (4 HCV-positive, 2 HBs positive and 1 with HBV and HCV positivity). Cryoglobulins were present in 5 patients, all of them anti-HCV-positive and with low grade NHL (32.2% of those anti-HCV-positive). The high incidence of HCV infection in low grade NHL is in agreement with several recent observations, most of them from Southern Europe and Asia. Although Romania is known to have a higher incidence of HBV and HCV infection than that observed in Europe (with the possible exception of Italy), the incidence of HCV in our NHL patients (30.7%) was significantly higher than that observed in a cohort of healthy blood donors. Only 0.7% was estimated at 3.4%. However, the presence of HBs positivity was not significantly higher than in the blood donors, which was estimated at 19%.

**PO-0625** Does amifostin (AMI) protect against doxorubicin (DOX)-induced cardiotoxicity in elderly patients?

Spital-Schwalbe E, Schanz J, Dietzmann A, Georgiessi E, Possinger K, Charité, Humboldt Universität, Medizinische Klinik m.s. Otolaryngologie/Hämatoologie, Berlin, Germany

Anthracycline-induced cardiotoxicity is believed to be related to the generation of free radicals. Preclinical studies have demonstrated cardioprotective activity for the cytoprotective aminothiol AMI and its active metabolite WR-1065, which has been attributed to the ability to scavenge superoxide anions and hydroxyl radicals [1]. We investigate, whether AMI pretreatment reduces cardiotoxicity of DOX in elderly patients (pts) receiving standard CHOP-polychemotherapy for advanced high grade Non-Hodgkin's lymphoma (NHL). To date, 12 pts with stages II (bulky), III or IV have received more than 200 mg/m^2 DOX (median 300 mg/m^2; range 237.5-400 mg/m^2). Median age was 71 years (range 60-79). Pretreatment Karnovsky score ranged from 40 to 100 (median 80). Six of the 12 pts had pre-existing coronary heart disease requiring medication, and 4 of these 6 pts had hypertension. There was normal renal and hepatic function. Pre-treatment echocardiographically estimated left ventricular ejection fraction (LVEF) ranged from 40 to 60% (median 50). LVEF was monitored every other cycle. A 740 mg/m^2 was administered as a 15 minute infusion immediately before chemotherapy. LVEF remained stable in 9 pts. In 3 pts there was a decline from 60 to 30%, from 50 to 30%, and from 45 to 30%. One of these pts developed symptomatic congestive heart failure grade III. Notably, these three pts had coronary heart disease and hypertension. Of
11 pts who are currently evaluable for response 7 obtained a complete and 4 a partial remission. In a recent study in a similar group of elderly pts with NHL who received CHOP without AMI, in 10 of 32 pts with serial measurements of UEF a absolute reduction of more than 15% from pretreatment values was observed (2). Although, overall our results compare favourably with this latter study so far, any conclusion regarding a protective effect of AMI against DOX-induced cardiomyopathy may be premature. It appears that elderly pts with several risk factors (3) have a high risk of developing DOX-associated cardiomyopathy even at moderate cumulative doses of DOX.

PO-0626 CEOP (Eribulin) treatment of non-Hodgkin's lymphoma. Prognostic factors and dose intensity

Rossini E, Rivolta F, Micocis I, Teruzzi E, Perego D, Isaila M, Pogliani EM, Corneo G

1Haematology Unit, University of Milan, S. Gerardo Hospital, Monza and (2) Medical Division, Hospital di Desio, Italy

Objective. We analysed prognostic factors and relative dose intensity (RDI) administered to patients affected by intermediate and high grade (Working Formulation type D+) non-Hodgkin's lymphomas (NHL), the patients were treated with CEOP (Cyclophosphamide 750 mg/m2 on day 1, Epirubicin 75 mg/m2 on day 1, Vincristine 1.4 mg/m2 on day 1 and Prednisone 60 mg/m2 days 1 to 5) with courses to be repeated every three weeks. Results and methods. We reviewed the data concerning 116 patients, treated in our Unit since 1983. Median age of patients was 61 (range 14-87). All patients were aged over 14, HIV negative and without active cardiac disease. Median follow-up is 60.3 months. RDI was calculated for epirubicin, cyclophosphamide and vincristine. Results. One hundred and nineteen out of 116 patients achieved complete remission (CR); 125 patients are alive, 87 in first CR. Therapy was well tolerated. Fifteen patients died within the first five months; 3 patients had to stop therapy because of cardiac toxicity (two with cardiac failure and one with arrhythmia). When RDI was calculated over the first 4 courses, it was over 0.5 in 48% of patients. No significant differences between the two groups could be shown, although patients with higher RDI have a higher rate of CR and a lower age. Stage was the most important prognostic factor for achieving CR. The achievement of CR, stage, age and Working Formulation type were related to overall survival. Stage was related to the duration of CR.

PO-0627 Serum soluble TNF-α receptor as a factor of disease activity in malignant lymphomas

Kaselko-Słowik K, Urbaniaik-Kudzia D, Jawiec B, Kilcikowski K

Dept. Of Haematology, medical University of Wrocław, Poland

It was found that in Hodgkin's disease and non-Hodgkin's lymphoma lev- el of soluble TNF-α receptor (sTNF-αR) is elevated and correlates with dis- ease activity. Measuring the plasma concentration of this receptor is use- ful for diagnosis, monitoring and prognosis of these diseases. The purpose of this project was to measure levels of sTNF-αR in patients with lym- phomas before, during treatment and in remission from the disease. In 46 newly diagnosed lymphoma patients sTNF-αR was determined in serum by the ELISA method. These 46 patients included 30 non-Hodgkin's lym- phoma and 16 with Hodgkin's disease. Median sTNF-αR plasma value was 2360±1312 pg/mL in lymphoma patients versus 1056±282 pg/mL (p=0.00057) in healthy controls. The presence of sTNF-αR L level greater than 3000 pg/mL was associated with poor performance status, B symp- toms, bulky tumors and short overall survival. Comparison of sTNF-αR con- centrations in sera of patients at presentation and in complete remission showed significantly higher values of receptor in active disease (1814±1010 pg/mL vs 1119±271 pg/mL p=0.024). Our results indicate the main role of sTNF-αR R concentration as a one of the prognostic factors in lymphoma patients. High sTNF-αR R concentration could be regarded as an indicator for more aggressive treatment.

PO-0628 A pilot study of anti-CD20 rituximab treatment after high-dose chemotherapy and autologous peripheral blood progenitor cell transplantation for newly diagnosed follicular non-Hodgkin's lymphoma

Brügger W, Manz M, Grünbeuch F, Rödder W, Scheding S, Kanz L
University of Tübingen, Dept. Of Haematology/Oncology, Tübingen, Germany

Objective. Based on the encouraging results of the anti-CD20 antibody Rit-uximab in patients with relapsed follicular lymphoma (FL), we initiated a pilot study to investigate the safety and efficacy of rituximab in a minimal resid-

ual disease situation in FL patients after high-dose therapy (HDT) and autol- ogous peripheral blood progenitor cell transplantation (PB/PCT). Methods. Eligibility included newly diagnosed FL, low grade malignant lymphoma patients with minimal residual disease (MRD) ≤10-4, WT (t(14;18))/bcl-2 positive FL. Up to now, 7 patients with a median age of 49 years (range 37-58) and a median IPI score of 2 (range 1-2) have been enrolled in this ongoing study. Initial chemotherapy consisted of 6 weeks of VACOP-B. Further cycles of VACOP-B were achieved by 2 cycles of CEOP sup- ported VIP-E chemotherapy (VP16 500 mg/m2, ifosfamide 4 g/m2, cis- platin 50 mg/m2, etoposide 50 mg/m2). PB/PCT were harvested after the first cycle of VP-E, and positive selection of CD34+ cells (purity >96%) as a purging strategy was performed with the CliniMACS device. HDT consisted of TBI (6x2 Gy) and cyclophosphamide (120 mg/kg), followed by CD34+ selected PB/PCT (3-7×10^6 CD34+/kg; range 3.2-20.3). Eight weeks after transplantation, Rituximab was given at a dose of 375 mg/m2 weekly for 8 weeks. Results. Before HDT, 4/7 patients achieved complete remission (CR) and 3/7 PR. Two of 6 patients were bcl-2 negative by PCR (PB, BM, CD34+ and PB/PCT). After HDT, 4/7 patients achieved complete remission and 3/7 PR. Two of 6 patients were bcl-2 negative by PCR (PB, BM, CD34+ and PB/PCT). After HDT, inclusions of CD34+ PB/PCT are safe, despite a prolonged treat- ment-induced immunodeficiency. Moreover efficacy of Rituximab treatment after HDT is demonstrated by the induction of bcl-2 negativity.

PO-0629 Comparative genomic hybridisation detects specific DNA gains and losses in different subtypes of non-Hodgkin's lymphomas


Servicios de Haematología y Anatomía-Patológica, Hospital Universi- tario de Salamanca; 3Servicio de Haematología y Oncología, Hospital Universitario de Valencia; 2Servicio de Anatomía-Patológica, Hospital Virgen de la Salud, Toledo, Spain

Background. Comparative fenomeric hybridisation (CGH) is especially help- ful to assess genomic copy number changes in tumour. Aims. To evaluate the utility of CGH in the analysis of chromosomal changes in non-Hodg- kin's lymphoma (NHL). Design and Methods. Thirty-five NHL patients with the following distribution of diseases were included in the study: 16 Mantle Cell Lymphoma (MCL), 9 Burkitt Lympho- ma (BL), and 10 Marginal Zone Lymphoma (MZL). Genomic DNA was extracted from lymph node (118 cases), spleen (9 cases) and bone mar- row (8 cases). The tumour and normal DNAs were labeled by nick-trans- lation with biotin-16-dUTP and digoxigenin-11-dUTP respectively and were hybridized to slides with metaphases from blood of a healthy donor. Thresholds for the identification of DNA imbalances (between tumour and normal DNAs) were defined as 0.75 (losses) and 1.25 (gains). Results. Chromo- somal changes were detected in 64% of NHL. The median number of changes was 2 per case (range 0-10). Overall gains were more frequent than losses (53 and 13 respectively). The most frequent imbalances were gains on chromosomes 12q (16%), 3q (13%), 8q (8%), 1q (8%), 11p (7%) and 10p (6%). All but one of MCL cases and all BL showed a genomic change. In MCL the most recurrent changes were losses on 15q (25%) and gains on 1p, 3q and 8q (13%). In BL amplifications on 12p-q24 and gains on X, 8q24, 10q12 and 11q22-q24 (22% each) were observed. Patients with MZL never showed amplifica- tions and in these NHL the most recurrent gains were detected in 9q34 (23%) and 12q24 (25%). None of the patients with more than 5 changes or amplifications reached a complete response. Conclusions. CGH is an useful tool for the diagnosis of chromosomal gains and losses in NHL and could contribute to identifying risk groups of patients.

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PO-0630 Discordant results of 4Gallium spect scintigraphy and com- puterised tomography in lymphoma.

Sojina J, Klein M, Lossos IS, Patiel O, Chissin R, Libson E, Pollack A

Departments of Radiology, Nuclear Medicine and Haematology, Hadassah University Hospital, Jerusalem, Israel

In patients with lymphoma (ly) Computerised tomography (CT) and 4Galli- um SPECT scintigraphy (4Ga) are used routinely to assess patients (pts) outcome. Discordant results (Di) between both studies do occur and cause confusion. We recently performed a cohort study during 1/94-6/98 of all pts in an attempt to determine the magnitude and characteristics of Di
Non-Hodgkin's Lymphoma II

PO-0631 Successful treatment of Langerhans cell histiocytosis with 2-deoxo-2-DCF
Lombardi A, Caniglia M, Caruso R, Miano C, Pinto RM, Rana I, De Rossi G
Children Hospital “Bambino Gesù”, Haematology Division, Rome, Italy

The clinical behaviour of Langerhans cell histiocytosis (LCH) is heterogeneous, varying from cases with spontaneously regressing solitary bone lesions, to cases characterised by multisystem, life-threatening disease. The nucleoside analogues 2-deoxycytomycin (2-DCF) and 2-chlorodeoxyadenosine (2-CDA) have been successfully used in the treatment of both Hairy Cell Leukaemia (HCL) and Chronic Lymphocytic Leukaemia (CLL). Recently, 2-DCF has been shown to be particularly effective in the management of mononuclear histiocytic disorders and has been successfully used in LCH; however, rare reports referred a clinical efficacy of 2-DCF in LCH. We treated three children with unresponsive and progressive LCH with 2-DCF at dosage of 4 mg/kg i.m., i.v. push, weekly for 8 weeks, and progressive LCH with and every second week thereafter. Two children (E.F., F.D.), aged 2 and 8 years, had been pre-treated using four different schedules including Vinblastine, Etoposide, Ara-C, Vincristine, Cyclophosphamide, Doxorubicin, and Cyclosporine; the third one (T.K.), four year old, had been pre-treated with Vinblastine, Prednisone and Etoposide. None of them reached a Complete Response and, all showed a significant disease progression. After 8 weeks of 2-DCF treatment, in all cases a significant reduction of initial lesions with an improvement of performance status was observed. In the first case (E.F.) the therapy has been carried on for ten months with a complete regression of cutaneous and pulmonary lesions, persisting bone lesions at MNR; in the second child (F.D.) we demonstrated a 30% regression of postobital mass and an improvement of exophthalmos just after the third administration of 2-DCF. In the third child (T.K.) the MMR control, following 8 administrations, showed complete regression of a postobital mass and involved lymphphodes, while NMR images of bone lesions persisted. E.F died 18 months after starting 2-DCF of hepatic cirrhosis and further disease progression; F.D. stopped treatment at the parents’ insistence; T.K is now on maintenance treatment. We never observed significant toxicity, or significant myelo-immunosuppression. We therefore obtained a good response in LCH, in three patients with progressive disease, heavily pre-treated, without any toxic effects, using 2-DCF which moreover has a very simple modal-ity of administration (i.v. push). Also considering the synergistic action of nucleoside analogues with alkylating agents and Interferon in HCL and LCH, it could be important to extend the use of 2-DCF (even in combination ther-apy) in LCH, as either second or first line treatment, the latter in aggressive cases with negative prognostic factors.

PO-0634 Post-transplant lymphoproliferative disorders (PTLDs) in solid allograft recipients: report of 18 cases from a single centre
Departments of Haematology and Pathology: Heart, Kidney, Liver and Lung Transplant Units, Niguarda Ca’ Granda Hospital, Milan, Italy

PTLDs are a severe complication arising in chronically immunosuppressed allograft recipients. The purpose of this study was to review the incidence and the clinical characteristics of PTLDs diagnosed at our Hospital. During the years 1973-1998, 2,002 transplants (tx) were performed: 483 heart, 49 lung, 412 liver and 1,058 kidney tx. All were managed with standard immunosuppressive protocols including ATG, cyclosporin A, prednisone and azathioprine: OKT3 was never used. PTLD was diagnosed in 18 patients (0.3%; 13 males, 5 females): heart 8/483 (1.6%); lung 1/49 (2%), liver 3/412 (0.7%); kidney 6/1,058 (0.6%). At diagnosis median age was 54 yrs (range 23-68 yrs); 11 patients were in stage III/IV; 1 patient had plas- macytic hyperplasia: 2 polyomaviruses; 14 high grade non-Hodgkin’s lymphomas and 1 myeloma. EBV was positive in 13/17 patients. In 5 patients (28%) PTLD developed within 1 yr from tx. In 5/18 diagnosis was at topic; 2/18 were lost to follow-up. In the remaining 11 patients reduced inmunite of immunosuppressive agents was undertaken: 3/11 did not receive further treatment because of deteriorated clinical status and died of pro-gressive disease; 2 received Ayclovir obtained remission and are alive at 600 and 575 days from diagnosis of PTLD; 6 were treated with chemother-apy; 4 died of progressive disease plus infection. 1 is alive and well at 90 days from diagnosis. I obtained remission but died of unrelated causes 426 days from diagnosis. Our data show that: 1) early diagnosis and management of PTLD are difficult because of the rapidity progressive clinical course; 2) therapy must be tailored to specific historical behaviour; 3) 20% of cases are autologous grafts. 31 in a high proportion of patients (78% in our series) PTLD developed later after tx (median 48 mos, range 17-174 mos) stressing the importance of long-term follow-up.
Objective. Age is a risk factor and a prognostic parameter in aggressive his-
tology NHL. Non-HL elderly patients can benefit from specific and adequate treatment
of curing a percentage of these patients. Design and Methods. Between January 1992 and September 1997, 350 previously untreated aggressive NHL patients >60 years old were treated with a com-
bination therapy including cyclophosphamide, melphalan, vincristine, etoposide, bleomycin, and prednisone (VNCOP-B). Results. Complete remission (CR) was achieved by 202 (58%) patients, PR by 87 (25%), whereas the remaining 61 (17%) patients were nonresponders. Overall response rate (CR + PR) was 83%. Clinical and haematological toxicities were modest, because 71% of the patients utilized granulocyte colony-stimu-
lating factor (G-CSF). The CR rates for the three age groups (60-69, 70-
79, and ≥80 years) were similar: 61%, 59%, and 56%, respectively. At 5 years, the relapse-free survival rate was 65% and the overall survival rate was 49%. In the multivariate analysis, prognostic factors associated with longer survival or longer relapse-free survival turned out to be localised dis-
ess (p=0.001) and good performance status (p=0.003). The interna-
tional Prognostic Factor index was significantly associated with the out-
come (p=0.001). Conclusions. These data confirm, in a large cohort of
patients, that the VNCOP-B regimen is effective in inducing good CR and
relapse-free survival rates with only moderate toxic effects in aggressive his-
tology NHL in the elderly.

2Dpt. Pathology, University Hospital, Olomouc, Czech Republic

PO-0636 Hepatic toxicity of chemotherapy among carriers of
non-Hodgkin's lymphomas (NHL) and hepatitis C virus (HCV)
Silveri F, Barillari G, Fanin R, Zaja F, Rogato A, Patriarca F, Marin L, 
Bertone A, Ermacora A, Damiani D, Baccarani M
Division of Haematology, University Hospital, Udine, Italy

While reactivation of chronic HBV infection induced by chemotherapy (CHT)
withdrawal is well known, reports on liver dysfunction among HCV carriers
undergoing CHT are lacking. Herein we report on the hepatic toxicity of CHT
among 26 patients with HCV-associated NHL. Their median age was 56y.
(37-75); 50% were male; diagnosis was CLL (2), marginal zone L. (5), 
immunocytoma (7), follicular L. (4), and diffuse large cell L. (8). All were
HCV Ab+, while RNA was positive in 80% of them. In 7 patients chronic
active hepatitis (CAH) was documented by biopsy; overall, 140 cycles of
CHT were administered and analyzed (62 CHOP, 45 Fludarabine, 28 F-
MACHOP and 5 BAVC as conditioning prior to autologous stem cell trans-
plantation - ASCT). Prednisone was part of 84 cycles in 16 patients. Liver
tests pretreatment) after the 2nd Fludarabine dose. After F-MACHOP, in 4
patients, that the VNCOP-B regimen is effective in inducing good CR and
relapse-free survival rates with only moderate toxic effects in aggressive his-
tology NHL in the elderly.

2Dpt. Haemat-oncology, University Hospital, Olomouc, Czech Republic

PO-0637 Response monitoring in relapsed non-Hodgkin lymphoma:
a comparison of different analytical methods using FDG PET
Zuilla IM, Jonkhoff AR, Hoekstra OS, Huijgens PC, Lammertsma AA
University Hospital Vrije Universiteit, Amsterdam, the Netherlands

Objective. The preferred treatment of patients with relapsed NHL after sys-
temic chemotherapy is reinduction chemotherapy followed by myeloabla-
tive therapy and stem cell transplantation. As outcome after high dose
chemotherapy is better for patients with chemosensitive disease, the pre-
sent study was performed to assess the value of positron emission tomog-
raphy (PET) with 18Fluorodeoxyglucose (FDG) for predicting outcome and
prognosis. In particular suitability of various analytical methods was
assessed, with intention to replace the laborious gold standard by a sim-
ple and reliable method. Methods. Dynamic FDG scans were performed before
and after each cycle of reinduction chemotherapy (IMVP and/or
DHAP) and before myeloablative therapy (17 scans in 4 patients)- Three
analytical methods were used: (1) semiquantitative standard uptake Val-
ue (SUV) corrected for body surface area and plasma glucose, (2) non-lin-
ear regression (NLR) using the standard two-tissue compartment model
(considered to be the gold standard) and (3) Patlak graphical analysis.
Methods were compared using the Bland-Altman method. Results. Com-
pared to MRgF as obtained with the NLR method, absolute statistical errors
in (normalised) SUV and Patlak were nearly constant, i.e. relative errors increased with decreasing MRgF. MRgF values (NLR) ranged from
0.03 to 0.30 mmol/ml/min. The 95% limits of agreement for the Patlak
method were -0.01 to +0.03, for the SUV method -0.17 to +0.22. There
was good correlation between clinical response and reduction in MRgF
(NLR). Reduction was >70% in 2 responders, but only in 2 non-respon-
ders. Conclusions. These result suggest that FDG PET might be able to dif-
fentiate between chemosensitive and chemoresistant disease. Given the
lack of agreement between SUV and NLR, further studies are required to
assess whether SUV can be used for response monitoring purpose. Ultimate
validation will require comparison with outcome in a larger study.

PO-0638 Mantle cell lymphoma: our experience with one of the worst
forms of non-Hodgkin's lymphomas
Papajíň, Z., Rádlí ě, L., Fabíř, E., Hubáček, J., Urbanová, R., Heczkó, M.,
Pikalová, Z., Kyčičková, E., Kovářková, L., Pálekova, P., Jarová, M., 
Dusík, J., Tichý, M., Indrák, K.

Mantle cell lymphoma (MCL) is generally accepted as clinically important
separate entity and incorporated in the REAL and WHO classifications.
The disease has distinct biological characteristics that are associated with its
aggressiveness and carry little hope of a cure by conventional cytotoxic
chemotherapy. We analysed 107 cases of non-Hodgkin's lymphomas
(NHLs) referred to our institution from regional haematological centers
between 1995 and 1999. Twenty-nine patients (27%) were selected and
compared with two other major subtypes of NHLs - follicular lymphoma
(FL - 23 pts. - 21%) and diffuse large cell lymphoma (DCLL - 29 pts. - 27%).

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apy. Our experience demonstrate the extremely unfavorable prognosis of MCL within the spectrum of NHLs and the need for aggressive first-line treatment with a perspective of high dose chemotherapy with autologous stem cell support or allogeneic transplantation in younger patients.

PO-0639 Natural killer cell mediated immunity in childhood malignant lymphoma
*University of Ankara Dept. of Pediatric Immunology - Allergy and Oncology, *Dr. Sami Ulus Children’s Hospital Oncology Unit, Ankara, Turkey

Malignant lymphomas - ML (Hodgkin’s disease - HD and non-Hodgkin’s Lymphoma - NHL) are regarded as a constellation of related entities each, however, possessing distinguished clinical, histologic, immunopathologic and molecular features setting it apart from other similar neoplasms. A total of 36 patients with MS; 17 HD (9 untreated, 8 in remission) and 19 NHL (11 untreated, 8 in remission) admitted to the University of Ankara Department of Pediatric Oncology and Dr. Sami Ulus Children’s Hospital Oncology Unit were studied. NK cell cytotoxic activity assays were performed at the Immunology Laboratory, Department of Pediatric Immunology-Allergy. Peripheral blood CD16 and CD56 molecule expression was also examined by using monoclonal antibodies and the indirect immunofluorescence method. No differences related to NK cell numbers or cytotoxic activity were detected at either stage of the disease in HD patients. However, in NHL patients cytotoxic activity was found to be slightly decreased in the active stage of the disease and significantly so during remission while the numbers of NK cells were normal at both stages. In NHL cases in which long remission has been achieved (>5 years). NK cell activity was found to recover in parallel with remission duration. Finally no relation between NK activity and aetiology, and prognosis of the disease other than remission duration has been established in children with ML.

This study was supported by Turkish Research Council (TUBITAK).

PO-0640 Expression of latent membrane protein-1 gene in tumour tissue of Burkitt’s lymphoma: correlation with prognostic criteria
Tacylidiz N, Cavdar AO, Unal E, Giziçaoğlu S, Etrem U, Iksicigullari A, Cin S
Ankara University, *Department of Pediatric Oncology, Hermatology, Immunology, Dr. Sami Ulus Children’s Hospital Department of Pediatric Oncology, Ankara, Turkey

This study was designed to investigate the molecular mechanisms underlying the pathogenesis of Burkitt’s lymphoma (BL) in Turkish children which leads to a high frequency in advanced-stage admission of the patients. It is known that BLs in Turkish children is commonly associated with EBV infection. To understand molecular mechanisms underlying EBV pathogenesis in BLs in Turkish children we analysed the 30-bp deletion region of the latent membrane protein (LMP-1) gene from paraffin-embedded tumour tissues of 28 BL patients (mean age: 5.9 years). Primer pairs spanning the 30-bp deletion region were designed for amplification by polymerase chain reaction (PCR). The PCR amplified products were analysed by gel electrophoresis, Southern blot hybridisation and DNA sequencing. Analyses of DNA sequence of 28 BLs have disclosed four patterns. The first (32%-29%) is identical to B95-8 with no deletion, second (13%-29%) is identical to Asian NPC CAO strain with 30 deletion, the third (46%-13/28) is prevalent in Turkish BLs with a longer deletion (69bp) and the fourth (11%-32/28) consists of a mixture of a 30bp and 69bp deletions. All 28 patients were infected by type-A EBV. Although the high frequency of the 69bp deletion appears to have no correlation with the disease site, it has a positive correlation with the stage. Frequency of 69bp deletion in advanced-stage patients was 54% while it was 14% in patients with earlier stages (p<0.02). The 5-year survival rate of the group with 69bp deletion of LMP-1 was 52.6% whereas the corresponding figure for patients without deletion was 77.5%. However, the difference was not statistically significant by log-rank test, probably due to improved survival rates or advanced-stage patients by intensive chemotherapy regimen. In conclusion, expression of 69bp deletion of LMP-1 gene in tumour tissues of BLs in Turkish children can be attributed to an aggressive pattern of disease in patients who are in advanced stage.

PO-0641 Rituximab: efficacy in clinical trials in combination with chemotherapy, biologicals, or radioimmunotherapy
Grillo-López AL, White CA, Shen D, Vams C, Alkuzweny B, Parker B, Dowden S
IDEC Pharmaceuticals Corp., San Diego, CA, USA

Clinical trials were conducted in low-grade or follicular (LGI/F) NHL patients (pts) to evaluate the activity of Rituximab (mAb) in combination with chemotherapy (CHOP), biologicals (interferon-α2a) and radioimmunotherapy (IDEC-Y2B8). In a study of Rituximab in combination with CHOP, (40 pts; 31 naive, 9 previously treated), CHOP was administered at standard doses q 3 weeks for 6 cycles along with 6 infusions of mAb (375 mg/m² dose). Two doses of mAb were given both at the beginning and end of therapy, as well as single doses before the 3rd and 5th cycles of CHOP. Pt characteristics included: 21 males, median age 48, stage III/IV at diagnosis (83%). All pts treated responded (58% CR and 42% PR). The overall response rate (ORR) for 35 evaluable pts completing all therapy was 100% (63% CR, 37% PR). Median duration of response was 35.3 months with median PFS not reached after 36.7 months observation. Twenty pts are still in remission beyond 36 and up to 53.4 months. Seven of 8 bcl-2 positive pts converted to PCR-negativity in blood and marrow (molecular complete remissions). Of these 7 pts, 6 remain in CR and 5 remain PCR-negative by serial analyses. The combination with interferon-α2a was investigated in 38 pts who received Rituximab (375 mg/m²) once weekly for 4 weeks (weeks 5-8) during concurrent treatment (3 times a week for 12 weeks) with subcutaneous injections of interferon-α2a (Roferon-A, 3 MIU injection). The ORR was 45%. The time to response in responders has not been reached after 25.2+ months. IDEC-Y2B8 is a murine IgG1 kappa monoclonal antibody directed against the CD20 antigen that is conjugated to MX-DTPA, and securely bound to the beta emitting radioisotope 90Yttrium (90Y). In this study of IDEC-Y2B8 in combination with Rituximab for relapsed or refractory NHL, IDEC-Y2B8 was given as a single dose following an infusion of Rituximab. IDEC-Y2B8 was used for imaging and dosimetry and was administered 1 week prior to IDEC-Y2B8, also following an infusion of Rituximab. Of the 39 pts with low-grade, intermediate-stage, or mantle cell lymphoma treated, 14 of 16 pts were CR or 0.4 mCi/kg, 61% responded. Of 25 pts with LG NHL, 76% (20 CR and 56%PR) responded. The response rate was 82% for the 11 LG/F NHL patients treated at the selected Phase II dose of 0.4 mCi/kg. Rituximab, the first monoclonal antibody approved for the treatment of cancer, is effective as a single agent in pts with relapsed or refractory CD-20 positive, B-cell, LGI/F NHL. The mechanism of action differs entirely from that of chemotherapeutic agents, thus presenting attractive opportunities for integration into combination regimens with chemotherapy biologicals, or radioimmunotherapies.

PO-0642 The role of CD79a in the immunophenotypic characterisation of lymphoplasmacytoid lymphoma in bone marrow trephine biopsies
Asplund SL, Miller ML, Flikkede AL
Department of Clinical Pathology, Cleveland Clinic Foundation, Cleveland, Ohio, USA

Objective. The CD79a (mb-1) protein is part of the B-cell receptor heterodimer complex formed early in B-cell development and found in most B-cell neoplasms. Its expression however in plasma cells is variable. Lymphoplasmacytoid lymphomas (LPL) contains variable proportions of small lymphocytes, plasmacytoid lymphocytes, and plasma cells. This cellular heterogeneity in bone marrow (BM) can make its distinction from chronic lymphocytic leukaemia (CLL) and plasma cell myeloma (PCM) difficult. This study evaluated the utility of CD79a, in conjunction with other B-cell antigens, in distinguishing LPL from CLL and PCM. Design & Methods. All bone marrows diagnosed as LPL (15) between 1994-1998 were studied. CD79a and PCM were selected for further study of the LPL group and were confirmed based on accepted morphologic criteria. LPL were morphologically subtyped as lymphoplasmacytic or plasmacytic variants and divided based on the serum M-protein present: IgM (7), IgA (2), light chain only (3), and nonsecretory (3). Clinical and laboratory data were reviewed for each case. B5 fixed BM trephine biopsies were stained for CD79a, CD45RB, CD20, and kappa and lambda light chains using standard immunohistochemical methods. An antigen was considered positive in a case if >30% of the cells stained. Antigen expression in each disease was based on % of positive cases and was designated as follows: + (>90%), +/− (50-90%), + (10-50%), and − (<10%). Results. The LPL cases exhibited variable antigen expression: CD79a (+5/12), CD548R+ (14/15), CD20+ (10/14) and monoclonal light chains (MCI) (15/15). The pattern of antigen expression in the LPL group was independent of the morphologic subtype or serum M-protein present. The CLL cases were very uniform demonstrating CD79a+ (8/8),...
CD45RB (8/8), CD20 (8/8), and (MLC) (8/8). The PCM cases had vari-able expression of CD10a (4/11) but CD45RB (11/11), CD20 (11/11), and MLC (11/11), which were constant in the other two groups. The pattern of CD10a, CD45RB, and CD20 expression in LPL and PCM may be useful in confirming a diagnosis of LPL. The LPL immunophenotype does not correlate with LPL morphologic subtype or serum M-protein type.

**PO-0643 Reduced dose CHOP therapy for elderly patients with aggressive non-Hodgkin’s lymphoma (NHL)**

Takagi T,* Morii M, Nitsu N, Tomiyama Y, Matsuo K, Nakagawa Y, Okamoto R,* Chiba Cancer Centre Hospital, Chiba; *Tokyo Metropolitan Geriatric Hospital, Tokyo; **Elderly Lymphaoid Study Group, Japan.

Background: The optimal dose schedule for elderly patients (pts.) with NHL remains uncertain. The with-grade dose intensity, the greater the tumour reduction induced, but the poorer the associated treatment compliance. Our own dose finding study has shown that five-sixths (5/6) and seven-twelfths (7/12) of the full dose CHOP have been most feasible for 65-79 year-old pts. (Group A) and pts. 80 years or older (Group B), respectively. We conducted a phase III study to verify the usefulness of the reduced dose CHOP therapy for the elderly pts. Design and Methods: Between June 1995 and Feb 1997, 58 pts. (44 in Group A and 14 in Group B) were enrolled in the study. Patients were eligible for the study if they were (1) aged 65 or older; (2) had PS 0-3 and PS 4 due to lymphoma; (3) had well-pre-served organ function; (4) had no previous treatment; (5) had PS 0-3 and PS 4 due to lymphoma; (6) had well-pre-served organ function; (7) had no previous treatment. Each RC member reevaluated 40 patients. The reevaluation was classified into one of eight mutually exclusive categories, which included CR uncertain (CRu) and PR. If progression had not occurred within one year, patients in the CRu group were referred to that of CR. Results. In five (7/12) of the full dose CHOP have been most feasible for 65-79 year-old pts. (Group A) and pts. 80 years or older (Group B), respectively. Fifipirin (50 mg m2 day) was admin-istered subcutaneously when WBC fell below 2,000/µL, and discontinued when WBC was over 10,000/µL. Results. 57 pts. (44 in Group A and 13 in Group B) were evaluable. The set dose schedule was completed in 76.7% of pts. in Group A and 66.7% in Group B. CR rate was 75.0% and 76.7% of pts. in Group A and 66.7% in Group B, respectively. Major toxicity: leu-copenia over grade 3, 73.7%; neutropenic fever, 45.6%; documented infection, 17.5%. Cardiac and renal failure, liver dysfunction of grade 3 were seen in each pt. Conclusions. 5/6 dose CHOP therapy can produce CR rate. OS and EFS in the Group A comparable with average adult pts. mainly due to the good treatment compliance.

**PO-0644 Reproducibility of treatment response evaluation in patients with high-grade malignant non-Hodgkin’s lymphoma**

Ödqvist E,* Cavallin-Stahl E,* Hagberg H,* Taube A,* Björklund M,* Department of Medicine, Karolinska Hospital and Institute, Stockholm, Departments of Oncology and *Uppsala University Hospital, Department of Statistics, Uppsala University, Uppsala, Sweden.

In contrast to survival, estimation of complete [CR] and partial response (PR) in non-Hodgkin’s lymphoma (NHL) is associated with a number of potential sources of error. In this report we have studied the reproducibil-ity of response evaluation performed by an independent review committee (RC). Design and Methods. In an open label, randomised multicentre Nordic study patients ≥60 years of age with high-grade malignant NHL were randomised in a bifactorial design to four groups: CHOP or CNOP alone or in combination with granulocyte colony stimulating factor (G-CSF, Neupogen®). In Sweden 35 institutions included patients. One major end-point of the study is the CR rate. Sixty patients who were already evalua-ted by the RC (consisting of three senior hematologists/oncologists dividen-d into three teams with two in each) were randomised to three groups and reevaluated by the RC. Each RC member reevaluated 40 patients. The assessment was classified into one of eight mutually exclusive categories, where the important borderline with regard to the end-point was between CR uncertain (CRu) and PR. If progression had not occurred within one year, patients in the CR group, were referred to that of CR. Results. In five cases the RC could not separate between CR and CR, with lesions < 2 cm (n=2). CRu with lesions 2-2 cm and PR (n=2), and PD and SD (n=1). Mis-classification was in two additional patients due to unclear definition of “CR, not assessable”. In two PR patients, major disagreements in tumour eval-uation occurred, depending on missed new lesions. After reevaluation the CR status was changed in 5 of 60 patients (8.3%). However, taking in con-sideration the status after one year of follow-up, the final end-point was changed in only two patients (3.3%). Conclusions. In our study, a good assessment of CR, only minor disagreement was seen between the two response evaluations performed by the independent RC. We conclude that an independent RC is a major prerequisite for the proper response evaluation in clinical phase III trials. However, the good reproducibility of the evaluation does not mot-iF a reevaluation of the RC result.

**PO-0645 PCR detection of clonality in low-grade follicular lymphomas and high-grade B-cell lymphomas**

Moreau E,* Marcelis L,* Philippe J,* Hartziekenhuis Roeselare H., Universitair Ziekenhuis Gent, Belgium.

Introduction. The detection of clonality in B-cell lymphomas has been facili-tated by polymerase chain reaction (PCR) analysis of the immunoglobulin heavychain gene (IGH) complementarity determining region 3 (CDR3) (IGH-PCR). The detection rate varies depending on the pathological sub-type and the choice of the primers used. Low-grade follicular lymphomas (FL) and high-grade B-cell lymphomas (HG B-NHL) are among the subtypes often leading to false negative results (no clonality detected). Design and Methods: We evaluated three known IGH PCR methods in a series of 18 FL and 26 HG B-NHL: 1. A consensus primer against the VH FR3 region (FR3 PCR), 2. A consensus primer against the VH FR2 region (FR2 PCR), 3. Six family specific VH primers (FR1 PCR). Results. By combining the three methods clonality was detected in 13 (72%) of 18 FL and in 21 (81%) of 26 HG B-NHL. Detection rates were different related to the primers used. In the FL series clonality was detected in 33%, 72% and 56% by the FR3, FR2 and FR1 PCR, respectively. Similar results were found in the HG B-NHL group with detection rates of 42%, 65% and 58% by FR3, FR2 and FR1 PCR, respectively. A combination of FR3 PCR and FR2 PCR detected clonali-ty in 14 (78%) of FL and in 19 (73%) of HG B-NHL. Conclusions. Our data suggest that clonality can be detected by the FR3 and FR2 PCR in the majority of FL and HG B-NHL. FR1 PCR will only slightly increase the sensi-tivity. Moreover FR3 and FR2 PCR can be performed successfully in paraf-fin embedded tissues because of the short length of the PCR products. The detection rate of the FL (14/18) translocation in these series of FL and HG B-NHL is now being investigated by real time quantitative PCR.

**PO-0646 Neuropsychological evolution of primary central nervous system lymphoma patients with long term complete remission**


Objective. Long term survivals are now obtained with high dose chemother-apy for primary CNS lymphomas. In this report, we studied neuropsycho-logical evolution of patients in long term complete remission (CR). Methods. 20 patients, median age 56 years (range 39-70), were treated between 09.15.89 and 04.23.97 with 3 courses of methylprednisolone 3 g m2 for 5 days and 15. DIL and D15, BCNU, teniposide, methyl prednisolone and 30 to 40 Grays in toto cerebral irradiation (POF LCP 88 Trial). Nine of the 13 patients with a CR longer than 20 months underwent a neuropsychological assessment which included a clinical interview, in order to obtain the patients self report of cognitive problems, a behaviour observation, and a battery of tests which mainly measured language, praxis, gnosia, memory processes, intellectu-al efficiency and executive functions. Most of the patients had several neu-ropsychological assessments. Four of them were tested at the time of diag-nosis, 8 patients were tested 4 months after the initial treatment and 7 had a follow up evaluation between 2 to 8 years after the treatment. Results. Moderate memory impairments (everyday memory problems, new-learning difficulties) without intellectual problems were observed in 7 patients. Six of them also showed deficits in oral verbal fluency. Intelligence scores were decreased in the patients tested at the time of diagnosis but recovered to the premorbid level 4 months after the treatment. Behaviour problems such as tiredness and attention fluctuations could also overestimate intel-llectual problems. For example, 4 patients (1 chartered accountant and 3 teachers), reported emotional problems with self esteem assessment, tiredness, and only 2 of them resumed a part time job. Two patients exhibited a moderate frontal syndrome (one had a frontal lobectomy). Conclusions. Neuropsychological problems did not increase as the delay post-treatment grew longer (mean 4.5±1.3-7.9 years). These problems seemed to be more frequent after 40 Grays (5 patients) and are overestimated by behaviour problems such as tiredness and self under-estimation in patients whose jobs require cognitive resources (4 patients).
PO-0650 Bilateral trephine biopsy in the diagnosis of lymphoma. Is unilateral biopsy enough?

Johannsen P, Stockelberg D, Eikman T, Ridel B

Department of Medicine, Haematology Section, Department of Oncology and Department of Pathology, Sahlgrenska University Hospital, Gothenburg and Department of Medicine, Uddevalla Hospital, Uddevalla, Sweden

Purpose. To evaluate the frequency of bilateral bone marrow biopsy performed in the staging of non-Hodgkin’s lymphomas compared with unilateral bone marrow biopsy design. Methods. In our area with about 300,000 inhabitants, 272 patients were given a diagnosis of lymphoma between January 1992 through December 1996. We examined all medical journals to find out how many bone marrow biopsies had been performed and in how many cases a bilateral bone marrow had been done. In all cases in which a bilateral bone marrow biopsy was performed an evaluation was made as to whether lymphoma involvement was found on both sides. In these cases a second evaluation was performed by a lymphoma pathologist. Results. Of the 272 patients a bone marrow biopsy was done in 153 patients (56%); of these a bilateral bone marrow biopsy was done in only 46 cases (17%). Involvement of the bone marrow was found in 12 of 46 bilateral biopsies, and of these all had lymphoma involvement in both of the biopsies. Conclusions. In the literature there is no consensus about the need to perform bilateral bone marrow biopsies. In this small pilot study lymphoma involvement was found in 12/46 bilateral bone marrow biopsies. In all 12 cases the involvement was bilateral. A study is under progress in a larger patient population to investigate this subject further.

PO-0651 CD30+ and CD30- large cell lymphoma of childhood: clinical, immunophenotypic and survival differences between the two groups

Marchi M, Filippa DA, Thaler RA, Polivak T, Wolliner N

Memorial Sloan-Kettering Cancer Centre, New York, USA

Pediatric diffuse large cell lymphoma (DLCL) patients (pts) treated with two consecutive regimens, LSA-Li and LSA+. Subgroups retrospectively identified as CD30+ and CD30- were compared. A total of 78 consecutive pts were treated for stage III (55 pts) and stage IV (23 pts) DLCL: 21 primary mediastinum, 14 intra-abdominal, 12 peripheral nodal, 9 subcutaneous, 9 bone, 7 nasopharyngeal, 3 epidural and 3 from other sites. Immunophenotypic data were obtained retrospectively for 52 using a panel of monoclonal antibodies against CD30, CD15, CD45, CD45 Ro, CD43, EMA, CD5, BCL-2, CYC-D and p53. Of the 78 treated pts, 56 are alive with a median follow-up of 10 years (2-26). The 5-year event free survival for the CD30+/CD30- were compared. The 5-year event free survival for the CD30+/CD30- were compared. The 5-year event free survival for the CD30+/CD30- were compared. The 5-year event free survival for the CD30+/CD30- were compared. The 5-year event free survival for the CD30+/CD30- were compared. The 5-year event free survival for the CD30+/CD30- were compared. The 5-year event free survival for the CD30+/CD30- were compared.

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Objective. The presentation of patients (pts) with refractory or relapsing non-Hodgkin lymphoma (NHL) is usually suboptimal, with refractory NHL (NHL) as salvage for patients with poor prognosis, achieving response rates of < 60%, most of them of short duration. We have performed a pilot study in which the efficacy of a combination of fludarabine phosphate (Flu) and cyclophosphamide (Cy) was evaluated in these pts (Blood 1999; 94: Suppl 1:195A). We report our experience in a larger cohort of pts. Design and Methods. Twenty seven pts (20 males and 7 females), mean age 53 years (range 16-71) were included in this study. Twenty two pts had indolent and 5 had aggressive NHL. Twenty pts had relapsing disease while 7 were refractory to treatment. All pts had previously been treated with 1 to 3 chemotherapy (CT) regimens (mean 1.7) including CHOP (Bleo), CV, VEP, DVP, CYBOM, PROMACE-MOPP and mini-BEAM. Eight pts had received radiotherapy, 5 had undergone splenectomy and 3 had relapsed after high dose CT followed by autologous (2) or syngeneic (1) BMT. Pts were treated with monthly, 3 day cycles of IV Flu (25 mg/m2) 4 hours prior to a 1 hour infusion of Cy (500 mg/m2, reduced to 250 mg/m2 in 3 pts). Pts received 1 or 2 cycles, while 21 pts completed 3-7 cycles. Results. The overall response rate (ORR) was 81% (11 CR, 12PR, 1CRi, 9NR, 2PD). All 3 pts (4 with aggressive disease) failed, dying within 9 months (mo). RR for pts with indolent NHL was 95%. The median duration of CR is > 9 mo with 6 pts still in unmaintained CR 6-20 mo after therapy. Eight pts with FR had disease progression after a mean of 7 mo (range 4-10), while 2 are still in stable PR for 4, 6 and 8 months. It is noteworthy that all 3 pts relapsing after BMT achieved a good, but shortlasting, PR. Myelo-suppression with febrile neutropenia occurred in 4 pts and 1 pt died of Flu-induced neurotoxicity, 1 of prolonged chemotherapy-induced aplasia and 1 of transfusion related GHD. The single opportunistic infection due to lymphocytosis was a non-fatal Pneumocystis carinii pneumonia. Conclusions. Flu-Cy is probably not salvage useful as salvage for aggressive NHL. However, response in previously treated indolent NHL is unexpectedly impressive. This easily administered regimen should be considered a therapeutic option in this difficult group of pts.

PO-0653 Diagnosis of mediastinal lymphoma: the value of CT-guided core needle biopsy

Skirair-Levy M, Libson E, Gillies S, Sherman Y, Pollicia A
Departments of Radiology, Pathology and Haematology, Hadassah University Hospital, Jerusalem, Israel

This retrospective study examines the accuracy, safety and subsequent value of CT-guided core needle biopsy (CNB) in the diagnosis of patients with mediastinal lymphoma (Myl). The results of 48 CT guided CNB in 41 patients (pts) were analyzed. CT guided CNB were performed with 18G or 20G, Turner core biopsy needles, under local anaesthesia in ambulatory pts. Results show, that a definitive diagnosis was obtained in 29 of the 41 pts; the overall success rate being 70.7%. In 5 other pts (12.2%) the biopsy was suggestive of lymphoma and the diagnosis was finally proved by surgery. In 6 pts the biopsy was not definitive (14.6%) and the diagnosis was established by surgery in 5 pts and in 1 pt by bone marrow aspiration. In 1 patient (2.5%), there was a false positive diagnosis of suspected Myl which on surgery proved to be inflammatory. The technique was successful in the diagnosis of Hodgkin’s by in 17/21 pts and in 12/19 of non-Hodgkin’s. The biopsy was diagnostic and yielded information on the basis of which therapy was started in 29 of 41 pts (70.7%). There were no major complications, except for mild hemoptysis in one pt. In conclusion, percutaneous CT guided CNB of Myl has been shown to be a safe procedure; an accurate and efficient alternative to open surgical excisional biopsy. It is indeed possible that it will become the preferred initial diagnostic procedure to be used for histologic sampling in pts with suspected Myl prior to performing a more complicated surgical procedure in the mediastinum.

PO-0654 VACOP-B, high-dose cytoxan and high-dose therapy with PBPC rescue for aggressive NHL with bone marrow involvement: clinical and prognostic significance


Aggressive NHL with BM involvement at diagnosis has a poor prognosis. Survival probability at 3-years is about 20% using conventional chemotherapy. From 1992 to 1994 a study which included VACOP-B until maximum response followed by high-dose cytoxan (HDCy) and PBPC autografting as front-line therapy in 40 successive patients (groups F-G-H-K/WF) was performed. Median age of patients was 51 yrs. (range 20-60); 25 were male and 15 female; median BM involvement was 35% (range 8%-90%). Patients received a median of 8 VACOP-B courses, followed by HDCy (7 g/m2 single dose) plus G- or GM-CSF (5 mcg/kg) to reduce tumour burden and collect PBPC. With a median number of 3 aphereses a median of 11.3x109/kg CD34+ cells were collected. Twenty-nine patients underwent PBPC autografting after Melphalan +TBI or BEAM regimen. According to intention to treat, 29/40 patients (72.5%) achieved CR/10/40 (25%), after VACOP-B treatment 8/40 (20%), after HDCy 11/40 (27.5%), after high-dose therapy. The statistical analysis shows a 3-year probability of survival of 47%, with a probability of DFS and PFS of 50 and 37%, respectively. A statistical analysis of morphology and extent of BM infiltrate, and clinical features according to the IPI, in terms of OS, DFS and PFS was performed. This analysis did not show any statistical factor predicting a poor outcome excluding the extent of BM infiltrate >50% in terms of DFS (p<0.05). This study suggests that high-dose sequential therapy may improve the outcome of these patients. The extent of BM involvement could identify a group of patients with high risk of relapse.

PO-0655 The International prognostic score (IPS) in advanced Hodgkin’s disease: its usefulness in evaluating overall survival (OS)

Haematology Department, University of Bari, Italy

Objective. The aim of our retrospective study was to evaluate IPS (Hansen- clever ASH 96) in advanced HD, the impact on OS and if it is able to identify subsets of pts requiring more aggressive treatment. Design and Methods. The presenting feature of 194 adults (<16 years) seen at our Department between 1988 and 1996 with clinical stage of lB (83 pts), lI (79 pts) and IV (32 pts) were reviewed. Median age was 35 years (range 16-64); 59 pts (30.4%) were <45 years old 103 pts (51.1%) were male. Histological subtypes were: nodular sclerosis in 104 (53.6%), mixed cellularity in 65 (33.5%), lymphocyte predominance in 11 (5.7%), lymphocyte depletion in 11 (5.7%) and unclassifiable in 3 (1.5%). Hemoglobin was >10 g/dL in 29 pts, serum albumin >4.0 mg/dL in 94 pts, leucocytes <10.5 g/dL in 27 pts and lymphocytes <600/µL in 13 pts. Overall Survival (OS) curves were calculated using the Kaplan Meier method and table death due to causes other than HD were not censored. Results. According to the IPS the pts were stratified into two prognostic groups: score 0 and score >3: results are summarised in Table 1.

Table 1. First-line therapy

<table>
<thead>
<tr>
<th>IPS score</th>
<th>N (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>NR (%)</th>
<th>OS (months)</th>
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<td>0-2</td>
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<td>66.4</td>
<td>70.9</td>
<td>35.2</td>
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<td>5.80</td>
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<tr>
<td>p</td>
<td>n.s.</td>
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<td>Log-rank &lt;0.0001</td>
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Seventy-four pts were treated with 2nd line therapy for relapse (REL) or PR: 48 pts (17 REL and 31 PR), received conventional polychemotherapy (group A) and 26 pts (6 REL and 20 PR) received stem cells transplantation (SCT) following high dose chemotherapy (group B). Twenty-four pts of group A had CR (53.3%), 7 PR (15.6%). Fourteen were NR (29.2%) and 3 NR. 21 pts of group B had CR (80.8%), 2 PR (7.7%) and 2 were NR (11.5%). A significant differences in RR (64.6 vs 88.5% - p <0.001) and OS (46.04 vs 66.03 months - log-rank 0.0001) were found between the two groups: we did not observe any difference in terms of OS if only pts with IPS score 0-2 were evaluated, while there was significant difference in pts with IPS score ≥3. Conclusions. IPS may be useful in predicting OS in pts with advanced HD and in selecting a subset of pts (IPS ≥3) needing more intensive treatment such as SCT.

PO-0656 Treatment of advanced-stage Hodgkin’s disease with COPP and COPP alternating with ABVD

Dimitrovskova A, Nikoloviva L, Mikhovska V, Tolevska C, Maneva L
Institute of Radiotherapy and Oncology, Skopje, Macedonia

Objective. To evaluate the results of treatment and toxicity of two chemotherapy regimens, COPP and COPP alternating with ABVD in advanced stage Hodgkin’s disease (HD). Methods. One hundred and eighteen patients
Hodgkin’s disease ranges from 0.5–2% per year. Only a few reports of acute lymphoblastic leukaemia as a second malignancy have been published. We report the clinical course of a female patient with acute lymphoblastic leukaemia (ALL/L3) occurring 12 years later. In 10/81 HD (nodular sclerosis) female (53.4%) and 131 pts (46.6%) were males. By stages 43 (15.3%) patients (pts) with HD, diagnosed and treated in Fundeni Hospital between 1970-1999, with a follow-up period until 1999. Their distribution by clinical stage (CS) was: I =14%; II=31%, III=44.2%, IV=12%. Pts were treated initially with chemotherapy (MOPP=95, ABVD=35, ABVMP=10) alone, with or radiotherapy (95) according to CS, and ABVD (21) in MOPP relapsed pts. The 18 yr survival rate was 50% for CS I and II, while it was 50% for CS III and IV. Second malignancies were diagnosed in 8 pts (5.4%), 1-13 (median 7 yrs) after the end of HD treatment. At HD diagnosis those pts were 17-58 (Median 40) yrs old, with III B in 4, II in 13 (1 B, 2 A) pts and I B in 1 pts. Acute myeloblastic leukaemia was diagnosed in 2 pts: 1. FAB M4 type in pt treated only with MOPP (10 cycles) after 5 yrs; 2. FAB M3 in pt treated with ABVD (3 cycles) and radiotherapy (mantle field and intrathoracic field, 30 GY) 1 yr after. All other second malignancies were seen in pts treated with MOPP and radiotherapy, in radiotherapy-involved-field: non-Hodgkin’s lymphoma ventriculi (1 pt, after 9 yrs), solid cancers: pancreas (2 pts, after 4 and 1.5 yrs), lung (1 pt, after 13 yrs), kidney (1 pt, after 12 yrs), breast (1 pt-after 9 yrs). Successful treatment of second malignancies with remissions were in AML-M3 (4 yrs), non-Hodgkin’s lymphoma (4 yrs), and breast carcinoma (1 yr). Our results confirm that CS is a bad factor for second malignancies in HD: advanced stage, advanced CS, irradiation, leukaeogenic influence of MOPP, but also ABVD and radiotherapy combination.

The analysis and characterisation of bad prognostic factors could be helpful when designing special treatment strategies for these patients.
PO-0661 Echocardiographic findings in long-time survivors of Hodgkin's disease

Nasibov O, Pivnik A, Sotnikov V, Shevelev A, Medvedev P, Margolin O Research Centre for Haematology, Research Centre for Diagnostic and *Surgery, Moscow, Russia

Purpose. To elucidate the late cardiotoxicity after curative treatment of patients with Hodgkin's disease (HD). Design and methods. 80 patients under 45 months (60 females, 20 males) were treated by mantle radiotherapy and chemotherapy (6-12 courses COPP/CHOP and MOPP) from 1973 to 1995. They were examined by echocardiography (ECHO) 5-20 years after the completion of the treatment. The median age was 42.7 yrs (range 21-66) at the time of the study. We determined the relation of revealed ECHO cardiac abnormalities to known cardiovascular risk factors. Results. Resting left ventricular ejection fraction was normal in all our patients. Vascular thickening was recorded in 23 patients (4 aortic, 9 mitral, 10 aortic+mitral) (29%), prominent changes (grade 3) in 3 patients (4%). Prolapse of the mitral valve with slight regurgitation was found in 18% of no risk factors were identified including the parameters of chemotherapy and radiation therapy. Conclusions. The late cardiac sequelae after treatment for HD assessed by ECHO appear to be minimal. A relatively frequent finding is a thickening of the aortic and or mitral valves, mostly of a minor degree and without significant disturbance of valvular function.

PO-0662 Interleukin 10 basal serum levels correlate with clinical features and outcome of patients with Hodgkin's disease


The aim of this study was to measure pretreatment serum IL-10 levels in adult HD patients (pts) and to correlate them with clinical and laboratory features and with outcome. IL-4 and IL-10 are cytokines produced by Th1-type, whereas IL-2 and interferon γ are produced by Th2 type lymphocytes. The major antineoplastic activity is played by host's Th1 type cells. A shift from Th1-type cytokines toward Th2-type is considered as an evidence and a possible cause of cancer progression. Recent studies suggested that IL-10 could be involved in the cell-mediated immunosuppression and a possible cause of cancer progression. Recent studies suggested that IL-10 could be involved in the cell-mediated immunosuppression in HD by inhibiting the IL-2 secretion. We evaluated pre-treatment IL-10 serum levels in 35 untreated pts. IL-10 level was measured by ELISA test specific for cellular protein. Pts were treated uniformly according to the stage of the disease. IL-10 was detected in 24/35 (68.57%) pts and in 2/20 (10%) of healthy volunteers (p<0.001). The mean ± SD concentration of IL-10 was significantly higher (p<0.001) in HD pts (5.53±5.02 pg/mL) than in healthy controls (4.75±4.7 pg/mL). The mean serum level of IL-10 was associated with several prognostic factors. IL-10 level was higher in pts with an advanced Ann Arbor stage (p<0.001), bulky disease (p<0.001), B symptoms (p<0.01). In addition serum levels of IL-10 also revealed direct linear correlation with erythrocyte sedimentation rate (r=0.688, p<0.001), C-reactive protein (r=0.738, p<0.001) and an inverse linear correlation with serum albumin (r=0.649, p<0.001), hemoglobin (r=0.623, p<0.001), and lymphocyte count (r=0.41, p<0.05). No significant differences between patients and either controls. At the HSP70-2-linked locus, despite a strong association of the two genotypes of each locus were similar between patients and either controls. As a separate group, the patients with the nodular sclerosing subtype of HD showed a higher frequency of the 183 bp allele, which was not associated with histological subtype.

PO-0663 Primary high dose chemotherapy for Hodgkin's disease

Bergzwa WR, Mooldy D, Ruff P, Hoffman L University of Witwatersrand, Johannesburg, South Africa

Although Hodgkin's disease (HD) is regarded as a curable malignancy a significant number of patients either relapse after initial remission or fail to achieve such remission. Identified poor prognostic factors include disease bulk, age, low hemoglobin concentration, lymphopenia and low serum albumin at presentation. The outcome following treatment has also been shown to be significantly poorer in black patients with HD. The use of high dose chemotherapy (HDCh) as initial treatment for patients with poor prognostic HD may obviate some of these adverse influences. Thirty-six patients with advanced poor prognosis HD were treated with high dose melphalan 140 mg/m² and VP16 1.5 g/m² given after either 0 or 1 cycle of ABVD. Haematologic rescue was with marrow (4 patients) or G-CSF stimulated PSC (32 patients). Thirty four of 36 patients achieved CR (20 following 1 cycle of treatment), one patient had PR and 1 patient had refractory disease (overall CR rate 94%). Six patients received a single HDC treatment course. Among 6 who had 1 cycle HDC there were 3 recurrences (at 18, 22 and 25 months). Thirty patients received double HDC with the second course administered at a median of 5.4 weeks after the first HDC. Among patients receiving double HDC the CR rate was 100%. At a median follow up time of 42 months there have been no recurrences among patients receiving primary double HDC. Primary HDC may be a suitable initial treatment option for poor prognosis HD.

PO-0664 Positron emission tomography with 18-F-fluorodeoxyglucose in the staging and follow-up of lymphoma in the chest

Bargjeter M,* Kotzerke J, Grieshammer M, Reske SW, Bergmann L. *Institute of Internal Medicine III and Nuclear Medicine, University of Ulm, Germany

To define accurate spread of disease in patients with lymphoma and to differentiate responders from non-responders are major problems. The role of Gallium-67 scintigraphy in staging remains controversial, because sensitivity is decreased especially in the abdomen. Specificity is also limited by uptake in normal tissue such as lung hila. The following retrospective study was undertaken to evaluate the accuracy of positron emission tomography (PET) with 18-F-fluorodeoxyglucose (FDG) in predicting lymphomatous involvement in the hilar and mediastinal regions in the staging and follow-up of lymphoma patients. One hundred and forty-seven Petoracic PET studies before and after treatment in 89 lymphoma patients were reviewed. Results of FDG-PET were compared to results of CT and clinical follow-up examinations. Forty-five patients suffered from Hodgkin's disease (HD), 43 from non-Hodgkin's lymphoma. Patients received 270 Mbiq FDG (mean) i.v. Eighty-nine of 147 (60%) PET studies showed no FDG uptake in the hilar or mediastinal regions. Eighty-eight (60%) PET studies showed FDG uptake. In 52 of 89 (59%) abnormal studies there was lymphoma in the hilar or mediastinal regions. In the remaining six abnormal PET studies, FDG uptake was considered as benign. In four patients benign FDG uptake was before treatment, in two patients after treatment. In two patients benign FDG uptake after therapy was caused by thymus hyperplasia. The remaining four cases of benign FDG uptake remain unresolved. Sensitivity of FDG-PET was 96%, specificity 94%, positive predictive value 90%, and negative predictive value 98%, respectively. The present study suggests that PET-FDG has potential value in identifying lymphomatous involvement in the hilar and mediastinal regions. PET can accurately assess disease activity and differentiate patients who respond to treatment from non-responders.

PO-0665 Tumour necrosis factor and heat shock protein 70-2 polymorphisms in Hodgkin's disease

Doran MT, Burnett AK, Poynton CH* Dept of Haematology, University of Wales College of Medicine, Cardiff, UK.

Hodgkin's disease was the first disease found to be associated with the HLA system. A number of studies have shown associations with HLA class I and class II polymorphisms. In this study, we examined two HLA class III polymorphisms in 119 patients with Hodgkin's disease, 121 adult controls, and 204 newborns. The bi-allelic polymorphism of the tumour necrosis factor B (Tnfb) locus was analysed by PCR-RFLP using NcoI and the polymorphism of a microsatellite locus linked to the heat shock protein 70-2 (Hsp70-2) locus by simple PCR analysis. The overall distributions of the three genotypes of each locus were similar between patients and either control group. Similar to the studies on non-Hodgkin's lymphoma, allele and genotype frequencies of the 183 bp locus were not different between patients and controls. At the Hsp70-2 linked locus, despite a strong association with non-Hodgkin's lymphoma of the commoner 183 bp allele, no difference between patients (87.3%) and control groups (81.9% in newborns and 87.6% in adults) was noted. There was no significant deviation in the frequencies of the four possible 183 bp Hsp70-2 haplotypes between patients and controls. As a separate group, the patients with the nodular sclerosis type (n=70) did not show any difference from the whole group. This study did not provide any evidence for an isolated association of the two telemic class III loci in Hodgkin's disease, but these polymorphisms may be useful in examining haplotypic associations in a larger segment of the HLA complex.

PO-0666 Significance of positron emission tomography in follow-up of patients with lymphomas

Ilyin LV, Lengyel Zs, Galuska L, Szakal J Jr, Sz Trón L,* Dept Haematol Univ Teach Hosp Markuszovszky, *Szombathely and *PET Centre Univ Med School, Debrecen, Hungary

Positron emission tomography (PET) using radiolabeled glucose analog fluorine-18-deoxyglucose (FDG) is considered to be highly specific for detection of active tumour tissue before and of residual mass after therapy of non-Hodgkin's lymphoma of the commoner 183bp allele, no difference between patients (87.3%) and control groups (81.9% in newborns and 87.6% in adults) was noted. There was no significant deviation in the frequencies of the four possible 183 bp Hsp70-2 haplotypes between patients and controls. As a separate group, the patients with the nodular sclerosis type (n=70) did not show any difference from the whole group. This study did not provide any evidence for an isolated association of the two telemic class III loci in Hodgkin's disease, but these polymorphisms may be useful in examining haplotypic associations in a larger segment of the HLA complex.
The purpose of this study was to assess the diagnostic value of FDG-PET compared with other methods in monitoring the response to therapy in lymphomas patients. The histological subtype: Nodular Sclerosis (NS) 15/41 (42%), Mixed Cellularity (MC) 12/41 (33%), Lymphocyte Predominant (LP) 5/41 (14%) and Lymphocyte Depleted (LD) 4/41 (11%). LP and LD were more frequent in the elderly patients. Stage I and II disease was present in 19/41 (46%), while 22/41 pts. (54%) were in advanced stage III and IV disease. There was a preponderance of stage IV in the group of patients over 60 yrs. (p<0.01). Elderly patients had a significantly higher frequency of B symptoms (p<0.01). The presence of B symptoms was significantly less common in the group of elderly patients (p<0.01). CR was achieved in 25/41 (61%) pts, PR in 3/41 (7%) and no response in 7/41 (17%). There was a significantly lower group of patients aged <60 (p=0.02). Within a median follow up period of 81 months, 7/25 patients relapsed 10/25 remained in CR. The relapse rate in the elderly was not significantly different from that in the group of patients aged <60. There were recurrences to first line chemotherapy; they had all previously been treated with conventional chemotherapy. Median age was 28 years (range 21-56), male/female ratio was 0.36 (8/21). Eighteen patients received salvage chemotherapy consisting of 3 cycles of IFN regimen (fludarabine 2500 mg/mq days 1-3, etoposide 150 mg/mq days 2-5, male/female ratio was 0.41 (8/21). Eighteen patients received salvage chemotherapy consisting of 3 cycles of IFN regimen (fludarabine 2500 mg/mq days 1-3, etoposide 150 mg/mq days 2-5). The others 11 patients underwent 3 cycles of BEAM regimen, thereafter they were stimulated with granulocyte colony-stimulating factor (G-CSF) to collect peripheral blood progenitor cells by apheresis. The latter 11 patients received a conditioning regimen (BEAM) followed by peripheral blood stem cell transplantation (PBSCT). Results. Of the 18 patients treated with salvage chemotherapy, 8 (44%) are alive (5 in CR and 3 with disease), 9 (50%) died of disease progression and 1 of non-haematologic toxicity. After a median follow-up of 24 months (range 3-73 months), overall survival (OS), relapse free survival (RFS) and, event free survival (EFS) are 18%, 44%, 22% respectively, in the elderly patients who underwent autograft, autograft (90%) are alive (7 in CR, 3 with disease) and 1 patient has died of progressive disease. After a median follow-up of 29 months (range 3-73), OS, RFS, EFS are 91%, 71% and 56% respectively. Haematologic and non-haematologic toxicity were acceptable in both groups of patients. Conclusions. In accordance with the literature, our results confirm the data that patients with relapsed or resistant HD achieve CR with a higher probability (p<0.001) if treated with high dose therapy and autograft. Moreover, this group of patients have a significantly better OS (p<0.04) and EFS (p=0.04). However, the optimal timing of this procedure remains an open question whose solution deserves prospective randomised study.

PO-0667 Salvage chemotherapy vs high-dose therapy and autograft for recurrent or refractory Hodgkin's disease (HD) patients


Objective. Despite progress in the cure rate of HD, patients with a brief duration of the first complete remission (CR) or resistant to first-line chemotherapy still have a poor outcome. Many studies suggest that these patients might have a better outcome if treated with high dose therapy and bone marrow autograft. In an attempt to confirm these data, we retrospectively reviewed data from 29 patients with HD in first relapse or refractory to first line therapy. Design and Methods. Of 29 patients, 14 were in first relapse with a CR lasting less than 36 months (range 2-36 months), 15 patients were refractory to first line chemotherapy; they had all previously been treated with conventional chemotherapy. Median age was 28 years (range 21-56), male/female ratio was 0.36 (8/21). Eighteen patients received salvage chemotherapy consisting of 3 cycles of IFN regimen (fludarabine 2500 mg/mq days 1-3, etoposide 150 mg/mq days 1-5). The others 11 patients underwent 3 cycles of BEAM regimen, thereafter they were stimulated with granulocyte colony-stimulating factor (G-CSF) to collect peripheral blood progenitor cells by apheresis. The latter 11 patients received a conditioning regimen (BEAM) followed by peripheral blood stem cell transplantation (PBSCT). Results. Of the 18 patients treated with salvage chemotherapy, 8 (44%) are alive (5 in CR and 3 with disease), 9 (50%) died of disease progression and 1 of non-haematologic toxicity. After a median follow-up of 24 months (range 3-73 months), overall survival (OS), relapse free survival (RFS) and, event free survival (EFS) are 18%, 44%, 22% respectively. In the 11 patients who underwent autograft, autograft (90%) are alive (7 in CR, 3 with disease) and 1 patient has died of progressive disease. After a median follow-up of 29 months (range 3-73), OS, RFS, EFS are 91%, 71% and 56% respectively. Haematologic and non-haematologic toxicity were acceptable in both groups of patients. Conclusions. In accordance with the literature, our results confirm the data that patients with relapsed or resistant HD achieve CR with a higher probability (p<0.001) if treated with high dose therapy and autograft; moreover, this group of patients have a significantly better OS (p<0.04) and EFS (p=0.04). However, the optimal timing of this procedure remains an open question whose solution deserves prospective randomised study.
Hodgkin’s disease

phamide is substituted for methotrexate, a major toxic agent. Design and Methods. Seventy-three patients (37M; 36F; median age: 35 y) diagnosed of nodular sclerosis (stage III/IV, b, bulky disease, HIV+), adenopathies or ESR ≥ 40 mm h−1 were treated with C-MOPP/ABV during a 6-yr period. The histologic distribution was: 2 patients (3%) LF, 47 (64%) NS, 18 (25%) MC, and 1 (1%) LD. Treatment consisted of 8 courses of C-MOPP/ABV (cyclophosphamide 650 mg/m2 IV day 1, vincristine 3 mg/m2 IV day 1, procarbazine 100 mg/m2 PO days 1 to 7, prednison 40 mg/m2 IV day 1 to 4, adriamycin 35 mg/m2 IV day 8, bleomycin 10 mg/m2 IV day 8 and vinblastine 6 mg/m2 IV B) administered every 4 weeks. Radiotherapy (RT) was given in patients with initial bulky disease and in those with residual masses. Results. Sixty-five patients (90%) received 8 courses of C-MOPP/ABV, with 49 of them (70%) receiving the full prescribed doses. After chemotherapy, 57 patients (78%) achieved CR, 10 (14%) PR, whereas in 4 (5%) treatment failure was observed. Seven PR patients with residual masses achieved CR after complementary RT, the overall CR rate being 88%. After a median follow-up of 3 years, 12 patients have relapsed, with a 4 yr FS of 66% (95% CI: 54%-78%). Six patients have died, 2 due to sepsis during chemotherapy, and 4 because of progression. 4-yr overall survival was 92% (95% CI: 86%-98%). Grades 3 and 4 neutropenia were seen in 57 cycles (14%). Seventy-three febrile episodes were recorded, 29 of them requiring hospitalisation. Older age (>60 yrs) and bone marrow involvement were factors related to severe infection (p<0.05 in both cases). The most frequent non-haematological side-effect was paresthesia. At the time of this report, with a median follow-up of 3 months, no late toxicity has been encountered. Conclusions. C-MOPP/ABV induces prolonged CRs in an high proportion of poor-risk HD patients, with acceptable toxicity.

Salvage chemotherapy with mini-BEAM for relapsed or refractory Hodgkin’s disease

Department of Haematology, Hospital “La Paz” Madrid, Spain

Objective. Although Hodgkin’s disease (HD) is curable in many patients, 20% to 50% of patients either fail to enter remission or relapse after a complete response. The best therapy for this group is not defined. The purpose of this study was to evaluate our experience with the mini-BEAM regimen as salvage therapy in patients with relapsed or refractory HD who were potential candidates for a BMT. Design and Methods. From September 92 to June 98 twenty-four patients received mini-BEAM for resistance or relapse of their HD. Three patients had obtained no response with initial chemotherapy (refractory), eleven had obtained an incomplete response, and the remaining nine patients had previously been exposed to a median of one salvage regimen. Most of the patients received three cycles of mini-BEAM treatment. Results. The overall response to mini-BEAM was 79% with partial remission and complete remission rates of 16% and 84% respectively. A total of eighteen patients proceeded to intensive therapy, and ABMT. We observed no significant differences whether this regimen was given to the refractory or initial therapy, to those who were partial responders or to those who had relapsed. No difference in response was observed between patients treated as first salvage and those who had failed to respond to a previous salvage therapy. In the patients with no response to mini-BEAM, the response rate to other salvage therapy was 20%. No treatment-related deaths were observed. Conclusions. The mini-BEAM regimen is a safe and effective form of salvage therapy in HD. In our series it produced a high response with a high complete remission rate.

Common “pre-tumoral” cells for Hodgkin’s disease and acute lymphoblastic leukaemia

Paulova B,a Hopfinger G,a Vesely M,b Mihlberger H,b Hirschl J,a Hanak H,b Pittermann E,a Herz R,b *Ludwig Boltzmann Institute for Leukaemia Research and Haematology; †3rd Med. Dept. and ‡Dept. of Pathology, Hanusch Hospital, Dept. Of Pathology, Jakob Ertheim Institute, Lainz Hospital, Vienna, Austria

Objective. In order to investigate the biology of tumour cell development and the differentiation potential of populations of pre-tumour cells in a case of acute lymphoblastic leukaemia (ALL/L3) occurring 12 years after Hodgkin’s disease (HD) or an HD patient, we established a PBL from the blood of a patient who had a potential for activation of further differentiation towards H/S or ALL/L3 cells. The presence of EBV may provide the basis for tumorigenic activity of a common progenitor cell for both HD and ALL/L3 cells.

Comparison of two prognostic systems - V. Diehl and J.M. Andrieu’s systems - among 180 homogeneously treated patients with Hodgkin’s disease


We treated 180 patients (pts) - 106 males and 74 females - aged between 15 and 75 years (median 35.5) by 3 courses of ABVD or ABVD-like regimen before an “adapted” irradiation according to the H 81 and H 90 trials. Clinical stages were 99 I+IIA, 24 I+IIB, 31 IIIA, 31 IIIB and 17 IV. We noted 2 toxic deaths, 13 failures and 165 CR (92%) and, with a 9.5 yrs median follow-up, 20 relapses and 33 deaths of which 2 were due to ARDS; 5 myocardial infarction, 1 CVA, 3 second tumors, 2 NHL and 1 AML. The 12 yr survival rate was 77 ±4% with 2 later deaths (NHL and myocardial infarction at the 168th & 169th month). In opposite DFS curve shows a plateau after the 89th month at 79 ±3% level. Among the 7 prognostic factors identified by Diehl et al. (J Natl Cancer Inst 1993; 1506-14), six and age are without prognostic value for DFS and value of stage IV is borderline (p=0.07). The Cox model keeps anaemia, hyperleukocytosis and lymphopenia. This system can define 3 prognostic groups: 93 pts with score ≤ 1, 68 pts with score 2 or 3 and 17 pts with score ≥ 4. DFS rates are 88, 70 and 65% respectively (p=0.003). The same computation among the 88, advanced stages as defined by Diehl keeps anaemia and age in multivari- ate analysis but DFS rates of the 3 prognostic groups are not statistically different: 76% (30 pts), 65% (52 pts) and 63% (16 pts). Andrieu’s system (Ann Oncol 1998; 9:195-203) is based on 3 parameters: number of involved area according to Ann Arbor system: 1 or 2 vs 3 or 4 vs ≥ 5; mediastinal mass ratio: to 0.32 vs ≥ 0.33 to 0.44 vs ≥ 0.45; number of involved viscera: 0 vs 1 vs ≥ 2. Thus we can identify 3 groups, well-balanced size wise but with very different prognosis when looking at DFS (p=0.01) and at 12-year overall survival (p=0.10):

<table>
<thead>
<tr>
<th>Tumour mass</th>
<th>N. pts.</th>
<th>DFS</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>64 pts (≤36%)</td>
<td>98±2%</td>
<td>95±3%</td>
</tr>
<tr>
<td>Middle</td>
<td>67 pts (≥37%)</td>
<td>79±6%</td>
<td>81±6%</td>
</tr>
<tr>
<td>High</td>
<td>49 pts (≥27%)</td>
<td>53±7%</td>
<td>58±7%</td>
</tr>
</tbody>
</table>

This system is very effective among the 98 DIEHL’s ‘advanced’ stages for DFS and survival (p=0.001 and .005) and also effective among the 133 Hill stages (p=0.002 for DFS and .005 for survival) while the EORTC system is only significant for DFS (p=0.02).

Do biological markers add to prediction of outcome achieved by the international score in Hodgkin’s disease?

Axdorph U, Landgren O, Grimfors G, Sjöberg J, Porwit-MacDonald A, Björkholm M
*Department of Medicine, Division of Haematology and °Department of Pathology, Karolinska Hospital and Institute, Stockholm, Sweden

Background. Factors predictive of progression-free and overall survival following multistage chemotherapy in advanced stage Hodgkin’s disease (HD) have recently been established by an international prognostic factor study. This prognostic score is also relevant for HD patients with limited disease. However, the discriminatory prognostic power of this score does not allow identification of a group of patients who are at very poor outcome who may benefit from aggressive upfront treatment. Objective. To define biological factors which may add to the international score in predicting outcome of previously untreated patients with HD. Design and Methods. One
hundred and forty-nine patients (>15 years; 84 males, 65 females) with HD diagnosed 1974-1992 were included. Their median age was 36 years (range 14-87); stage I (n=68), stage II (n=48), stage III (n=33) and stage IV (n=30). Fifty-eight patients had 8 symptoms. Histology was lymphocyte predominance in 12, nodular sclerosis in 78, mixed cellularity in 53, lymphocyte depletion in 1 and unclassified in 5. Seventy-three patients were treated with radiotherapy alone, 57 with MOPP/ABVD or similar chemotherapy and 19 with combined modality treatment. Median follow up for surviving patients was 143 months (range 28-272). Apart from factors included in the international score, routine chemistry, serum levels of sCD4, sCD8, sCD25, sCD30, sCD54, interleukin (IL-10, β3-microglobulin and thymidine-kinase were analysed as well as spontaneous and concanavalin A induced blood lymphocyte DNA synthesis (lymphocyte function). Results. The following variables significantly predicted cause specific survival in univariate analysis; serum sCD30, lymphocyte function, hemoglobin, albumin (p<0.001); lymphocyte count, serum IL-10, age (p<0.01); stage (p<0.05). Conclusions. Serum levels of sCD30 and IL-10 and lymphocyte function were the strongest biological predictors of prognosis. Multivariate analysis of these and clinical prognostic factors will be presented.

PO-0675 Molecular epidemiology of lymphoma: role of oncogenes and human cytokines

Hassan HT, Murray PG
Division of Biomedical Sciences, School of Health Sciences, University of Wolverhampton, England, UK

The recent sophisticated molecular biological techniques including the PCR-based tests: long-distance PCR, in situ PCR, microsatellite PCR the FISH-based tests: Fibre FISH, FICTION, multiplex FISH, the comparative genomic hybridisation (CGH) and the oligonucleotide ligation assay (OLA) used to identify genetic mutations, amplifications and deletions in lymphoma, provide reliable valuable assays for use in human mutational surveillance of the risk of lymphoma. Molecular epidemiological analyses of the changes in cell cycle control genes (p16, p21, p27 cycl-1, CDK4), apoptosis-related genes (Mcl-1, bcl-2, MDM2), transcription factor genes (REL, bcl-6, c-myc) tumour suppressor genes (p53, ATM) observed in lymphoma, in populations with varying exposure to environmental causes would allow the identification of putative risk factors. Several recent reports have shown bcl-2 translocation similar to that seen in follicular lymphoma in B-cells from some healthy individuals. Age, male gender, cigarette smoking and exposure to radon showed a trend towards higher bcl-2 translocation frequency. Since B-cell lymphomagenesis involves successive oncogenic steps (1), comprensive large studies of the existence and frequency of these genetic changes and clinical prognostic factors will be presented.

PO-0076 Interferon-α in the treatment of hairy cell leukaemia variant

Lepekov SV, Volkova MA
Cancer Research Centre, Moscow, Russia

Hairy cell leukaemia -variant (HCL-V) is a rare form of HCL which is considered unresponsive to INF-α treatment. Among 61 patients (pts) with HCL who have been treated since 1987 in our Cancer Research Centre we had 8 pts with HCL-V, 7 males and 1 female, age range 35-85 (median 48) years. WBS count in this group was 11-40 x 10^9/L (median 29 x 10^9/L) platelet count 29-83 x 10^9/L. Hairy cells (HC) in bone and blood marom amounted to 90% and more. Splenomegally was observed in every case, and splenectomy was performed in 8 pts. After operation a high count of WBC and high percent of HC persisted. After splenectomy 5 pts. received 2-DCF 4 mg/m2 every other week. After 4-5 injections complete remission (CR) was achieved in 3 patient and partial remission (PR) in 4 pts. Only 1 patient was in durable CR after 2-CDF treatment. As we no more 2-DCF and 2-CD (Cadiodrine) is not registered in Russia, we had to use INF-α for the treatment of 3 previously untreated pts and 4 pts with relapse of HCL-V after 2-DCF treatment. The therapy with INF-α 3 MU daily during 1-2 months was ineffective. After escalation of dose of INF-α up to 6-9 MU daily there was a minimal response during 3 months of therapy. We continued the treatment with these doses, and after 12 months of therapy we received CR in 3 pts and PR in 3 pts. Only 1 patient was unresponsive. The duration of remissions at the moment is 6-12 months. Thus INF-α can be used for the treatment of HCL-V, but the duration of the treatment should be not less than 12 months.

PO-0677 Flow cytometric immunophenotyping in B-chronic lymphocytic leukemia - correlation between bone marrow and peripheral blood samples

Kardum MM, Siftar Z, Nazor A, Kardum-Skelin I,* Jakasic O. * Maric-Basic K,* Pleger-Mesic Z, jakasic B* Institute of Clinical Chemistry and *Department of Medicine, University Hospital “Merkur”, Zagreb, Croatia

Objective. Results of flow cytometric immunophenotyping of peripheral blood (PB) and bone marrow (BM) samples were compared in a series of B-CLL patients to establish whether they match at a sufficiently acceptable level for routine diagnostic purposes. Design and Methods. PB and BM samples of 138 consecutive B-CLL patients (66 females 72 males, median age 61.5 years) were analysed by flow cytometric method on Coulter Epics XL Primary panel of MoAbs used for diagnosis of B-CLL consisted of CD19, CD20, CD5+B+ly, CD23 together with the expression of either κ or λ-light chain on B-cells, amended with CD22-CD24 and HLA DR determination. Results. As expected, the majority of cases expressed monoclonal κ-light chains (840/9 K positive related to 33% λ positive). Mean expression and probability of difference in paired samples is shown on the table, along with correlation coefficients:

<table>
<thead>
<tr>
<th>MoAbs</th>
<th>CD19</th>
<th>CD20</th>
<th>CD22</th>
<th>CD23</th>
<th>CD45</th>
<th>HLA DR</th>
<th>κ</th>
<th>A</th>
<th>CDS</th>
<th>D5</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>85.0</td>
<td>82.0</td>
<td>78.1</td>
<td>25.3</td>
<td>60.9</td>
<td>70.0</td>
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We confirmed that expression tended to be higher in the BM compartment, but the mean was statistically significantly higher level only for light chains. A highly significant correlation (p<0.0001) was observed between individual samples with high respective correlation coefficients. Factor analysis performed on 12 variables disclosed two factors at a significant level. The first factor had a significant (r>0.7) associated with CD19, 20, 23, 24, 5 and CD5+B, HLA DR, while the second factor is associated only with the expression of light chains. Only CD22 did not correlate with either factor 1 or 2, so that its role in routine diagnosis remains to be elucidated. Conclusions. This study has confirmed the high correlation of routine MoAb expression in PB and BM. Addition of more markers listed, did not improve the diagnostic process. However, for research of adhesion molecules which are expected to be responsible for tumour cell re-circulation and distribution pattern, simultaneous evaluation of PB, BM and lymph node compartments may be essential and, therefore warranted.
PO-0678 Successful treatment of pure red cell aplasia associated with B chronic lymphocytic leukaemia with fludarabine

Grigor E, Serra S, Real E, Pastor E
Department of Haematology. Hospital Lluis Alcanyís, Xativa, Spain

A variety of treatments including prednisolone, cyclophosphamide, and cytotoxic chemotherapy are sometimes effective in inducing remission of Pure Red Cell Aplasia (PRCA) in patients with B Chronic Lymphocytic Leukaemia (CLL). Fludarabine itself can induce severe PRCA and haemolytic anaemia. We successfully treated a patient with B CLL-associated PRCA with fludarabine. A 56-year-old man had stage C CLL in August 1997 with lymphocyte count of 5700/μl. (mature CD5 CD19 lymphocytes). haemoglobin 9.5 g/dl, reticulocytes count 0% and platelets 159,000×10^3/L. He had disseminated lymphadenopathy. Bone marrow examination showed interstitial infiltration by small lymphocytes and a total absence of red cells precursors despite adequate myelopoiesis. He was given chlorambucil, 2 courses of CVP (cyclophosphamide, vincristine and prednisone), 3 courses of CHOP(cyclophosphamide, doxorubicin, vincristine and prednisone), and cyclosporin A (5 mg/kg) with prednisone, without success. Since diagnosis he has been dependent on red blood cell transfusions, two units every 2 weeks. Fludarabine was started in May 1998, and was given intravenously 5 days every month (25 mg/m^2 per day) for 5 months. The last transfusion was done after the first cycle of fludarabine on May 11, 1998. The reticulocyte count increased progressively and reached normal values after the second course of fludarabine. Haemoglobin increased to 10 g/dl, and 14.9 g/dl after the third and fourth course respectively. Two months after the last course of fludarabine, the patient is in remission from PRCA without any manifestations of CLL. Our case confirms the utility of fludarabine in the treatment of B CLL-associated PRCA. The mechanism of this success is unknown, but it may be caused by a decrease in the T cells population involved in the suppression of erythropoiesis. We believe that fludarabine can be an effective and safe therapeutic approach, at least in refractory cases.

PO-0679 Clinico-biological features of B-cell chronic lymphocytic leukaemia (CLL) expressing the B29 protein of B-cell receptor (CD79b)

Malika S, Levato D, Dottolo A, Lentini M
Divisione Ematologia-Azienda Ospedaliera "Pugliese-Ciaccio"
Catanza, Italy

In order to get more information on the clinico-biological features of "immunologically typical" (i.e., CD5+ CD23-) B-cell chronic lymphocytic leukaemia (CLL) expressing the B29 protein of B-cell receptor (CD79b), we analysed, by flow cytometry, 69 consecutive and previously untreated patients with a panel of monoclonal antibodies (Mo Abs) which included CD19, CD20, CD22, CD3, CD7, CD23, CD10, CD4, CD8, kappa and lambda chain surface immunoglobulins (Smig). Eleven out of 69 (16.9%) patients expressed CD79b (Smig) in more than 30% of CD19+ positive cells. When patients were stratified into two subgroups on the basis of expression of CD79b, no differences could be found with respect to main clinical features such as clinical stage (P =0.974), histopathologic pattern (LDT) (P= 0.495), and absolute peripheral blood (PB) lymphocytosis (112 vs. 88; p=0.0002). The same applied when correlations were performed by radioimmunoassay using recombinant TNF ("Sigma", LDL=0.67 ng/ml) and murine fibroblast cell line L929 as a target. TNF activity in peripheral blood of 15 healthy donors served as a control. Our investigations showed that plasma TNF level was significantly increased (1.203±0.160 ng/ml) in chronic lymphocytic leukaemia (CLL) contrary to Hodgkin's disease (HD) and non-Hodgkin's lymphomas (NHL). TNF production by blood mononuclear cells differed more appreciably in LDL; the highest est concentrations were detected in supernatants of patients with acute lymphoblastic leukaemia and high grade NHL (0.158±0.027 mg/l and 0.143±0.054 mg/l). Immunoreactivity to low normal levels in HD, low-intermediate grade NHL. Moreover TNF activity in lymphoid tissues was notably higher in cells supernatants from extirpated primary lymphoma affected spleen (0.116±0.036 mg/l), high grade NHL (0.114±0.014 mg/l) and HD (0.092±0.016 mg/l), especially in comparison with low grade NHL, inflammatory or metastatic lymphophones. Our studies revealed that ratio between TNF production in lymph nodes and its plasma level was considerably higher in intermediate-high grade NHL (0.085±0.017) than in low grade NHL, HD or non-specific inflammation of lymph nodes. On the other hand relationships between TNF production by blood mononuclear cells and TNF plasma content were similarly low in HD and NHL of different grades in comparison with CLL (0.264±0.074) and particularly with healthy donors (0.633±0.035). Thus the data so obtained may be evidence of some relationship between TNF production and clinical course of certain LPD, especially those with aggressive behaviour.

PO-0680 Correlation between blood lymphocyte count and spontaneous apoptosis in chronic lymphocytic leukaemia

Kapic M, Boskovic D, Bumbarevic V
*Institute of Histology, Medical School, University of Belgrade
**Institute of Haematology, Clinical Centre of Serbia, Belgrade, Yugoslavia

Chronic lymphocytic leukaemia (CLL) is slowly evolving disease that behaves clinically as though it involves the accumulation of long-lived cells rather than expansion of actively proliferating cells. Promotion of cellular survival and the induction of cell death are closely related, but separated activities. The mechanism for the progression of CLL in more advanced clinical stages
PO-0683 Evaluation of minimal residual disease in patients with hairy cell leukaemia after treatment with 2-chlorodeoxyadenosine

Tomaszewska A, Dwilewicz-Trojaczek J
The Medical University of Warsaw, Warsaw, Poland

Hairy cell leukaemia (HCL) is a rare chronic lymphoproliferative disorder (about 2% of all leukaemias) of B cell origin. The hallmark of this disease is 2-Chlorodeoxyadenosine (2-CDA). In some patients complete remission of HCL after treatment with 2-CDA minimal residual disease (MRD) can be detected. The purpose of this study was the presentation of results of treatment with 2-CDA in HCL patients treated in Department of Haematology, Oncology and Internal Medicine of the Medical University of Warsaw between 1994-1998. The study comprised 49 patients (41 men, 8 women) aged 24-76 years (median 54). 2-CDA was given intravenously as a single dose of 0.15 mg/kg d in 2-hour infusion for 5 days. Twelve courses of chemotherapy were administered (in 6 patients, 2 courses; in 2 patients, 3 courses; in 1 patient, 4 courses). The diagnosis was based on clinical, morphological and histological features, TRAP presence and by immunophenotyping of peripheral blood and bone marrow sections using a panel of monoclonal antibodies against antigens: CD19, CD25, CD103, HLA, DR, HCL, FMC7. The same investigations has been performed in all patients every six months since completing active treatment. All 49 patients achieved complete remission according to conventional criteria (absence of hairy cells in the peripheral blood and bone marrow biopsy specimens, normalisation of peripheral blood counts and 20%, respectively, of bone marrow cells). Positive clinical response of pts with normalisation of glycosylation level of nuclear proteins was observed in 20% of patients.

PO-0685 Glycosylation of leukocyte nuclear proteins in leukaemias

Bybakov LA, Golota ZN, Samuskevich IG
Russian Research Institute of Haematology and Transfusiology, St.Petersburg, Russia

Studies on glycosylation of nuclear glycoproteins (GNP): histones (H), non-histone proteins (NHP), which take part in regulation of functional activity of chromatin were carried out in 23 healthy subjects, 45 patients (pts) with CML, 21 with CLL before and after chemotherapy. Biochemical and radiological methods were used. Metabolic labelling of cells was performed using H-[^3H]glucosamine. It was found that in healthy persons incorporation of label into nuclear proteins of myeloid cells was higher than of lymphoid ones. Specific activity (SA) of histones in granulocytes was 2 times higher than that into total NHP. In pts with CML and in healthy subjects this tendency was noted, but in CML pts the highest SA was observed. However, the level of incorporation of H-glucosamine into transcriptionally active HMG-14 and HMG-17 was decreased in CML and CLL by 15% and 20%, respectively, in comparison with healthy controls. Positive clinical response of pts with normalisation of glycosylation level of nuclear proteins in leucocytes. Results of studies show the presence of differences in the rate of glycosylation in leukaemic and normal leukocytes. Results could be used in the evaluation of the effectiveness of treatment.

PO-0686 Second neoplasms in patients treated with interferon-α-2b for hairy cell leukaemia

Gaman GS, Gaman A
Haematology Clinic, University of Medicine, Craiova, Romania

The introduction of IFN-α has improved response and survival rates in HCL. Because of reports of the development of second neoplasm in patients who had received IFN-α for HCL, the incidence of secondary tumors was determined in a cohort of patients with HCL treated with IFN-α. Methods. Our study comprises 49 patients with HCL treated with IFN-α-2b (Intron A) during 5 years (1993-1998); available histologic studies on the secondary neoplasms. Results. The observed number of secondary neoplasms was compared with the expected number using average annual cancer incidence rates but also with a sample of 92 patients with HCL who never received IFN. A second neoplasm was identified in 13 of the 49 patients (22.4%), at a median of 84 months after IFN-α treatment. Adenocarcinoma was diagnosed in 5 patient, haematopoietic neoplasms in 3 patients and in the other 2 cases epithelial neoplasm. One pt. developed myeloblastic leukaemia and another 2 developed non-Hodgkin's lymphomas (intermediate malignancy). The number of malignancies was expected to be 4, resulting in an excess frequency of 6.33. The only significant difference between patients with a secondary neoplasm and those without was age at diagnosis and the starting of IFN-α therapy. Total IFN-α dose and duration of treatment with IFN did not have a significant influence on the overall or the secondary survival. From the studied patients 22% died during the treatment with IFN-α: 11% from complications related to the disease (other than neoplasias) and 11% of a second neoplasm (average life

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time =7.7 months). Conclusions. This study shows an increase incidence of second neoplasms in patients with HCL treated with IFN-α 2b compared to average annual cancer incidence rates and versus the sample of conventionally treated pts. (8.93%); this incriminates IFN and the natural evolution of the disease, probably related to the prolongation of the mean life-time as a result of IFN therapy.

PO-0687 Expression of thrombospondin receptor (CD36) in B-cell chronic lymphocytic leukaemia as an indicator of tumour cell dissemination

Rumi C, Rutella S, Sica S, Leone G
Dept. of Haematology, Catholic University School of Medicine, Rome, Italy

We investigated the expression of CD36 on B-cell chronic lymphocytic leukaemia (CLL) and correlated these results with laboratory and clinical features. CD36 could be detected on 3% (range 2-5) of normal CD19+ B-lymphocytes and on 45% (30-75) of neoplastic CD19+B-cells. When CLL patients were arbitrarily stratified according to CD36 staining intensity (weak – score 0, moderate – score 1, intermediate – score 2 or strong – score 3), significantly higher Ho level platelet count and significantly lower lymphocyte counts were recorded in patients assigned to score 0 compared to patients scoring 1-2 or 3. CLL patients expressing CD36 at intermediate-to-strong intensity were more frequently assigned to Rai stages III-IV than stages I-II (p=0.005) and stage 0 (p=0.001). Interestingly, bone marrow - BM-diffuse histology was strongly associated with higher CD36 expression (relative fluorescence intensity - MFI = 8.7; 4.7-15.9) compared to non-diffuse patterns of BM infiltration (MFI = 6.7; 5.2-9.3; p=0.0019). When the significance and independence of potential predictor variables with respect to BM histology (ie. percentage of CD19+ CD36+ leukemic B-cells, CD36 fluorescence intensity, PB lymphocytosis, Ho level and Ptt count) were evaluated in multivariate regression analysis, none of these covariables significantly altered the association between diffuse pattern of BM infiltration and brighter CD36 staining intensities (p=0.033).

In conclusion, the present study provides the first evidence of CD36 expression on CD19+B-cells from CLL; the correlations with clinical parameters strongly support the view that CD36 might favour tumour cell spreading. Whether high CD36 expression levels identify an aggressive disease subset remains to be confirmed in larger series of patients.

PO-0688 Trisomy 12 is a poor prognostic factor in atypical chronic lymphocytic leukaemia

Howe D*, Bromidge T,* Ewings M,* Phillips M,* Rule S,* Johnson S,* Leukaemia Research Unit and *Department of Haematology, Taunton and Somerset Hospital, Musgrove Park, Taunton, UK

Aim. To study the prognostic impact of trisomy 12 (+12) in groups of patients with carefully classified sub-types of CLL. Excluding CLL-FL, Methods. the presence of +12 was determined using fluorescence in situ hybridisation on peripheral blood smears from 125 patients who were then classified using strict immunophenotypic and morphological criteria as described previously (1). Clinical staging was according to the Binet system and treatment decisions were made without prior knowledge of the +12 status of the patients. Kaplan-Meier plots were calculated to examine overall survival and the length of time between diagnosis and treatment. Results and Discussion. A total of 91/125 patients were found to have +12 of these 4/96 were typical CLL and 5/29 atypical CLL. Trisomy 12 did not affect the overall survival of the CLL patients (p=0.61) but it did significantly reduce the time between diagnosis and first treatment (p=0.02). Classification of patients into atypical and typical CLL revealed that this finding remained significant only, in the atypical group (p=0.03). The time from diagnosis to treatment of patients without trisomy 12 was not significantly different for atypical or typical CLL (p=0.56); +12 therefore appears significantly different for atypical or typical CLL (p=0.56), +12 therefore appears remaining significant only, in the atypical group (p=0.03). The time between diagnosis and first treatment (p=0.02).

These results confirm our previous finding that the "normal" T cells of patients with B-CLL are dysfunctional. Reduced expression of the CD4 antigen suggests an inability to respond effectively both to and during antigen presentation as CD4 plays a vital role in strengthening the antigen induced signal through the TCR. Whist TCR expression is not significantly downregulated, reduced CD4 could affect the ability of the former antigen/receptor to function efficiently. Failure to adequately express CD25, CD28 and CD152 implies reduced capacity to activate both surrounding T-cells as well as other key immune responders. Failure of this normal T-cell compartment to recognise and react to the malignant B-cell may be of importance in the pathogenesis of B-CLL and its associated auto-immune disorders.

**PO-0691** Campath-1h in T-prolymphocytic leukaemia (T-PLL)

Czepiel B, Wetterwald M, Bonnevie F, Cambier N, Taillefer MF, Joutet JP, Bauters F

Service des Maladies du Sang, Hôpital Huriez, CHU Lille, France

T-PLL is a rare and severe lymphoproliferative disease resistant to conventional chemotherapy. We report on 7 patients with T-PLL treated with human CD52 antibody, CAMPATH-1H supplied for compassionate use by Glaxo Wellcome and Ilex from April 97 to January 99. The 5 males and 2 females, aged from 52 to 77 (median 64) years had a diagnosis of T-PLL confirmed by morphology, immunophenotypic profile and cytogenetics. All 7 received treatment for their disease before CAMPATH, one with chlorambucil and CHOP, all 7 with pentostatin (3 or 8 cycles, median 5) with poor response (1 PR, 6 SD). All 5 had CD52 positive tumour cells in the blood. CAMPATH-1H was delivered 3 times a week by iv infusion with a maximal daily dose of 80 mg (median 30). Total dose ranged from 410 to 1160 mg (median 650) during 4 to 8 weeks. Results: Three pts entered in CR. One remains in CR 6 months from CAMPATH. One pt in first CR was achieved in March 1998. Of B-CLL.

**PO-0692** Expression of 44 kDa non-histone protein in the lymphocytes of B-cell chronic lymphocytic leukaemia

Chrusciel J, Niewiadomska H, Bronisz J, Robak T, Kilianka ZM

*Dept of Cytobiochemistry, University of Lodz; Dept of Otolaryngology and Dept of Haematology, Medical University of Lodz, Lodz, Poland

As was shown by our previous studies non-histone protein with a mol.wt of 44 kDa is present in SDS-PAGE profiles of nuclear fractions from malignant lymphocytes (B-CLL, PO-ALL, CALL) but not in normal ones. Rabbit polyclonal antisera raised against this electrophoretically specific component was used in immunoblot and immunocytochemical investigations. Using the immunoblot method it was noted that this antisemum reacted with a 44 kDa antigen of nuclear fraction from most studied B-CLL lymphocyte preparations (40 of 44). The above antisernun revealed no cross-reactivity with nuclear fraction preparations (11) of normal lymphocytes. P44 antisemum was applied for immunocytochemical staining of lymphocytes from normal peripheral blood and from blood of patients with B-CLL. This antisemun recognised p44 antigen in only malignant cells. The expression of p44 polypeptide was detected in most B-CLL lymphocyte probes (14 of 17 studied). Staining of leukemic lymphocytes with p44 antisemun was observed in 5 to 45% of examined cells. The degree of immunostaining seems to correlate with progression of the disease. Our results suggest that p44 non-histone protein may be considered as a potential biomarker of B-CLL.

**PO-0693** Cladribine - an effective but not curative therapy of hairy cell leukaemia

Raida L,* Papajik T,* Faber E,* Hubáček J,* Heczko M,* Pikalova Z,* Indrak K,* Dusek J*

*Haematology- oncology Department of University Hospital, Olomouc, Czech Republic; *Department of Pathology, Medical School of Palacky University, Olomouc, Czech Republic

Introduction. During the nineties Cladribine (2-chloro-2-deoxyadenosine, 2-CDA) has become established as the treatment of choice in patients suffering from B-hairy cell leukaemia (HCL). Some studies confirmed the impossibility of total eradiation of the malignant clone but the question of clinical disease free survival and an effective treatment of relapses still remains open. Design and Methods. A group of 30 patients (24 males and 6 females) with the median age of 49 years received Cladribine for HCL in the period of 4 years were retrospectively analysed. All patients received one cycle of 2-CDA. The dose of 0.1 mg/kg/day was administered in 7- or 5-day continuous infusion in 7 (23%) and 23 (77%) patients, respectively. The median follow-up of the groups was 34 months at the time of analysis. Results. Complete remission (CR) was achieved in 26 (87%) patients, 3 (10%) partial remission (PR) and no response was registered in 1 (3%) case. Neutropenia was developed or worsened in 26 (87%) cases but no severe infectious complication or haematological toxicity related death occurred. The non-haematological toxicity was minimal. Generalised rash developed in 9 (30%) patients and resolved spontaneously over several days. Five (16%) relapses and 1 (3%) progression from CR occurred at a median of 7 months from CAMPATH. One (3%) relapsed patient died of progression of disease, 3 (10%) received another cycle of 2-CDA and achieved a second complete response. Finally, 2 relapsed patients remained asymptomatic and without development of HCL. HCL therefore has not required any therapeutic intervention. Conclusions. The analysis confirmed the high therapeutic efficacy of Cladribine in HCL. One cycle of 2-CDA can eliminate the huge number of tumour cells, resolve the symptoms of disease and increase the quality of life for a relatively long time despite the fact that even such effective drug cannot cure the disease. Minimal residual disease (MRD) can be identified by special methods and presents a potential source of clinical relapse. There is still, however, the possibility of achieving a second remission with 2-CDA again.

**PO-0694** Expression of the p53 protein in lymphoid malignancies by flow cytometry

Kaczmarek D,* Morilla R,* Gruszka-Westwood A,* Lens D,* Brito-Babapulle V,* Matutes E,* Catovsky D

*Department of Haematology, Medical School, Wroclaw, Poland; *Department of Academic Haematology, RHM Trust, London, UK

The p53 protein plays an important role in determining the fate of cells being involved in both cell cycle arrest and programmed cell death. Aberrant expression of the p53 protein as a result of mutation or other mechanisms is used as a marker of bad prognosis in several neoplasms. Although less common in low-grade lymphomas it seems to bear prognostic significance as it usually correlates with aggressive course of the disease. We studied by flow cytometry peripheral blood from 81 CLL patients and 30 samples from patients with other lymphoproliferative disorders (20 B-NHL, 3 B-PLL, 3 T-PLL, 3 HCL and 1 LGL). To assess reproducibility of the flow cytometry method we analysed frozen samples from 33 patients with various lymphoproliferative disorders which had been previously analysed for the p53 protein expression by immunocytochemistry. Results. Flow cytometry detected p53 protein in 11 out of 81 CLL patients (13.6%). This included seven out of 11 patients diagnosed with typical CLL and 4 out of eight with CLL/PL. A variable proportion of cells (12% to 92%) expressed p53. Only a minority of cases with B-cell NHL were p53 (+) i.e., 2 of the 20 cases. In the group of other lymphomas we found two positive cases out of 6 of lymphoproliferative leukaemias (1 B and 1 T) and the only case with LGL. In the group of frozen samples analysed, we found a good correlation between flow cytometry and immunocytochemistry but the results depended heavily on the good viability of the frozen cells. Conclusions. The incidence of p53 positive CLLs in our study was 13.6%, similarly to that reported by other authors who used immunocytochemistry as a method of detection of the protein. Reproducibility and simplicity of the flow cytometric method make it very useful in determining abberant p53 expression.
To determine the clinical and prognostic significance of these findings.

PO-0695 Treatment of advanced chronic lymphocytic leukaemia with fludarabine
Giraldo P, Palomera L, Moneva JJ, Rubio-Félix D, Pardo M, Rabasa P, Diego P, Mayayo P, MENCHACA C, AMUTO E, HERNÁNDEZ M CAIHN and ASO VASNA cooperative study group, Spain

Purpose: To determine the efficacy of fludarabine (FLD) as second line therapy in refractory chronic lymphocytic leukaemia (CLL). Patients and methods. From January 1996-December 1998, 34 previously treated CLL patients, were included in one arm prospective co-operative study. Treatment schedule: FLD 25 mg/m² daily for 5 iv monthly administered as the only drug. Adjuvant therapies included: corticosteroids, uracil, and cytotoxic and hyperhydration. G-CSF was administered as prophylaxis of infectious state if ANC <0.5-10⁹/L. Response was evaluated after 3 (R3) and 6 (R6) courses of therapy. Results. Mean age 64.7 (range 45-80); M/F ratio 11/21. RA1 stage: in 16 patients and IV in 18. Mean time from diagnosis 59.4 (range 5-186). Response status (ECOG) in 19, 2 in 12 and 3 in 3 previous. Previous therapies: chlorambucil 18; chlorambucil + prednisone 4; chlorambucil + polychemotherapy 12. Number of previous schemes: 1, 17 (50%); 2, 12 (35.3%) and 3 or more 5 (14.7%). For R3, 31 patients were evaluable (CR 3/14.7.8%, PR 26/83.5%; F 2/6.4%). For R6 21 patients were evaluable (CR 7/33.4%; PR 13/61.9%; F 1/4.7%). Toxicity: myelosuppression 4 cases AIHA 2 cases; severe opportunistic infections 6 cases (2 Candida, 2 herpes zoster, 2 staphylococci). The number of previous chemotherapies did not have any influence on the response. Three patients died of different causes unrelated to CLL; two patients were out of study (1 severe myelotoxicity, 1 immune anaemia). For responders the relapse free-time was 13.5 months. Comments. In our experience, fludarabine as a single therapy seems to be effective and a good therapeutic choice for patients with advanced non responsive CLL.

PO-0696 Review of an immunophenotyping B-CELL scoring system.
Milen T, Macey MG, Annes J, Dale C, Clarke R, Newland AC, Cavenagh JD Department of Haematology, Royal London Hospital, London, UK

B cell chronic lymphocytic leukaemia (B-CLL) is the commonest lymphoproliferative disorder (CLD). It is distinct from other types of B cell non hodgkin lymphoma such as mantle cell lymphoma and splenic lymphoma with villous lymphocytes which can present in a leukaemic phase. B-CLL typically expresses the following antigens: CD5+, CD22 weak++, CD23+, FMC7-, SmIg weak+. Each of these antigens scores one point giving a highest possible score of 5/5. By including CD3 and CD19 in this panel of antigens the total number of T and B lymphocytes can be assessed. During 1998 we processed 64 new B-CLL cases (B-CLL score 5/5 or 4/5) and 33 B-NHL (B-CLL score <4/5). All the 64 cases with a score of 4/5 or 5/5 had typical B-CLL morphology. Of the 33 cases scoring <4/5 one were morphologically typical B-CLL. We noted that 1 of the 64 typical B-CLL group was CD3- and that 3 of the 64 typical B-CLL group were FMC7+. In contrast 23/33 of the non B-CLL group were CD23- and 25/33 were FMC7+. The most unreliable antigen in the scoring system in our laboratory was SmIg with 23/64 typical B-CLL group showing a moderate intensity. This contrasted with the other 4 antigens in the scoring system which showed typical reactions for B-CLL in the following number of cases: CD5 63/64, CD22 60/64, CD23 63/64 and FMC7 61/64. Our laboratory found a FITC conjugate essential for the estimation of CD22 (high expression conjugates gave too sensitive a reaction for the scoring system). We conclude that the B-CLL scoring system is an accurate and fast way to assess whether or not a possible B-LPD is B-CLL (requiring no further immunophenotyping) or a B-NHL in leukaemic phase or T-NHL in leukaemia phase requiring further characterisation. Given the relatively low frequency of CD23, B-CLL this group should always go under further analysis to exclude mantle cell lymphoma. Also FMC7+B-CLL appears to be relatively infrequent. These two groups of B-LPD patients require further study to determine the clinical and prognostic significance of these findings.

PO-0697 Mantle cell lymphoma in leukaemic phase: a lymphoproliferative disorder with a broad cytological spectrum
Wong KF, Chan JC, So CC, Yu PH Department of Pathology, Queen Elizabeth Hospital, Hong Kong, China

Objective. Mantle cell lymphoma is a mature, virgin B-cell neoplasm characterised immunologically by a panB+, CD5+, CD23-, cyclin D1+ phenotype, and genetically by t(11;14)(q13;q32) with over-expression of the cyclin D1 (bcl-1) gene. It usually presents as high-stage disease, involving lymph nodes, spleen, bone marrow and extranodal sites, particularly the gastrointestinal tract. However, leukaemic presentation is not common, and can pose significant problems in distinction from other chronic lymphoproliferative disorders. This study aims to characterize the morphological spectrum of leukaemic mantle cell lymphoma. Methods. During the period July 1994 through October 1998, fourteen patients with mantle cell lymphoma in leukaemic phase were diagnosed at the Department of Pathology, Queen Elizabeth Hospital, Hong Kong. The diagnosis of mantle cell lymphoma was based on histological and immunocytochemical findings, and confirmed by cyclin D1 immunoreactivity in all cases. The clinical records and laboratory results were reviewed. Peripheral blood smears, bone marrow and other tissue biopsies were examined, with particular attention to the cytological features of the leukaemic mantle cells. Results. Mantle cell lymphoma in leukaemic phase showed a very aggressive clinical course despite the use of aggressive chemotherapy, with eight patients dying at a mean of 13 months and only one patient being disease-free. The leukaemic mantle cells exhibited a broad morphological spectrum, with several cytological patterns being identified: (1) mixed small and medium-sized cells, (2) predominantly medium-sized cells, (3) predominantly large cells, and (4) giant cells. Despite variations in size and nuclear shape, the leukaemic mantle cells could often be recognised by their nuclear irregularity and clefting, moderately dense but evenly distributed chromatin, small nucleoli, and scanty cytoplasm. Conclusions. Recognition of the characteristic cytological features of leukaemic mantle cells can help in the distinction from other chronic lymphoproliferative disorders. In contrast to the latter, the clinical course of leukaemic mantle cell lymphoma is aggressive and response to conventional chemotherapy is poor.

PO-0698 Telomere length in peripheral blood cells from patients with B-cell chronic lymphocytic leukaemia (B-CLL)
Tsilika A,° Papageorgiou E,° Tsirigotis P,° Dervenoulas J,* Pappa M,* Raptis S* *Second Department of Internal Medicine, Propaedeutic, Evangelismos Hospital, University of Athens; °Fifth Department of Internal Medicine, Evangelismos Hospital, Athens; °School of Biology, University of Athens, Greece

Objective. Somatic cells do not express the enzyme telomerase, hence telomeres progressively shorten with each cell division leading to chromosomal instability and cell senescence. B-CLL is characterised by the accumulation of monoclonal B lymphocytes with an extended life span. The aim of the present study was to determine the mean telomere length (TRL) in peripheral blood from patients with B-CLL and to associate any possible alterations with clinical and laboratory parameters. Design and Methods. DNA from the peripheral blood of 17 patients with B-CLL was digested and transferred by Southern Blotting. The blots were hybridised with a biotinylated probe specific for the telomeric repeats (Telquont, Pharmin- gen). The mean TRL values were calculated by integrating the signal intensity above background over the entire TRL distribution as a function of TRL length. The controls used included an age matched population of healthy volunteers the HL60 and 293 cell lines with short and long telomeres. Results. The mean TRL values in patients with B-CLL overall, did not signifi- cantly differ from an age-matched control population (p=0.483). How- ever the mean TRL of patients with advanced stage disease (Rai III, IV) was significantly shorter than that of age matched controls (p=0.02). Moreover this was also true for patients with stage C according to the Binet staging system (p=0.05). Patients without B symptoms had also shorter telomeres in comparison to those of the control population (p=0.05). Interestingly patients with abnormal liver function tests had also shorter TRL values in comparison to those of the age matched controls (p=0.01). Patients who received treatment had significantly shorter TRL values than controls (p=0.03). Conclusions. The above data indicate that patients with advanced stage CLL (stage III and IV, stage C), abnormal liver function tests and receiving chemotherapy are characterised by the presence of shorter telomeres, suggesting an association with aggressive disease. Shorter telomeres may simply reflect genetic instability predisposing to disease evolution. Analysis of larger number of patients and determination of telomere- erase activity, would help to shed light in the biology of the disease.
The value of clinical and biochemical parameters in the prognosis of chronic myelocytic leukaemia

*Dept of Haematology, *Dept of Paediatrics, Clinical Hospital Centre, Rijeka, Croatia; Institute of Haematology, University of Zagreb, Croatia

Chronic myelocytic leukaemia (CML) is characterised by considerable variability in its course and prognosis. A number of clinical and biochemical parameters have been identified as prognostic factors. The aim of this study was to evaluate the prognostic significance of 20 initial physical and laboratory findings on the overall survival of CML. A total of 205 patients treated at the Department of Haematology, Rijeka and Institute of Haematology, Zagreb between 1983 and 1996 were included in the study. Their median age was 44 years. The male to female ratio was 107:98. The patients were divided in two groups according to the therapy administered: patients (109) treated with busulphan (BU) and those (72) treated with hydroxyurea (HU) An univariate analysis was done. The median follow-up was 31 months. Sex showed no influence on the outcome of the disease. In the group of patients treated with BU age >60 years, marked splenomegaly, thrombocytosis and elevated lactate dehydrogenase were associated with statistically significant shorter survival rate. In the HU group, patients with a high proportion of blast cells in the peripheral blood had a poorer outcome. In both groups of patients low hemoglobin level, basophilia (>5%), >5% of promyelocytes in the peripheral blood, and >5% myeloblasts and promyelocytes in the bone marrow correlated with shorter survival. Our results indicate the prognostic significance of simple, readily available clinical and biochemical parameters in patients with CML.

Poster Discussions Chronic myeloproliferative disorders II

PO-0699

PO-0700 Polycthemia vera: long-term efficacy of pipobroman therapy

Passamonti F, Brunasolino E, *Kersy C, Baratè C, Lazzarino M, Bernasconi C
Institute of Haematology, *Biometry, IRCCS S. Matteo, Pavia, Italy

Objective. We evaluated the efficacy and long-term complications of pipobroman (PB) therapy in 163 patients (pts) affected by polycythaemia vera (PV) and treated from 1975 to 1996 in our institution (median follow-up: 113 mos). Methods. Diagnosis and response evaluation were according to PVSG criteria; 37% of pts were asymptomatic at diagnosis, 32% had neurological problems, 10% cardiovascular symptoms, 31% hematopoietic disorders and 15% thrombocytosis. All pts received phlebotomy at the onset of disease. PB was given at the dose of 1 mg/kg/day until haemolastic response was achieved and of 0.50-0.25 mg/kg/day as maintenance. A minority of pts received busulphan (7%) or hydroxyurea (10%), as well, to maintain the haematologic control. Results. Median age at diagnosis was 56 yrs and M/F ratio 1:5. 70% of pts were alive at the time of this analysis. Pipobroman induced haemolastic responses in 160 pts (98%); the median time to response was 3.5 yrs and maintenance therapy was required in 79% of responders. Median overall survival (OS) was 232 mos, with a standardised mortality ratio of 1.726; age over 65 yrs at diagnosis significantly worsened the OS (p=0.0005). Eleven patients (6.7%) developed acute leukemia (AL) in a median time of 158 mos from diagnosis; incidence of AL was 6/10,000 person-months and cumulative risk was 0.8% at 5 yrs and 3% at 10 yrs (in two cases myelodysplasia preceded AL). Six pts had been given PB alone, four PB and BU, one PB and HU. Univariate analysis indicated female gender and age over 65 yrs as the only significant risk factors for AL; the overall duration of therapy did not significantly influence the risk of AL, while the addition of other drugs to PB showed a trend towards a higher risk. Six pts (4%) developed myelofibrosis (MF) in a median time of 110 mos from diagnosis; the incidence was 3/10,000 person-months and cumulative risk was 4.2% at 10 yrs. Eleven patients (6.7%) developed solid tumors (ST) after a median time of 85 mos from diagnosis; the incidence was 6/10,000 person-months and cumulative risk was 3.6% at 5 yrs and 7.6% at 10 yrs. Univariate analysis failed to demonstrate any significant risk factor for the occurrence of MF or ST. Conclusions. This study confirms the efficacy of PB in the long-term control of PV, with a risk of metastatic neoplasms comparable to that of other drugs. The duration of PB therapy did not significantly influence the risk of subsequent leukaemia.

PO-0701 Plasma homocysteine levels in polycythemia vera

Cattedra di Ematologia, Istituto di Scienze Biomediche, Ospedale San Paolo, and *Laboratorio Analisi Clinica e Microbiologia, Ospedale San Paolo, Milan, Italy

Objective. Elevated homocysteine levels are an independent risk factor for atherosclerotic vascular disease. Since subjects with Polycythemia Vera (PV) have an increased risk of thrombotic events, we investigated plasma homocysteine in PV patients. Design and Methods. Plasma homocysteine was measured in 13 patients with PV, ranging between 41 and 79 years, and 12 healthy controls, matched for sex and age. Patients were selected according to the diagnostic criteria of the Polycythsemia Vera Study Group. All patients underwent chronic treatment with phlebotomy. 9 out of 13 also had aspirin, and only 1 out of 13 with cytodereactive therapy. Plasma homocysteine was determined by FPIA Homocysteine Reactent IMX System (ABBOTT). Results. The mean haematocrit in PV patients was 0.47±0.02 and haemoglobin levels were 15.8±1.1 g/dL. All patients had normal serum folate and cobalamin levels. Plasma homocysteine levels in our patients with PV were significantly higher (23.95±14.35 μmol/L, p=0.006) than in healthy controls (11.13±2.8 μmol/L). There was no statistical relationship between genotype of methylene tetrahydrofolate reductase and homocysteine levels. Seven out of 13 patients had thrombotic events in the remote past. No statistical relationship between homocysteine and age, sex, or the use of aspirin was observed. Conclusions. Our data show that in PV homocysteine levels might contribute to thrombotic risk.

PO-0702 Platelet count above 600x10^9/L is not an absolute requirement for essential thrombocythemia diagnosis

Sacchi S, Vinci G, Rupoli S, Garantini L, Martinelli V, Baravelli S, Lazzarino R, Tinazzi G, Gugliotta L for the Gruppo Italiano Malattie Mieloproliferative Croniche (GIMIC); Modena, Italy

Essential Thrombocythemia (ET) is universally recognised as a distinct entity within chronic myeloproliferative disorders (CMD), evolving with a specific natural history. However, ET still remains a diagnosis of exclusion although a platelet count >600x10^9/L is commonly considered a necessary diagnostic criterion. Here we describe the long-term follow-up of 31 patients, who presented at diagnosis all updated PVSG criteria for ET, but platelet count. Patients. An ongoing, retrospective cohort study of GIMIC enrolled 2316 ET patients diagnosed between 1976 and 1996. Patients not fulfilling all PVSG criteria were excluded from the subsequent analysis. Of these 2316, 68 patients had a platelet count <600x10^9/L; 37 out of 68 were also excluded due to a follow up shorter than 2 years and/or because of treatment with myelosuppressive agents. The remaining 31 patients had a median age of 49 years (range 16-74 years) and 22 were female (70%). At diagnosis the median platelet count was 512±10^9/L (range 394-600±10^9/L). Three patients had thrombotic events before and at diagnosis; no patients had haemorrhagic episodes. Follow-up, After a median follow-up of 4.36 yrs (range 1.29-11 years), none of the 31 patients had a spontaneous decrease of platelet to the normal range. Transformation to a different CMD was never observed and no patients developed a condition known to produce reactive thrombocythemia. During follow-up, 23 patients (74%) were treated with antiplatelet drugs, mainly aspirin. No patients’ disease evolved into an acute leukaemia, 3 had thrombotic events and none had haemorrhagic episodes. Median platelet count during follow-up was 534±10^9/L (range 398-716±10^9/L). Conclusions. Long-term follow-up has documented that our 31 patients were correctly diagnosed, although platelet count was ≤600x10^9/L. Our patients were probably in an early phase of their disease and following PVSG criteria they would have been misdiagnosed, leading to an incomplete recognition of the natural history of ET. Further, because early diagnosis could also be clinically relevant, our results outline the need for new criteria for the diagnosis of ET, in order to avoid the exclusion of patients with a platelet count ≤600x10^9/L.

PO-0703 Intensive treatment of chronic myelogenous leukaemia (CML) in accelerated and blastic phase (AP/ BP)

*Department of Medicine, Karolinska Hospital and Institute, Stockholm, Sweden

In 1989 the Swedish CML-Group started treating CML pts in AP/BP with intensive induction therapy including autologous stem cell transplantation (ASCT). Pts ≤65 years who reached a second chronic phase (CP2) with no donor were planned to receive high-dose therapy x 2 and ASCT (cells harvested at diagnosis). In pts >65 years and in most pts who had undergone Haematologica vol. 84 (EHA4 Abstract Book); June 1999
leukaemic transformation in which we do not detect ras mutations in the one out of 34 patients with ET developed acute leukaemia. No ras mutations results. The HL-60 cell line was used as a negative control and the HCT116 products derived from normal alleles. Sequencing was also used to verify the on was administered for 81 months (median time). DNA was extracted from G, Loukopoulos D Greece

order to determine the utility of EEC assay in the prediction of polycythemia ined in patients presenting with idiopathic marked thrombocytosis (IT) in Chang Gung Memorial Hospital, Chang Gung University, Taipei, Taiwan PO-0705 Endogenous erythroid colony assay can predict outcome of patients with polycythemia vera and essential thrombocytethymia Mavroganis D, Vinious N, Michailly C, Yataganas X, M eteisis, J, Yapopoulos G, Loukopoulos D First Dept of Medicine, Laikon General Hospital, University of Athens, Greece Polycythemia Vera (PV) and essential thrombocythemia (ET) are haematologic diseases which may progress into acute leukaemia. The ras proto-oncogenes encode proteins which function in signal transduction and which acquire transforming properties via activating point mutations in codons 12, 13 and 61. The aim of this study was: 1) the detection of leukaemogenic action of hydroxyurea (HU); and 2) the detection of mutations in codons 12 and 13 of K- and Ras during the course of the disease. Thirty-APF pts hosts with ET and 34 patients with PV were studied. Median time for follow-up peri-od was 67 months for patients with PV and 50 months for patients with ET. Thirty out of 35 patients with PV and 28 out 34 with ET had received therapy with HU. Median time of therapy was 93 months in 13 patients and 26 months in 14 patients with PV. A combination of HU and busulphan was taken by one patient for 44 months. Ten out of 34 ET patients had received HU alone for 68 months (median time) and 18 out of 34 ET patients had received N1 for 35 months (median time). In 5 patients with ET α-interferon was administered for 81 months (median time). DNA was extracted from peripheral blood with the proteinase K/Chloronform method. Ras genes were PCR-amplified using mismatched primers to introduce restriction sites into products derived from normal alleles. Sequencing was also used to verify the results. The HL-60 cell line was used as a negative control and the HCT116 cell line was used as a positive control. Two out of 35 patients with PV and one out of 34 patients with ET developed acute leukaemia. No ras mutations were detected during the early and late chronic phase of the disease. Our results indicate that 1) leukaemogenic risk after treatment with HU is not increased comparing with the results of other studies (6.6% for PV and 3.4% for ET); and 2) PV and ET are clonal disorders with intrinsic potential for leukaemic transformation in which we do not detect ras mutations in the chronic phase of the disease.

PO-0705 Endogenous erythroid colony assay can predict outcome of patients with idiopathic marked thrombocytosis Shih-Li L, Lee-C-T, Dunn P Chang Gung Memorial Hospital, Chang Gung University, Taipei, Taiwan Objective. The ability to form endogenous erythroid colonies (EEC) was exam-ined in patients presenting with idiopathic marked thrombocytosis (IT) in order to determine the utility of EEC assay in the prediction of polycythemia vera (PV) evolution or development of vascular complications. Design and Methods. One hundred patients with IT were evaluated at initial presenta-tion, they all had sustained platelet counts >500 x 10^11/L, without known causes of reactive thrombocythemia or increased red cell mass. The methyl-cellulose semi-solid culture technique was used to assay the EEC formation from bone marrow and peripheral blood. Results. EECs were found in 59

patients of them 32 had PV evolution in a median time of 7 months (group A) and the remaining 26 EEC(+1) patients did not progress to PV in a median follow-up of 34 months (group B). Twenty-three of 34 patients developed PV in a median follow-up of 41 months (group C). Thrombosis and/or haemorrhage occurred in 81%, 77% and 31% of patients in group A, B, and C, respectively. Twenty-three of 32 patients in group A, 17 of 26 patients in group B, and 11 of 42 patients in group C required myelosuppressive ther-aPy during the course. In group B, 7 patients had renal insufficiency with 2 requiring maintenance hemodialysis, 5 had myelofibrosis and moderate splenomegaly with possible hypersplenism, and 2 had transfusion dependent anemia. All these disorders in conjunction with myelosuppressive therapy in most patients might preclude them from PV evolution. No significant difference in platelet count among the three groups was found, and we also failed to find a correlation between vascular complications and platelet count. The vascular complications and the need of chemotherapy were significantly higher in group A than group B and than in group C. The incidence of splenomegaly or ele-vations of leukocyte alkaline phosphatase was also higher in group A than group B and than in group C, but there was no correlation between vascular com-plications and splenomegaly, or LAP score. Conclusions. This study showed that it patients, who form EEC, will likely evolve to PV and are at high risk of vascular complications.

PO-0706 Detection of bcr/abl fusion gene in chronic myeloid leukaemia and other myeloproliferative disorders: detection of a new two colour probe for fluorescence in situ hybridisation Lazareidou A, Mirantisi Ch, Korantzis I, Papaioannou M, Gavrielidis G, Christakis P Dept. of Haematology, "Theagenio" Cancer Centre Thessaloniki, Greece BCR-ABL transcripts, at low level, have been reported in patients with myeloproliferative disorders (MPD) other than chronic myeloid leukaemia (CML), such as essential thrombocythemia (ET) and polycythemia vera (PV). In order to explore this interesting finding further we looked for the bcr/abl fusion gene in bone marrow samples of 53 patients with MPD (CML 28, ET 10, PV 10 and 5 classified 5) and 15 haematologically healthy individuals, using fluorescence in situ hybridisation (FISH). This technique, until recently, had a problem of relatively high false positive rates (FPR) and false negative rates (FNR), but newer refinements have min-imised this. For that purpose, we used a new commercial kit (LSI bcr/abl ES; Wyss, UK) where the spanning abl probe is approximately 650 kb and the abl probe 300 kb. This dual color translocation probe in a nucleus possessing the bcr/abl translocation produces, instead of three, four sig-nals, i.e. one additional smaller orange signal from the residual abl gene on chromosome 9. Standard bcr/abl probes cover approximately 200 kb only of the abl breakpoint area. In our CML group the FNR was reduced to 0.52% (0.1-1.2%) as compared to a mean of 5% (5.1-12.5%) when the standard bcr/abl probe was used. The FPR in the control group was 0.33%, instead of 2.2% (5-8%). Three ET patients had bcr/abl fusion gene signals (in 6%, 9.8% and 14.8% cells). In the other 7 the FPR ranged from 0.3 to 0.8%. None of the metaphases examined was bcr/abl positive. Two patients with PV had very high numbers of bcr/abl signals and the fusion gene was also present in metaphases. In the other 8 patients the FPR ranged from 0.1 to 0.8%. Three unclassified MPD patients had 14.2%, 62%, and 8% cells with positive bcr/abl signal. In CML, such as essential thrombocythemia (ET), and polycythemia vera (PV), the bcr/abl (+), Ph1(+) and bcr/abl (+) Ph1(-) CML and to assess the role of PO-0707 Improvement of cytogenetic response by GM-CSF and interferon-α in CML patients Thiebaud A, Belhaibr A, Charain C, Liu J, Fière D, Michallet M Service d’Hématologie, Hôpital Edouard Herriot, Lyon, France Patients with chronic myelogenous leukaemia (CML) who achieve a major cytogenetic response (MCR) when treated with interferon-α (IFN-α) have a survival advantage compared to patients with no cytogenetic response. For these patients, different strategies of polyclonal expansion thymic and IFN-α therapy alone: 4 females, 5 males, median age of 44 years (25-66). These patients had shown sensitivity to IFN-α by achievement of a haema-
tological or transient cytogenetic response but failed to achieve MCR. At inclusion, 5 patients were 100% Ph+ and 2 had 95% Ph+ and 1 patient 70% Ph+. GM-CSF was given at an initial dose of 0.25 mg/kg and subcu-
taneously, continued over 8 days and secondarily adapted to blood count. INF-α was continued at the same dose and escalated when possible. All patients were evaluable. The median intervals between beginning of INF-
α and start of GM-CSF and between diagnosis and inclusion in the study were 29 months (range 12-48) and 31 months (range 13-51) respectively. Two patients achieved MCR (1 complete and 1 very good partial response: 2% Ph+) and 1 patient remained in partial response (62% Ph+). The medi-
ad dose of INF-α received varied from 3 to 10 MIU/d for 5 to 7 days week-
ly. The dose of GM-CSF was escalated from 11.5 to 470 µg/day. No tox-
icity could be attributed to the addition of GM-CSF.

Addition of GM-CSF to INF-α in CML patients sensitive to INF but who fail to achieve MCR, may improve cytogenetic results obtained by INF-α alone and perhaps survival of CML patients.

PO-0708 Two chronic myelogenous leukaemia patients with micro bcr abl gene (e19-a2 junction)
Haikovcová C, Koza V, Polák J*, Zemanová Z, Michalová H, Cermák J*
*Institute of Haematology and Blood Transfusion, Prague; "Dept. Haema-
tology, Oncol, Faculty Hospital, Pizen; *Third Medical Dept, General Facul-
ty Hospital, Prague, Czech Republic

In most CML patients the BCR/ABL gene resulting from the Philadelphia (Ph) translocation - t(9;22), has breaks in the BCR gene in the major bcr region and the e13 or 14-a2 junction in the BCR/ABL gene. There are a few CML cases published so far that have originally been taken as having a mild course of the disease (also called chronic neutrophilic leukaemia), which have a break in the BCR gene in another region, called micro bcr resulting in e19(c)-a2 junction. Among about two hundred BCR/ABL-positive CML patients tested in our laboratory, there were two with the micro bcr and e19a2 BCR/ABL gene. The e19a2 junction was verified by nested RT-PCR with e19a2a primers in the second round, by positive hybridization with the e 19a2 junctional probe and by sequencing of the PCR Product. Patient A progressed into an accelerated phase of CML in which he was treated by bone marrow transplantations and at present he is in a complete haematological and molecular remission. Patient B, originally diagnosed as Ph+ AML, but evidently a case of myeloid blast crisis of CML (two Ph chromosomes, i(7q), +8, del(7), +19 with a silent chron-
ic phase of the disease) was refractory to chemotherapy and died three months after the diagnosis. In addition these two CML patients show that among the micro bcr CML cases there can also be those in accelerated or blast phase. This study was supported by grants of IGA MHCR 3563-3 and GACR 30298-007.

PO-0709 The profiles of Th and Tc-cell subsets in chronic myelogenous leukaemia
Tadahiro Yamazaki
Division of Clinical Haematology/Immunology, Kumamoto City Hospi-
tal, Kumamoto, Japan

T cell immunity is believed to play an important role in the control of cell growth in chronic myelogenous leukaemia (CML) although information regarding the characteristics of T lymphocytes in CML patients is limited. Using flow cytometric analysis of intracellular cytokine expression at the sin-
gle-cell level, we analysed helper-T and cytotoxic-T cell subsets in six CML patients (12 samples). The percentage of INF-γ single-positive CD4 cells (Th 1) and of IL-4 single-positive CD4 cells (Th 2) were markedly increased in CML compared to in normal controls. In addition, the per-
centage of IFN-γ double positive CD4 cells (Th 0) was also reduced in CML. Consequently, the percentage of IFN-γ/IL-4 double-negative CD4 cells was markedly increased. Similarly, a reduction in IFN-γ single-posi-
tive CD8 cells (Tc1) and an increase in IFN-γ/IL-4 double-negative CD8 cells were observed in CML. Imbalance of all these parameters was improved to a great extent by cytokine therapy. Our findings directly indicate the anergic states in CML patients. Determination of the factors that affect Th and Tc profiles may lead to further understanding of immuno-
ological states and the development of effective immunotherapy.

PO-0710 Is AC 133 selection able to enrich Philadelphia chromosome-negative progenitor cells?
Waller CF, Martens U M, Lange W
Department of Internal Medicine 1, Haematology/Oncology, Albert-Lud-
wigs University, Freiburg Medical Centre, Freiburg, Germany

Objective. PH negative haematopoietic progenitor cells have been shown to be present in bone marrow patients during the early course of CML. The phenotype of these PH+ cells has been previously demonstrated to be CD34+HLA-DR. They can be mobilised into the peripheral blood which offers the potential for autologous transplantation. A new stem cell mark-
er, AC133, has been described recently. AC133 has been shown to be selectively expressed on CD34+ hematopoietic stem cells. In recent years, progenitor cells derived from human fetal liver, bone marrow and blood while other more mature blood cells lack expression of this surface antigen. We have tested the clinical usefulness of this marker for subselection of CD34+ cells with regard to PH positivity. Design. PBPC from 8 patients with CML (7 CP, 1 BC) and 3 controls with malignant disease other than CML without bone marrow involvement were isolated from leucapheresis samples. AC133 enrichment or depletion of CD34+ cells was performed using MACS separa-
tion columns (Miltenyi Biotech, Germany). Different cell fractions were examined for the presence of the PH-chromosome by interphase FISH. Results. It could be demonstrated that neither the CD34+AC133+ nor the CD34+AC133- fraction was enriched by or depleted of PH+ cells. Conclu-
sions. AC133 is not able to distinguish PH+ from PH- PBPC in CML.
PO-0712 The changing profile of Ph+ chronic myeloid leukemia at presentation: possible impact of earlier diagnosis
Hernández-Boluda JC, Cervantes F, Ferrer A, Cid J, Montserrat E
Haematology Department, Hospital Clinic, Barcelona, Spain

Objective. To ascertain whether the initial features of chronic myeloid leukemia (CML) have changed over time, and the possible impact on survival. Design and Methods. The initial features of 167 patients diagnosed as having chronic phase Ph+ CML from 1972 to 1985 were compared for with 174 such patients diagnosed at the same institution from 1985 to 1998. The survival of the two groups was also compared. Results. CML patients diagnosed since 1985 were significantly older at presentation (mean age 47±17 vs 43±17 years, p=0.04), were more often asymptomatic (36% vs 19%, p=0.0013), less often had constitutional symptoms (30% vs 45%, p=0.0034), displayed splenomegaly less frequently (59% vs 75%, p=0.008) and hepatomegaly (35% vs 49%, p=0.01), had less marked leukocytosis (mean WBC count 139±124×10^9/L vs 179±132×10^9/L, p=0.007), with 30% of them showing an initial WBC count below 50×10^9/L (vs 19%, p=0.02), and showed less marrow blast cell infiltration (p=0.003). No significant differences were observed in the distribution of Sokal’s risk groups. Median survival of patients diagnosed since 1985 was 5.3±3 years (95% CI: 4.3–6.36), vs 4.06 years (95% CI: 3.28–4.84) for patients diagnosed before (p=0.07). Finally, a longer survival was observed for patients asymptomatic at diagnosis (median survival: 5.7 years, 95% CI: 4.5–6.9, vs 4.1 years, 95% CI: 3.4–4.7, p=0.03). Conclusions. A substantial proportion of CML patients are currently diagnosed early in the course of the disease. The effect of earlier diagnosis on survival prolongation in such patients should be taken into account.

PO-0713 Ph+ non-Hodgkin’s lymphoma or two malignancies: a problem solved by RT-PCR in situ
Balatzenko G, Guenova M, Ganeva P
National Centre of Haematology and Transfusiology, Sofia, Bulgaria

Objective. A novel method for intracellular detection of bcr-abl transcripts by reverse transcription and polymerase chain reaction (RT-PCR) is reported. This allows simultaneous molecular analysis and morphological evaluation of the blast population. This approach was applied in a case with Philadelphia (Ph+) chromosome and a non-Hodgkin’s lymphoma (NHL). Case report. Mrs. T.P.N, 57 yrs. old was admitted with a histological diagnosis of NHL (diffuse large cell) after chemotherapy. In the course of staging, myeloproliferation in the bone marrow and leukocytosis was found and cyto genetic analysis was performed which appeared Ph+(+). B2a2 transcripts were detected by RT-PCR in peripheral blood, as well as on material from formalin fixed and paraffin-embedded spleen tissue. In order to identify the source of bcr-abl positivity in the spleen precisely and to distinguish a NHL from extramedullary blast crisis of chronic myeloid leukemia (CML), a two-step RT-PCR in situ was performed on 5 μm sections, previously deparaffinised and enzymatically permeabilised. The PCR products were detected by biotinylated primers during the second reaction and revealed by streptavidin-HRP complex and ABC. A red end-product was detected by light microscopy in the cytoplasm of the granulocytic population in the spleen circulation, the lymphoma being negative. Conclusions. This preliminary case report is a rare example of co-occurrence of two malignancies - NHL and CML - distinguished on the basis of RT-PCR in situ method.

PO-0714 Major thrombotic complications in adults younger than 40 years with essential thrombocythaemia
Randi ML, Rossi C, Fabris F, *Giroliami A
Dept. of Medical and Surgical Science, II. Chair of Internal Medicine and *Chair of Medical Semeiotics, University of Padua Medical School, Padua, Italy

Although ET is primarily a disorder of middle age, it is also now often observed in young adults and the real risk of thrombosis in these patients has not yet been clearly established. We report the results of a prospective study in 68 patients (28 males and 40 females, mean age 30.8±7 years) with ET diagnosed in agreement with the criteria of Polycythemia Vera Study Group over the last 15 years (median follow-up 99.1±4 months). Asymptomatic patients did not receive any treatment, while those with positive atherosclerotic risk factors, microvascular disturbances and/or major thrombotic complications received ASA (100 mg/day). Only patients with a platelet count over 1000×10^9/L or a solitary thrombosis (HU) with the aim of reducing the platelet count to less than 600×10^9/L were included in the study. Ten patients (6 males and 9 females) (22%) had major thrombotic complications: 1) life threatening thromboses are quite rare in young people with ET 2) most thrombotic complications occur at the time of diagnosis and 3) ASA seems to be an effective and safe antithrombotic agent reducing not only microvascular disturbances but also thrombosis and re-thrombosis.

PO-0715 P190 bcr-abl transcripts in chronic myeloid leukemia: possible correlation with disease evolution
Balatzenko G, Guenova M, Arnaudov G, Galabova I, Ganeva P, Avarova D
National Centre of Haematology and Transfusiology, Sofia, Bulgaria

Objective. There is a controversy concerning the incidence and clinical significance of the p190 bcr-abl transcript, resulting in the production of a 190 kD protein (P190) in chronic myeloid leukemia (CML) patients. The available data varied from lack of p190 transcripts to a common finding in almost all patients at the time of diagnosis. The aim of this study was to determine the occurrence of p190 in CML patients and its possible relationship with the clinical course. Design and Methods. Multiplex reverse transcription polymerase chain reaction (RT-PCR) for M-BCR-ABL and M-BCR-ABL (sensitivity 10^-1) was performed to study the splice types in 38 CML patients both in chronic phase (CP) (n=33) and advanced stages (AS); accelerated phase (n=2) and blast crisis (n=3). Results. All studied CML patients were M-BCR-ABL(+); b3a2 (n=22); b2a2 (n=13) and b3a2/b2a2 (n=3). Expression of P190 bcr-abl transcripts in addition to P210 was found in 21 patients in CP (6.3%), while in the group of AS patients 13/33 transcripts were identified in 23 (50%) (p<0.05). In 2 cases the initial testing revealed only P 210 transcripts, the p190 being negative. Conclusions. The simultaneous expression of P210 and P190 bcr-abl transcripts, mainly in patients in AS-CML, as well as the absence of p190 at the time of diagnosis and their appearance during the clinical course support the hypothesis that expression of p190 correlates with the progression of the disease.

PO-0716 Serum β2-microglobulin, TNF-α and interleukins in myeloproliferative disorders
Bourantas KL, Hatzinicola E, Makis A, Chaidos A, Kapalis E, Tsirata S, Karalis P, Christou L, Mavridis A
Haematology Unit, Department of Internal Medicine, University of Ioannina Medical School and *Microbiology Laboratory, Ioannina General Hospital (Chatzikosta G.), Ioannina, Greece

Objective. Whereas β2-microglobulin (β2M) has mainly been used as a prognostic factor in patients with lymphoproliferative disorders, there are few data on its value in myeloproliferative disorders (MPD). In order to investigate a potential role in the pathogenesis of MPD and to find a possible value as indicators in monitoring the course of the disease, we measured β2M and TNF-α, IL-1α, IL-1β, IL-2, IL-6, IL-10 and soluble IL-2 receptors in MPD patients at diagnosis and during the course of the disease. Design and Methods. Fifty-five patients were included in the study (28 males and 22 females, mean age 62.9±10.7). Ten patients had polycythemia vera, fifteen had chronic myelogenous leukaemia, ten had myelofibrosis and twelve had essential thrombocythaemia. All patients underwent hydroxyurea and/or interferon-α2b treatment. We measured β2M, using rate nephelometry. Commercial enzyme immunoassay kits were used for the quantitative measurement of cytokines. Using the Mann-Whitney U-test, values detected in the patients were compared to those obtained from 14 healthy controls. The Wilcoxon signed rank test was used for the evaluation of change in β2M and interleukins during the course of the disease. Results & Conclusions. In progressive disease and particularly when transformation to acute leukaemia occurred, high levels of β2M, IL-2 and sIL-2R were found in all patients; the elevation was progressive which suggests a potential usefulness in the individual patient.

PO-0717 Venous thromboembolism in patients with polycythemia vera and essential thrombocythaemia is not associated with factor V Leiden mutation
Department of Haematology, S.Bortolo Hospital, Vicenza, Italy; Department of Internal Medicine, University of Padua Medical School and Department of Laboratory Medicine, Division of Molecular Biology, University of Vienna, Vienna, Austria

Polycythemia Vera (PV) and Essential Thrombocythaemia (ET) are chronic myeloproliferative disorders characterised by a high incidence of thrombotic complications. Extensive studies of plasma coagulation and the
platelet system failed to demonstrate a consistent pattern of abnormali-
ties associated with thrombosis. Recently, a poor anticoagulant response to
activated platelet factor 4 (PF4) has been identified as the most frequent hereditary disorder associated with
venous thrombophila. We investigated, in 304 patients with PV and ET, whether the presence of the PV Leiden mutation could be a risk factor for
thrombosis, before, at diagnosis and during follow-up. PV Leiden was found in
14/304 patients (4.6%); in 5/18 patients with venous thromboem-
bolism (VTE) before diagnosis (28.72%, odds ratio 11.3), in 1/20 (5%) and
1/23 (4.3%) patients with VTE at diagnosis and during follow-up (odds
ration, 1.2 and 0.73 respectively). The frequency of VFE Leiden in patients
with and without arterial thrombosis was similar (5/79, 6.3% and 9/225, 4%,
respectively, p=0.367). This study indicates that the prevalence of the
PV Leiden mutation in patients with PV and ET is comparable with that
observed in the general population; furthermore, PV Leiden mutation is not
an independent and additional risk factor for VTE and AT thrombosis in this
setting. Diagnosis and dual antiplatelet therapy as it acts as a risk factor
diagnosis, as in the normal population.

PO-0718 Spontaneous megakaryocyte colony formation in patients with
essential thrombocythaemia successfully treated with anagre-
lide
Gotic M, Basara N, Rolovic Z, Sefér D, Jankovic G, Elezovic I, Bogdànovic A, Boskovic D
Institute of Haematology, Clinical Centre of Serbia, Belgrade, Yugoslavia
It has been demonstrated that spontaneous megakaryocyte colony for-
mation (CFU-Mk) occurs in a large proportion of patients (pts) with
essential thrombocythaemia (ET). Anagrelide is a new platelet-lowering
agent which has been shown to be efficacious in the control of thrombo-
 cytosis in pts with ET. In our study we applied the in vitro culture assay in
pts with ET successfully treated with anagrelide who achieved long lasting
hematological remission. According to our knowledge there has been no
such a study in pts with ET treated with anagrelide. We enrolled eleven pts
into present study: 9 with ET and 2 with polycythemia rubra vera (PRV)
with thrombocytopathy. They were diagnosed and treated according to an
open protocol for the use of anagrelide for pts with thrombocythaemia pro-
posed by Roberts Pharmaceuticals. The median age at the initiation of anagrelide treatment in these
pts was 69.5 years (range 46-87). The median platelet count prior to anagrelide was 1130×10^9/L (range
800-2550). All eleven pts achieved complete haematological response. The
median maintenance dose of anagrelide was 2.5 mg/d. The median
duration of the treatment was 45 months (range 30-68). The median
platelet count was 370×10^9/L (range 240-570) at the time of in vitro
culture. In vitro culture assays of spontaneous megakaryocyte colony
(CFU-Mk) and erythroid (BFU-E) progenitors were done according to the methods that we have
previously published (1). Our results showed that spontaneous CFU-
Mk were seen in bone marrow cultures in 3/9 pts with ET and in 1/2 pts
with PRV who were in stable haematological response induced by anagre-
lide. Spontaneous BFU-E were seen in bone marrow cultures in 6/9 pts with
ET and 2/2 pts with PRV who were in stable haematological response
induced by anagrelide. Further investigation of megakaryocyte number, size
and ploidy and correlation with CFU-Mk in bone marrow cultures are need-
ed to evaluate the significance of our findings.

PO-0719 Haematological and cytogenetic efficiency of single interferon
alpha 2b therapy and combined interferon alpha 2b plus low
 dose cytarabine as a first-line treatment in chronic myeloid
leukaemia
Tóthová E, Kraúglajic A, Nandic V, Lazarrevic V, Jankovic G, Colovic M
Institute of Haematology, Clinical Centre of Serbia, Belgrade, Yugoslavia
The aim of the study was to analyse the clinical features of patients with blast crisis in chronic myeloid leukaemia (BC-CML), and to evaluate their karyo-
typic (cytogenetic profiles) and response to therapy in various types of
blast crisis in CML. The study included the selection of 39 pts with
BC-CML, out of whom 29 pts had megakaryoblast crisis, 9 had lymphoblast cri-
is and 1 had biphrenoblast crisis. Cytogenetic analysis was performed by
the G-banding technique, and immunophenotypic investigation was carried out
from flowcytometry. Thirty-nine pts were investigated; 24 M 15 F, median age 44
years. Mean Hb value 73 g/L, WBC 84×10^9/L, platelet count 63×10^11/L. Cytogenetic analysis: 16 pts had classical (19;22), 2 pts had complex (19;22),
7 had additional structural and numerical aberrations and 9 pts had no
abnormalities. The blast crisis with the most frequent karyotypic
abnormalities was in 3 pts. A significantly higher prevalence of hyperploidy was found in pts with
megakaryoblast crisis (57%). Immunophenotypic investigation: 
myeloblast crisis in 17 pts (HLA-DR, CD34, CD13, CD33, CD11 b+,
myelomonoblast crisis in 4 pts (HLA-DR, CD13, CD34+, megakary-
oblast crisis in 7 pts (HLA-DR, CD34, CD13, CD33, CD61+), and erythroblastic cri-
is in 1 patient (GPA, CD17, CD36, CD33, CD61+). Nine patients had 8-limp-
phoblast crisis (HLA-DR, CD34, CD19, CD10)+, out of whom 2 patients
manifested aberrant expression of CD13+. One pt had a hybrid variant of BC-
CML (HLA-DR, CD34, CD38, CD19, CD22, CD10, CD13, CD33)+. The
patients were treated according to a conventional chemotherapeutic proto-
col. The median survival of the entire group was 4 mos (1-26), while the pts with
megakaryoblast crises had significantly longer survival - mean 8.3 mos. Longer survival of pts with megakaryoblast crises in CML was probably due to
the absence of haemotrophic complications of chemotherapy and possibly due
to the favorable effect of hyperploidy in the course of the blast crisis. These
results deserve further study in larger cohorts of pts.

PO-0721 Monoclonal gammopathies (MG) in myeloproliferative
 disorders (MPDs)
Randi ML Rossi C, Luzzago G, Girolami A
Dept. of Medical and Surgical Science, II Chair of Internal Medicine,
University of Padua medical School, Padua, Italy
Both MPDs and multiple myeloma (MM) are regarded as clonal disorders of
hemopoietic stem cells, expressed as uncontrolled proliferation of
myeloid progenitors and mature-appearing plasma cells respectively. The
simultaneous presentation of both disorders in the same patient is con-
sidered to be rare. The retrospective analysis of our cohort of patients with
MPDs (438 cases, 183 males and 247 females, mean age 54.75±1.61
years) allowed us to detect 12 patients with MG (2.8%): 1 male with MM
together with polyclonal gammopathies of unknown significance (MGUS).
At the time of development of MG, 4 patients were younger than 55, 4 were
between 55 and 70 years and 2 were older than 70, representing respec-
tively 1.8%, 2.7% and 2% of all MPDs patients of the same age range. In
4 patients the diagnosis of MG was contemporary to that of MPD while in
the remaining 8 patients the diagnosis of MG was made 6.5±1.7 years after
that of MPD. Nine patients developed the MG when the diagnosis for
the MPD was performed while 2 subjects received radiophosphorus and 1
hydroxyurea. The incidence of MG in MPDs seems to be slightly higher than
in the general population (0.9-1.25%), in particular in subjects over
50 years of age (1.5%). No relation seems to exist between the occurrence of
MG and the treatment performed for the MPD. We surmise that the high-
er incidence of MG in MPDs could be secondary to the strict haematolog-
ical control performed in such subjects.

Chronic myeloproliferative disorders II

Haematologica vol. 84 (EHA-4 Abstract Book); June 1999
Between 1988-1998 we followed up 668 cases of chronic myeloid leukaemia (CML); out of these cases 65 received aIFN in low doses (3 MU x 3/week) and a total of 15 cases in high doses (10-20 mg/m² per day) in courses of 7-10 days/month (mo). Twenty-nine cases were evaluable: 12 males and 17 females with a median age of 39 years (25-62). Clinical, haematological, cytological and cytogenetical data established that 16 patients were in chronic phase (CP), 7 in late chronic phase (LCP) and 6 in accelerated phase (AP). Prognostic estimation was performed using the Sokal score responses were recorded in 50% of low risk cases, 40% in intermediate risk and 33% in high risk responses. According to the Synthesis model responses were correlated subsequently with the risk groups and survival. According to the Sokal score responses were recorded in 50% of low risk cases, 40% in intermediate risk and 33% in high risk responses. According to the Synthesis model the following responses were registered: st.I = 50%, st.II = 28% and st.III = 28%. The patients were divided into two groups: A: st.i + CHR + Hy = 70% CHR + 40% cytogenetic responses out of which 11% major responses; IFN + Hy + CHR = 85% CHR + 28% cytogenetic responses with 14% major responses. The cytogenetic responses were correlated subsequently with the risk groups and survival. According to the Sokal score responses were recorded in 50% of low risk cases, 40% in intermediate risk and 33% in high risk responses. According to the Synthesis model the following responses were registered: st.i = 50%, st.II-III = 44% and st.IV = 0%.

**PO-0723 Low dose interferon-α-2a therapy in treatment of chronic myeloid leukaemia**

Gaman GD, Gaman A. Haematology Clinic, University of Medicine, Craiova, Romania

Recombinant human interferon-α (IFN-α) is the treatment of choice for patients with CML. Although low doses of IFN-α are generally used, they are associated with significant toxicity. Our work contained a prospective study of 21 patients with CML, who received low doses of IFN-α. Complete remission, median survival and degree of toxicity were evaluated. Methods: We included 26 patients with newly diagnosed CML in chronic phase, and 5 patients in advanced phase. Therapy consisted of IFN-α-2a (Roferon) in a daily dose of 2 x 10⁶ units/m² body surface area for 30 days, and then 3 times/week. Haematological remission and toxicity were evaluated monthly (physical examination, complete blood counts). Bone marrow cytogenetic analysis were undertaken to assess karyotypic response in patients with complete haematological remission. Survival curves and median survival values were also determined. We also studied a group of 11 patients treated with hydroxyurea, and 1 group consisting of 49 patients who received high dose IFN-α-2a. Results were compared. During 20 months of diagnosis, 80% of the patients achieved complete haematological response, with 18% having a major karyotypic response. Kaplan-Meier estimated 5-year survival rate was 78%. Approximate annual cost of low dose IFN therapy is 4 times lower than with high dose IFN-α-2a. Conclusions. Using low doses of IFN-α-2a in the treatment of CML may be a good strategy, the rate of complete haematological remission was nearly the rate of that when using high doses of IFN-α (80-70%). The rate of cytogenetic response was also near the rate we found in our study (16-30%). The lower cost and minimal toxicity (p<0.001) support the use of this low dose therapy.

**PO-0724 Treatment of chronic myelogenous leukaemia with a combination of interferon-α-2a and chemotherapy**


Forty-two outpatients with early chronic phase of chronic myelogenous leukaemia were included in the CML-MRG-97 Protocol (Moscow Research Group - 97) and randomised to either interferon-α-2b alone (Intron-A, Schering Plough, 5 MU/m² per day) or to a combination of Intron-A with 10-15 days courses of cytosine-arabinoside (Ara-C 10 mg/m² per day) monthly. Median follow-up was 11 months (3-20 months). Complete haematological remission was achieved in 74% of cases, cytogenetic response in 74% (major in 28.6% of cases). The majority of patients (20) belonged to low, 11 to intermediate and 11 to high risk groups (Sokal’s criteria, 1987). The treatment effectiveness was equal in both regimens and did not depend upon risk group. A significant decrease of the P<0.05 cells percentage allow us to predict improvements in future therapeutic regimens. Our previous experience of low IFN-α doses (1-3 MU/m² per day) in 66 patients in combination with chemotherapy (monotherapy or polychemotherapy) showed good tolerance of these regimens and a significant increase of patients survival as compared with the control group treated with CT only, especially in patients with the poor prognosis. According to this protocol, patients with poor prognosis receive repeated courses of polychemotherapy as the induction therapy with subsequent standard IFN-α dose injections. The authors express their gratitude to representatives of “Schering Plough” firm in Russia for sponsoring our research work.

**PO-0725 Initial characteristics associated with long-term survival in chronic myelogenous leukaemia (CML)**

Mantzourani M, Stavrouyianni N, Vinious N, Belesi C, Kyriazopoulos P, Sagriotis A, Loukopoulous D, Yataganas X. First Dept. of Medicine, University of Athens, Greece.

The purpose of this retrospective analysis was to identify the initial characteristics associated with long-term survival in a series of 53 adult Philadelphia-positive CML patients with very long follow-up (median duration of chronic phase: 69 months, median overall survival: 73 months). Clinical and haematological laboratory data at diagnosis before treatment were available in all 53 patients; 25/53 patients carried a b3a2 and 28/53 patients a b2a2 bcr-abl chimaeric transcript. A multivariate analysis by the Cox proportional hazard model was performed in order to ascertain possible associations between demographic data, clinical and haematological parameters/treatment with interferon-α (IFN-α) and type of chimaeric transcript and duration of CP and OS. The median duration of OS of patients treated with IFN-α was 89 months (confidence interval, CI, 61-117 months), while that of patients who did not receive IFN-α was 70 months (CI: 47-93 months). The results are summarised in the following table.

<table>
<thead>
<tr>
<th>p-values</th>
<th>Chronic phase</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M: 23 F: 30</td>
<td>0.0411 0.1807</td>
</tr>
<tr>
<td>IFN-α</td>
<td>Yes: 21 No: 32</td>
<td>0.0450 0.0165</td>
</tr>
<tr>
<td>Transcription</td>
<td>b₂a₂: 25 b₂a₁: 28</td>
<td>0.2542 0.1560</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean: 53.3 Median: 53.0</td>
<td>0.6213 0.6919</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.9</td>
<td>0.0005 0.0002</td>
</tr>
<tr>
<td>WBC (x 10⁹/L)</td>
<td>101.0 60.0</td>
<td>0.0591 0.0367</td>
</tr>
<tr>
<td>PLT (x 10⁹/L)</td>
<td>444.0 340.0</td>
<td>0.9436 0.7494</td>
</tr>
<tr>
<td>Spleen size (cm)</td>
<td>3.7 2.0</td>
<td>0.0007 0.0002</td>
</tr>
</tbody>
</table>


In conclusion, low haemoglobin, lower WBC counts and larger spleen were identified as significant negative predictive factors. No patients treated with IFN-α was positively associated with better outcome; as in other studies, the type of chimaeric transcript does not seem to affect prognosis.

**PO-0726 Philadelphia chromosome negative chronic myeloid leukaemia without breakpoint cluster region rearrangement: a survey of 5 cases**


Objective. Little information is currently available about patients with Philadelphia chromosome negative and bcr abl negative chronic myeloid leukaemia (CML). The aim of this study was the investigation of the natural history, characteristics at presentation and clinical outcome of a series of 5 patients with typical clinical and haematological features of CML, not showing either t(9;22) or the bcr abl hybrid gene. Design and Methods. From 1992 and 1997, 90 new patients with typical clinical and haematological features of CML were observed at our institution. We describe here the disease characteristics, treatment response and prognosis of 5 of them (5.5%), in whom t(9;22) and bcr abl hybrid gene were undetectable. Results. All patients were observed in early chronic phase at diagnosis. Their
PO-0727 Towards European criteria, classification and staging of the myeloproliferative disorders
Michiels JJ, Thiele J*
Goodheart Institute, MDD Centre Europe, Rotterdam, The Netherlands and *Institute of Pathology, University of Cologne, Cologne, Germany

The clinical, haematological and morphological characteristics from bone marrow smears and biopsy material, in particular megakaryocytopenia and bone marrow cellularity features that enable a clear-cut distinction to be made between essential thrombocythaemia (ET), polycthyma vera (PV) and anetogenic or essential myeloid (megakaryocytic/granulocytic) metaplasia (AMM or EMGM). The characteristic increase and clumping of enlarged megakaryocytes with mature cytoplasm and multilobulated nuclei and their tendency to cluster in a normal or only slightly increased cellular bone marrow represent the hallmark of ET. Granulopoiesis and erythropoiesis are normal in ET. The characteristic increase and clustering of enlarged mature and pleomorphic megakaryocytes with multilobulated nuclei and proliferation of erythropoiesis in a moderate to marked hypercellular bone marrow with hyperplasia of dilated sinuses are the specific diagnostic features of untreated PV. Megakaryocytic/granulocytic metaplasia may or become a prominent feature of PV, EMGM, including the early prefibrotic stages as well as the various myelofibrotic stages of AMM appear to be a distinct neoplastic dual proliferation of abnormal megakaryopoiesis and granulopoiesis. The histopathology of the bone marrow in prefibrotic EMGM and in classical AMM is dominated by atypical, enlarged and immature megakaryocytes with cloud-like immature nuclei, which are not seen in ET at diagnosis or during follow-up. Myelofibrosis is secondary to essential megakaryocytic/granulocytic proliferation on various MPDs and can be graded into no reticulin fibrosis (F0), early reticulin fibrosis (F1), advanced reticulin sclerosis with minor collagen fibrosis (F2) and advanced collagen fibrosis with or without osteosclerosis (F3). Myelofibrosis is not a feature of ET. Myelofibrosis and myeloid metaplasia may be present in PV at diagnosis and usually appear apparent during long-term follow-up. Myelofibrosis constitutes a prominent feature of EMGM and AMM during the natural history of the disease.

PO-0728 Immunophenotyping of bone marrow CD34+ cells in myelodysplastic syndromes
Haeomatología and Cytometry Department, University Hospital and University of Salamanca, Salamanca, Spain

Immunophenotyping of bone marrow cells in myelodysplastic syndromes (MDS) has been done in a few studies. Most of these studies did not use combinations to explore the phenotype of CD34+ cells from MDS. Objective: The aim of the present analysis was to characterise phenotypically the different CD34+ subpopulations in bone marrow, (BM) cells from MDS patients. Design and Methods. BM cells from 72 MDS patients were studied. Unseparated BM cells were stained by direct immunofluorescence with triple markers (CD34/CD33/CD38; CD15/CD34/HLADR) and analysed with a two-step procedure. In the first step, 15,000 cells were acquired and in the second, 300,000 CD34+ cells were selected using an appropriate live gate. Results. MDS patients showed a significant increase in the proportion of CD34+ cells compared with that of normal controls, patients with poor prognostic subtypes had a higher number of CD34+ cells (RA = 2.9± 6.6%; RAE = 4.5±4.5%; RAEI = 10.8±6.7%; CNML = 1.4±2.1%; Control = 0.8±0.5%). Neither percentage of CD34+ cells nor percentage CD34+/CD33+ correlated with blast cells proportion identified by morphological means. MDS patients displayed a significant increase in CD34+/CD33+ cells showing, that most CD34+ cells are already committed to myeloid differentiation (CD34+/CD33+/CD34+CD33+ ratio was; RA = 54; RAE = 42; Control = 24). In more than 25% of cases the existence of an aberrant cell subpopulation defined by the expression of CD34+ and CD15 in the absence of HLA-DR was observed; and this was more frequent among RAEB and RAEBI patients (p = 0.02).

PO-0729 Follow-up of allogeneic bone marrow transplantation in two children with myelodysplastic syndrome (MDS) who developed succeeding Fanconi's anaemia
*Unidad de Antropología; **Unidad de Biología Cellular, Facultad de Ciencias, Universidad Autónoma de Barcelona, Barcelona; *Servicio de Haeematología infantil, Unidad de Transplante de médula ósea, Hospital Materno-infantil “Vall d’Hebron”, Barcelona, Spain

Objective. Allogeneic bone marrow transplantation (BMT) is the treatment of choice for severe bone marrow failure in patients with Fanconi’s anaemia (FA). However, FA-patients who develop MDS or acute leukaemia submitted to allogeneic BMT have a poor outcome, being in need of tailored conditioning regimen. We present the follow-up of two children with FA who developed MDS with clonal chromosome abnormalities; Design and Methods. They received an allogeneic BMT from HLA-identical sibling donors after a conditioning regimen with a low-dose of cyclophosphamide and radiation. The follow-up of the BMT was performed by dual-colour FISH, using X-Y chromosome probes and by cytogenetic analysis. Results. In one patient FISH detected an increased of host cells six months before clinical and cytogenetic relapse. In both patients cytogenetic analysis showed new chromosome rearrangements along with evolution to acute leukemia. A second transplant using the same donor proved successful following a more intensive conditioning regimen. FISH and cytogenetic analysis confirmed the clinical remission three years after the second transplant. Conclusions. This study demonstrates the unfavorable prognosis of chromosome abnormalities in FA, particularly, the presence of monosomy 7 or complex karyotypes; the need to use a more intensive conditioning regimen for FA-patients with MDS; and the utility of the FISH in the follow-up of chimaerism, predictive of relapse when show an increase of host cells in serial studies. This study was supported by the “Fondo de Investigaciones Sanitarias” no: 98/1411.

PO-0730 CD34+ progenitors in myelodysplasia show cluster growth with increasing apoptosis in time in single cell-assay.
Stap LFD, Vierwinden G, Pennings A, Boezeman J, Raymakers RAP, Witte TUM
University Hospital of Nijmegen, Nijmegen, The Netherlands

Bone marrow (BM) cells of patients with MDS show increased levels of proliferation and premature apoptosis in vivo as well as in vitro. Aim. To explore the intrinsic clonogenic capacity and apoptotic propensity of CD34+ progenitors of MDS patients by excluding interference of accessory cells by culturing PO-0730 CD34+ progenitors in myelodysplasia show cluster growth with increasing apoptosis in time in single cell-assay.
Colonies 0.5 10.6 17.6 21.0 0.03 1.0 2.2 1.9
Clusters 35.2 32.7 25.6 24.2 39.2 46.4 44.0 48.8
mean numbers per plate (Cl/Col = Cluster-Colony ratio).

Annexin V+ cells of all cells of a cluster or colony.
done. The amount of apoptosis (A%) was defined as the percentage of
ing Annexin V-FITC. After incubation fluorescence microscopy imaging was
4, 7, 10 and 14 for clusters (Cl: 2-40 cells) and colonies (Col: >40 cells).

At each time point 2 plates were used for determining apoptosis by apply-

4, 7, 10 and 14 for clusters (Cl: 2-40 cells) and colonies (Col: >40 cells).

At each time point 2 plates were used for determining apoptosis by apply-

In 8 of the 13 cases tested, efflux of the Rh 123 was detected. In 2 cases, Pgp expression was detected without Rh 123 efflux. In 3 of the 8 CAM tests, MRP activity was detected, 3 cases were discordant (1 MRP+/CAM-; 2 MRP-+CAM+). Response to intensive chemotherapy (anthracycline + Ara C) was evaluated in 23 cases: 8 achieved complete remission including 4/14 MRP+ and 4/9 MRP- cases (p<0.05). Conclusions, MRP expression was more frequent in MDS-AML. However, MRP seems to be less implicated as a prognostic factor in MDS than Pgp. As for Pgp, discordant expression/function of MRP in some cases suggests the existence of a non-functional transport protein in MDS.

PO-0733 Downregulation of CD43 in myelodysplastic syndromes

Kyrilakou D,*, Alexandrakis M,*, Tsardi M,*, Stefanaki D,*, Eliakias P,*

Departments of Haematology and *Pathology of the University Hospital of Herakleion, Crete, Greece

CD43 (leukosialin, sialophorin) is a cell surface mucin expressed at high levels on most leukocytes and is reported to be involved in adhesion, anti-

signal transduction procedures. Regulation of its expression is thought to take place through DNA methylation in non-producing cells. We studied CD43 expression in patients suffering from myelodysplastic syndromes (MDS) in an effort to identify variations and correlate them with clinical outcome. Methods. Whole blood from 53 MDS patients was analysed by flow cytometry after labeling with FITC-conjugated anti-CD43 monoclonal antibody (DFT-1, Serotec). A Coulter EPICS PROFILE flow cytometer was used. RTPCR and Northern Blot hybridisation with radiola-

bient DNA probe was used to investigate CD43 expression. Western Blot, hybridisation with anti-CD43 (DFT-1) monoclonal antibody and enhanced chemiluminescence was used to investigate CD43 expression at protein lev-

ingle. Single strand conformational polymorphism and DNA sequence analy-
sis were used for mutation analysis of the CD43 gene. Results. In most cases CD43 was expressed in more than 60% of the lymphocytes and more than 80% of the neutrophils in peripheral blood. In three patients, one with RAEB and two with RAEB-C, CD43 was expressed in 3.8%, 7%, and 9.9% respectively. The downregulation was observed at both protein and RNA level while DNA analysis did not reveal any mutation within the gene or the promoter region. Further studies are needed to reveal the mecha-

ism of gene downregulation in these patients and investigate whether the phenomenon is related to the dysplastic population alone or not.

PO-0734 Idarubicin and cytosine arabinoside in induction and main-

tenance regimens for high-risk myelodysplastic syndromes


Medicina Interna ed Oncologia Medica, Università di Pavia, IRCCS Poli-

clinico S. Matteo, Pavia and Sezione di Ematologia, Spedali Civili, Brescia, Italy

We evaluated in a prospective study the efficacy and tolerability of an inten-
sive therapy with idarubicin (IDA) and Ara-C in patients with de novo RAEB and RAEB-t, and the efficacy of low-dose maintenance chemotherapy.

Adults ≤55 years old were treated with full doses (ARA-C 1 g/m2/d days 1-4, IDA 10 mg/m2/d days 1-3), elderly with lower doses (ARA-C 1 g/m2/d days 1-2, IDA 10 mg/m2/d days 1-2). Responders followed a consolidation course identical to the induction therapy. Patients in complete remis-

sion (CR) after consolidation regimen were treated for 2 years with mainte-

nance therapy (ARA-C 10 mg/m2/d 12 h.c. for 10 days a month; IDA 15 mg/m2/d p.o. for 3 days a month in alternate cycles). For patients ≤55 years old BM or PBSC transplantation was optional. From February 1994 to February 1998, 29 patients were enrolled (median follow-up 38 months), 23 M and 6 F, median age 61 years, 11 ≤55 years old, 7 RAEB and 22 RAEB-t in 9, treatment began more than 3 months after diagnosis. Thirteen cases (45%), 2 RAEB and 11 RAEB-t, 2 >65 years old, achieved CR (8, 28%); 6 >65 years old, PR (20%), 2 >65 years old, were resist-
ant. CR rate was significantly higher in younger patients than in RAEB-t. Two patients (7%), 1 >65 years old, died during the aplastic phase, one from sepsis and one from cerebral haemorrhage. Among CR patients median
times to recovery of neutrophils and platelets were respectively 21 (range 14-33) and 20 (range 13-24) days. No severe complications occurred during consolidation and maintenance courses. Two patients in CR underwent allogeneic BM and 2 autologous PBSC transplantation. Seven of the 9 patients treated with maintenance therapy and both PBSC transplanted patients relapsed. Median duration of DFS was 10 months, 3 patients (1 allografted) in continuous CR lasting 24 to 55 months. The other allo-grafted patient died in CR of acute GHD. Median survival duration of patients achieving CR was 19 months, but only 11 months in the PR and resistant patients (p=0.05); overall median survival was 12 months. Younger age, normal karyotype, presence of Auer bodies and intermediate IPSS were associated with longer survival. In conclusion, in de novo RAEB and RAEB-1 intensive chemotherapy is associated with a satisfactory frequency of response with acceptable toxicity. Maintenance therapy prolonged, at least in a subgroup of patients, the period of CR and survival.

PO-0738 Low dose melphalan for treatment of high risk myelodysplastic syndromes in elderly patients

Denzlinger C, Benz D, Bowen D,* Gelly K,* Brugger W, Karz L. Abt. II Med. Klin. Univ. Tübingen, Tübingen, Germany; *Molecular and Cellular Pathology, University of Dundee, Dundee, Scotland

Since myelodysplastic syndromes (MDS) are predominantly diseases of elderly people, therapeutic options that are well tolerated in this group of patients are urgently needed. We treated 16 elderly patients with high risk MDS with 2 mg melphalan orally once a day, according to Omoto et al. [1]. Patients were 59 to 81 (median 71) years old. All patients were at intermediate-2 or high-risk (International Prognostic Score System values 1.5 to 3.5, median 2.5). They had CMLM (n = 1), RAEB (n = 4), RAEB-T (n = 7) according to the FAB-classification and 4 patients had secondary AML. We observed 5 (31%) complete peripheral responses occurring within 2 to 10 weeks. Responses lasted for 16 to 40 + weeks. Complete responders had RAEB (n = 2), RAEB-T (n = 2) or secondary AML (n = 1), a normal or reduced cellularity in bone marrow and a normal karyotype. In addition, 1 patient had a transient increase in platelet counts and a significant reduction in bone marrow blasts. In all responding patients, improvements in blood counts were noted before week 5 of treatment. One patient relapsed twice i.e. 12 or 42 weeks following discontinuation of low dose melphalan; in this patient second and third remission were obtained upon retreatment. Ten patients (63%) had no significant response or disease progression. Treatment was generally well tolerated apart from an initial worsening of cytopenia observed in most patients. Our data essentially confirm those of Omoto et al. We conclude that low-dose melphalan is a well tolerated out-patient regimen with promising activity in elderly patients with high-risk MDS. A normal or reduced bone marrow cellularity may be predictive of a beneficial response. The drug is currently being tested in a randomised double-blind, placebo-controlled clinical trial.

PO-0739 Absence of HTLV-I in myelodysplastic syndromes in an area non endemic for the HTLV-I infection


Department of Medical Sciences, Section of Haematology, Modena, *Department of Biomedical Sciences and Advanced Therapies, Section of Hematology, Ferrara, Italy

Recently Karlic et al. (1) reported the detection of human T lymphotropic virus type 1 (HTLV-I), in patients with myelodysplastic syndromes (MDS), in a non endemic region of central Europe, indicating a 19% incidence of HTLV-I. Moreover, cytogenetic analysis showed the presence of del (5)(q) in 3 out of 8 HTLV-I positive MDS cases, but only in 1 of 38 HTLV-I negative cases. The same authors, having observed similar results in acute myeloid leukaemia (AML) cases evolved from MDS (3), suggested that allelic deletions of 5q-located genes, that typically occur in MDS, might be associated with HTLV-I infection in central Europe. We analysed by PCR 39 MDS cases from northern Italy, for the presence of HTLV-I, using primers for the pol and tax genes. The amplified products were then hybridised with a specific oligonucleotide probe, as described (1,2). The cytogenetic analysis showed the presence of del (5)(q) in 4 out of 18 patients. In 8 cases, long-term bone marrow cultures were also obtained, and DNA was extracted from the adherent cell population, composed of fibroblasts (95%), endothelial cells (2%) and macrophages (3%). In contrast with the data reported by Karlic et al. (1), we found no evidence of HTLV-I tax and pol sequences in all the 39 patients with MDS from our non endemic area. In particular this was true also for MDS patients with documented del (5)(q). An involvement of HTLV-I infection in human diseases in non endemic areas is not clearly established. It is often acknowledged that regions of distant origin may have crossreactivity with HTLV-I antigens. Our study provides no evidence for a role of HTLV-I infection in MDS. However, the presence and pathogenic relevance of still unknown factors, related to or not to HTLV-I remain a possibility.


PO-0740 The importance of magnetic resonance imaging for diagnostic differentiation in patients with pancypnetia

Santiago GF, Torniani M, Lima CSP, Lorand-Metze IL

Faculty of Medicine, State University of Campinas, SP - Brazil

Distinction between aplastic anemia (AA) and myelodysplastic syndrome, hypoplastic form (h-MDS) is not always easy. Evolution of AA into a clonal disorder has been documented in patients in whom a bone marrow transplantation has not performed. Magnetic resonance imaging (MRI) has proven to be a useful tool in documenting the proliferative character of MDS and other hematopoietic clonal disorders. We studied 22 patients with pancypnetia and hypocellular marrow who had not been transplanted. Median time between diagnosis and MRI evaluation was 16 months. Coronal T1-weighted images and STIR were performed on both femora in order to assess areas of bone marrow extension. The following criteria were used for image analysis: 1. focal or diffuse involvement; 2. extent of haemopoiesis and signal intensity. Among 21 cases with AA, 7 patients showed few hematopoietic foci, 3 had several foci on the femoral heads and diaphyses and 1 patient showed a faint diffuse pattern. Among patients with h-MDS 5 had a focal pattern and 6 had a diffuse one (4 faint and 2 bright), documenting active hematopoesis in both femora. Moreover, in h-MDS, an irregular distribution of hematopoesis in bone marrow histological sections was associated with a focal pattern in MRI in 8/11 cases. We can conclude that MRI of the femora is a useful tool for the differential diagnosis between AA and h-MDS.

PO-0741 Abnormal function of stromal microenvironment and macrophages in myelodysplastic syndromes

Manakovs T, Gerasimova L, Tsvetaeva N

Haematoalogical Scientific Centre, Moscow, Russia

Dysregulated haemopoiesis in MDS is caused by abnormalities in haemopoietic progenitors and disturbances in interactions with the bone marrow microenvironment. The role of the microenvironment itself in MDS has not been well characterised. We examined the capacity of MDS stroma to support the growth of CFU-GM obtained from normal bone marrow. The growth of normal CFU-GM on MDS stroma from long-term bone marrow cultures was significantly reduced (46.4±4.5) compared with that of normal stroma (95.8±13.2). The addition of growth factors did not stimulate the normal CFU-GM to control values. We estimated also the effect of macrophages that originate from marrow haemopoietic progenitors and form part of microenvironment on the growth of haemopoietic progenitors. MDS and normal macrophages supported the growth of normal CFU-GM equally well (95.8±40.8 and 73.2±23.0). However there were differences in the growth of normal CFU-GM on the macrophage feeder from the different groups of MDS. These studies demonstrate the defect of stromal microenvironment and macrophages in MDS.

PO-0742 t(7;9)(p15;q34) in childhood myelodysplasia

Pappe B,* Van Limbergen H,* Van Roy N,* Van der Cruys E,* De Paepe A,* Benoit Y,* Speleman F*

*Department of Medical Genetics, and *Department of Pediatric Haematology and Oncology, University Hospital Ghent, Ghent, Belgium

We describe the finding of a t(7;9)(p15;q34) in a 15-month old girl with de novo MDS. She was admitted to our hospital for further investigation of increased bruising. A RAEBt was diagnosed. The bone marrow examination. Karyotypic analysis on bone marrow cells revealed an abnormal karyotype: 47, XX, +6, t(7;9)(p15;q34), +10, -17,-20. The constitutional karyotype was normal. After an induction course according to the EORTC-CLG-92 protocol a complete remission was obtained. The patient died due to infectious complications (RSV pneumonitis) while in aplasia after the first intensification course. Childhood myelodysplastic syndromes are rare and comprise a heterogeneous group of mononuclear stem cell disorders, characterised by the association of cytopenia and abnormalities of erythroid, myeloid and/or megakaryocytic maturation. The clinical course is highly variable, ranging from spontaneous remission to rapid transformation to therapy resistant AML. Confusion exists over classification and nomenclature since FAB classification for adult MDS often is inadequate for stratifying these young patients. Frequent nonrandom cytogenetic abnormalities encountered in childhood MDS include monosomy or deletion of chromosome 7 and trisomy 8. To our knowledge this is the first report on a balanced translocation involving chromosomes 7 and 9 in childhood MDS. We excluded ABL rearrangement by FISH, with a probe which spans the ABL locus, indicating that the translocation breakpoint on 9q34 lies telomerically of the ABL gene. Balanced translocations involving 7p15 and 9q34 have been reported in AML, leading to genomic fusion between the HOXA9 and the NUP98 genes and between the DEK and the NUP214 genes in t(7;11)(p13;q22) and t(6;9)(p23;q34), respectively. We are currently investigating whether the t(7;9) in this patient may have lead to a novel chimaeric transcript involving the HOXA9 and the NUP214 gene. Also, further collection and molecular analysis of additional cases of t(7;9) in childhood MDS and other haematological malignancies is warranted.

PO-0743 Expression of Gα16 and 5-lipo-oxygenase reflect growth and differentiation processes in MDS and MPD

Pfleidercker M, Karlic H, Moeldt M, Pittermann E, Heinz R

Ludwig Boltzmann Institute for Leukaemia Research and Haematology, 3rd Medical Department, Hanusch Hospital, Vienna, Austria

Objective. The G-protein subunit Gα16 plays a specific role in signal transduction pathways which mediate the proliferation of haematopoietic cells. During differentiation blood cells acquire the capacity to synthesise leukotrienes for which 5-lipo-oxygenase (5-LO) is a key enzyme. Data exist relating expression of 5-LO to maturation of blood cells. Thus the objective of this study was to test the diagnostic potential of Gα16 and 5-LO for monitoring growth and differentiation processes in myelodysplastic syndromes (MDS) and myeloproliferative diseases (MPD). Design and Methods. RT-PCR mediated monitoring of Gα16 and 5-LO expression was performed from MNC isolated from blood and bone marrow samples of patients with MDS and MPD. Thirty-six MPD patients were analysed for Gα16 including 13 cases who were also analysed for 5-LO. Of 20 MDS cases (including 5 secondary AML) screened for Gα16, 10 cases were analysed for 5-LO. In addition, 3 MDS patients were tested for both parameters before and after treatment with amifostine. Results. Among MPD patients, most cases were positive for Gα16 whereas expression of 5-LO was rare (4/13 cases, all of them positive for Gα16). Among 21 MDS cases, 17 expressed Gα16 and 15/10 cases were 5-LO positive. In 2 MDS patients, Gα16 negativity/5-LO positivity was reversed during treatment with amifostine. Conclusions. These data indicate that proliferation and differentiation of haematopoietic cells may occur simultaneously in MDS and MPD. Determination of Gα16 and 5-LO expression may provide a tool for observing regulation of growth and maturation in these diseases especially under different treatment conditions.
**PO-0744** Serum levels of endogenous haematopoiesis-regulatory cytokines in patients with myelodysplastic syndromes

Marijana Brereton, MD; Sefer D; Suvajdžić N; Jelušić V; Bošković D; Colović M

Institute of Haematology, Clinical Centre of Serbia, Belgrade, Yugoslavia

Serum concentrations of erythropoietin (Epo), tumour necrosis factor α (TNF-α), interleukin-1 β (IL-1β), interleukin-6 (IL-6) and granulocyte/monocyte-colony stimulating factor (GM-CSF) were measured in patients (pts) with myelodysplastic syndromes (MDSs) to characterise dysregulation of the cytokine network in these disorders. Cytokines were measured using commercially available enzyme-linked and radio-immunoassays. All 43 tested pts showed increases of TNF-α and IL-1β (47% 70 pts (67%) and 31/59 pts (52.5%) showed increase of Epo and IL-6, respectively, while only 6 of 29 (20.7%) tested pts had a moderately elevated serum concentration of GM-CSF. TNF-α was in positive correlation with IL-6 (r=0.415; p=0.06) and GM-CSF serum concentration (r=0.676; p=0.00001). Epo level correlated negatively with hemoglobin level (r=-0.501; p=0.0001). But no relationship was found between serum levels of cytokines and cytogenetic abnormalities.

**PO-0745** Megakaryopoiesis in myelodysplastic syndromes (MDS) and AML

Breereton ML, Adams JA, Briggs M, Liu Yin JA

University Dept Haematology, Manchester Royal Infirmary, UK

Ineffective megakaryopoiesis resulting in thrombocytopenia is a major problem in patients with MDS and AML, thus recombinant growth factors capable of increasing megakaryopoiesis may have a role in treatment. We have used two in vitro culture systems to assess the effect of pegylated recombinant human megakaryocyte growth and development factor PEGrHuMGDF both alone and in combination with IL3, IL6, G-CSF, GM-CSF, SCF or Epo on the megakaryopoietic potential of bone marrow from 40 patients with MDS (RA=19, RAS=7, RAEB=10, RAEBt=4), 33 with AML (M1-M6) and 16 normal subjects. Direct effects on CFU-Mk and CFU-GM were measured using standard assays, day 14 colonies stained for CD61. RA + RAS and RAEB + RAEBt were grouped together for analysis. PEGrHuMGDF only stimulated CFU-GM in 19/25 RA+RAS, 11/14 RAEB+t, 29/33 AML and 16/16 normal BM. (Response defined as 1.5 fold increase over the no PEGrHuMGDF control).

Responders included 5/7 MDS and 6/7 AML patients with plt counts <20x10^9/L. The addition of other GFs with PEGrHuMGDF further increased CFU-GM growth in 3/35 MDS (cluster formation in one) and in 1 of 31 AML, BM was unaffected. Increases in CFU-GM cells in suspension cultures with the addition of PEGrHuMGDF were seen in 22/25 RA+RAS, 11/14 RAEB+t, 29/33 AML and 16/16 normal BM. (Response defined as 1.5 fold increase over the no PEGrHuMGDF control). Responders included 5/7 MDS and 6/7 AML patients with plt counts <20x10^9/L. The addition of other GFs with PEGrHuMGDF increased CFU-GM growth in 3/35 MDS (cluster formation in one) and in 1 of 31 AML, BM was unaffected. Increases in CFU-GM cells in suspension cultures with the addition of PEGrHuMGDF were seen in 22/25 RA+RAS, 11/14 RAEB+t, 29/33 AML and 16/16 normal BM. (Response defined as 1.5 fold increase over the no PEGrHuMGDF control). Responders included 5/7 MDS and 6/7 AML patients with plt counts <20x10^9/L. The addition of other GFs with PEGrHuMGDF increased CFU-GM growth in 3/35 MDS (cluster formation in one) and in 1 of 31 AML, BM was unaffected. Increases in CFU-GM cells in suspension cultures with the addition of PEGrHuMGDF were seen in 22/25 RA+RAS, 11/14 RAEB+t, 29/33 AML and 16/16 normal BM. (Response defined as 1.5 fold increase over the no PEGrHuMGDF control).

**PO-0746** Thrombocytosis and acquired sideroblastic anemia. Can this association be considered a new entity?


Hospital Príncipe de Asturias, Alcalá de Henares, Madrid, Spain

Introduction. Myelodysplastic syndromes (MDS) are usually associated with pancytopenia but patients with some chromosome abnormalities (5q-syndrome, del(3) and +8) may have a normal or even a raised platelet count. We have reviewed the charts of 49 patients diagnosed as having MDS between 1994 and 1998 in our hospital to evaluate platelet counts at diagnosis and chromosome abnormalities trying to correlate them to FAB subtype. Results. Of the 49 patients, 13 were given a diagnosis of sideroblastic anemia (SA). Five of the 49 presented with platelet counts >400x10^9/L (one of them having a 5q-syndrome and the other 4 a SA). These 4 patients with SA and thrombocytosis showed at diagnosis Hb between 8-12 g/dL, platelet counts between 415-966x10^9/L and normal WBC. BM studies showed >40% ringed sideroblasts and dysplastic megakaryocytes without dwarf forms in all cases. Myelodysplasia was found in 5 patients with chromosome abnormalities. BM karyotype was normal in the three patients in whom it was studied. Of the four patients, an 83-year old lady was lost to follow up. Three patients (males, aged 64, 68 and 57) were followed up for 12, 18 and 20 months. One has persistently platelet counts around 500x10^9/L. The two remaining cases showed increasing platelet counts reaching 700 in one case and 1200x10^9/L in the other, who has been treated with hydroxyurea. Discussion. Our cases have not shown the chromosome abnormalities usually associated with raised platelet counts, but for a 5q-syndrome. Moreover, we have also observed a surprisingly high incidence, and not previously reported, of thrombocytosis among SA (4/13 cases), which was not the case among the non-SA-MDS (1/36, one 5q-syndrome). Our SA-thrombocytosis did not show any features of myeloproliferative disease. We conclude that a case can be made to define better SA-thrombocytosis as this association could be considered a new MDS subtype.

**PO-0747** Myelodysplastic syndrome in patients who suffered from the Chernobyl accident

Klymenko VI, Dyagil II, Bebeshko VG, Klymenko SV, Bazyka DA, Lubarets TF

Scientific Centre for Radiation Medicine, Kyiv, Ukraine

Objective. Identifying the features of myelodysplastic syndromes (MDS) in the patients who suffered from the Chernobyl Accident. Design and Methods. Twenty-seven patients who suffered from the Chernobyl Accident were included in this study. Nineteen patients were men and eight were women; the mean age was 52 years (38-69 years). The diagnosis was established according to the French-American-British classification (FAB). All patients were diagnosed in the years from 1991 to 1998. Diagnosis of MDS was made according to a modern classification. The diagnosis of MDS patients shows the long period before developing overt MDS. Tranchet bone marrow fibrosis was found in about 50% of the patients. Analyses of the clinical records were found in all cases. Myelodysplasia was found in 50% of the patients. Of the 49 patients, 13 were given a diagnosis of sideroblastic anemia (SA). Five of the 49 presented with platelet counts >400x10^9/L (one of them having a 5q-syndrome and the other 4 a SA). These 4 patients with SA and thrombocytosis showed at diagnosis Hb between 8-12 g/dL, platelet counts between 415-966x10^9/L and normal WBC. BM studies showed >40% ringed sideroblasts and dysplastic megakaryocytes without dwarf forms in all cases. Myelodysplasia was found in 5 patients with chromosome abnormalities. BM karyotype was normal in the three patients in whom it was studied. Of the four patients, an 83-year old lady was lost to follow up. Three patients (males, aged 64, 68 and 57) were followed up for 12, 18 and 20 months. One has persistently platelet counts around 500x10^9/L. The two remaining cases showed increasing platelet counts reaching 700 in one case and 1200x10^9/L in the other, who has been treated with hydroxyurea. Discussion. Our cases have not shown the chromosome abnormalities usually associated with raised platelet counts, but for a 5q-syndrome. Moreover, we have also observed a surprisingly high incidence, and not previously reported, of thrombocytosis among SA (4/13 cases), which was not the case among the non-SA-MDS (1/36, one 5q-syndrome). Our SA-thrombocytosis did not show any features of myeloproliferative disease. We conclude that a case can be made to define better SA-thrombocytosis as this association could be considered a new MDS subtype.
PO-0748 Coagulation activation in first-degree relatives of type 2 diabetic patients

Martinez-Brotons F, Fernandez-Castarlen M, Domenech P, de la Banda E
Haemostasis and Thrombosis Unit, CSU Bellvitge I Hospital de Llobregat, Spain

Cardiovascular diseases are the major cause of morbidity and mortality in patients with type 2 diabetes and are present at the time of diagnosis in a substantial number. First-degree relatives of type 2 diabetic patients are at increased risk of clinical disease. To evaluate whether or not activated coagulation is present in the preclinical phases of type 2 diabetes mellitus, we studied 46 non-diabetic first-degree relatives of type 2 diabetic patients and 21 matched controls with no family history of diabetes. We determined the plasma levels of prothrombin fragment 1+2, D-dimer, fibrinogen, plasminogen activator inhibitor type 1 (tissue plasminogen activator, von Willebrand factor and coagulation factors VII and VIII. Glucose tolerance, beta-cell function and insulin sensitivity were assessed in all subjects by a continuous glucose infusion of 5 mg/kg ideal body weight -1 min-1 for 60 min with model assessment of glucose, insulin and C-peptide values. Plasma levels of prothrombin fragment 1-2 (median 1.24 vs 0.68 mmol/L; P = 0.0001) and D-dimer (331 vs 254 μg/L; 1.06; P = 0.018) were higher in relatives, without significant differences in the other haemostatic variables. Relatives showed higher fasting (5.5 vs 4.9 mmol/L; P = 0.004) and post-infusion (9.3 vs 8.3 mmol/L, P = 0.02) serum glucose, no differences in insulin or C-peptide levels, lower β-cell function (122 vs 147%; P = 0.02) and no significant differences in insulin sensitivity. Our results suggest that first-degree relatives of type 2 diabetic patients have abnormal coagulation activation. These abnormalities may play a role in the pathogenesis of cardiovascular diseases frequently seen at diagnosis of type 2 diabetes.

PO-0749 Diagnosis and clinical significance of antithrombin III deficiency

Loginska Z, Gayda G, Gayda A, Loginsky V
Research Institute of Haematology & Blood Transfusion, Lviv, Ukraine

Antithrombin III (ATIII) level is one of the most informative tests in the investigation of blood coagulation disorders during pregnancy pathology. ATIII activity was studied in 23 healthy subjects and 139 pregnant women by diagnostic test kits developed by the authors. Low ATIII activity was observed in pregnant women (last trimester), in 71% of patients with vascular disorders, complicated by thromboses (ATIII level was decreased to 57%), in 100% of patients in acute renal failure after shock (ATIII level was decreased to 61%), and in 50% of the patients with chronic renal diseases (ATIII level was decreased to 65%). ATIII activity was also decreased in 77% of patients with immune disorders (ATIII level was decreased to 59%) and malignant diseases, especially haematological malignancies, in 67% patients with acute lymphoblastic leukaemia (ATIII level was decreased to 62%), in 71% of patients with chronic lymphoproliferative disorders (ATIII level was decreased to 60%). Thus, the results strongly indicate the important diagnostic and therapeutic significance of ATIII level determination in complex coagulation tests in a number of diseases.

PO-0750 A Protein C pathway screening test

van Voorst tot Voorst E, Van Berkel HP, Edelaar AE, van Putte A
Clinical Chemistry Laboratory, Isala Clinics/Weezenlanden, Zwolle, The Netherlands

We evaluated the Protein C pathway screening test (PCP-test) in order to establish whether this test can assist in the diagnosis of common venous thrombosis or pulmonary embolism. The PCP test is easy to conduct and relatively cheap. Our laboratory has studied the applicability of this test to differentiate healthy patients from those with a Protein C deficiency, a protein S deficiency or a Factor V Leiden mutation. A reliable screening test would differentiate healthy patients from those with a protein C deficiency, a protein S deficiency or a Factor V Leiden mutation. A reliable screening test would differentiate healthy patients from those with a protein C deficiency, a protein S deficiency or a Factor V Leiden mutation.

PO-0751 Haemorheological profile in chronic venous insufficiency after surgery

Azaceta G, Romero S, Azcona JM*, Moreno JA, Olave T, Palomera L, Domingo JM, Turbe T, Gutiérrez M
Servicios de Haematologia y Angiologia y Cirugia Vascular, Hospital Clinico Universitario de Zaragoza, Spain

Over the last years, the interest in the haemorheological contribution to vascular diseases has increased strikingly. Several authors have reported the presence of haemorheological abnormalities in chronic venous insufficiency (CVI), which probably take part in the pathophysiology of the disease. Nowadays, saphenous vein stripping is considered as one of the most successful surgical treatments for CVI. Objective: To analyze the haemorheological profile after stripping in 45 patients suffering from CVI. Furthermore, we studied the rheological conditions before surgery, by comparing them with a healthy control group. The follow-up included laboratory tests on the 7th, 60th and 180th days after surgery. EA was assessed with a photometric aggregometer (Myrenne - MA1) in stasis and low shear (3s-1). Fibrinogen, haemometric and biochemical tests were performed as well. Results show an increase of EA and fibrinogen in patients (p< 0.05). On the 7th day after surgery, the hyperaggregability was even higher, reaching statistical significance when compared with previous and subsequent measurements (p<0.001). Two and six months later, EA values return to those found prior to surgery. Plasma fibrinogen level changed in a parallel way statistical significance when compared with previous and subsequent measurements (p<0.001). Two and six months later, EA values return to those found prior to surgery. Plasma fibrinogen level changed in a parallel way. The association between rheological disturbances and thrombogenesis is well-known, so the hyperaggregability found supports the practice of antithrombotic prophylaxis in the early post-surgical period. The hemorheological abnormalities persist after stripping, so postsurgery treatment to inhibit EA may be beneficial.

PO-0752 Haemorheological profile in chronic venous insufficiency

Azaceta G, Romero S, Azcona JM*, Moreno JA, Olave T, Domingo JM, Palomera L, Gutiérrez M
Servicios de Haematologia y *Angiologia y Cirugia Vascular, Hospital Clinico Universitario de Zaragoza, Spain

In recent years haemorheological properties of blood have been considered as being of interest in pathophysiological studies and treatment approaches of vascular diseases, including chronic venous insufficiency (CVI).
Biological Department of Moscow State University and Institute of Bioorganic Chemistry RAS, Moscow, Russia

The known methods of leech therapy aim to prevent the blood from coagulating. The proposed method, a modification of M. Rigbi's method (1960), allows for collection of the leech saliva in high- and low-molecular weight fractions. The low-molecular weight fraction is divided into more than 20 main components as detected by Phase-Reversed HPLC-chromatography. In the report some mass-spectrometric characteristics of the leech saliva high- and low-molecular weight components will be introduced.

**Novel method for collecting medicinal leech saliva, a unique native antithrombotic agent**


*Biological Department of Moscow State University and Institute of Bioorganic Chemistry RAS, Moscow, Russia

The medicinal leech salivary gland secretion is a complex of biologically active substances which is injected by the leech during bloodsucking in order to prevent the blood from coagulating. The known methods of leech saliva collection do not exclude the presence of contaminants from the leech intestine. The proposed method, a modification of M. Rigbi's method (1960), allows collection of natural salivary gland secretion which the medicinal leech injects directly into the wound as it bites the victim's skin. Dialysis through a membrane which filtrates substances of molecular mass smaller 500 Da was used to separate the leech saliva into low- and high-molecular weight fractions. The high-molecular weight fraction preserves antithrombin and anticoagulating activities and the other properties unique to the leech saliva. It exhibits D-dimer monomerising activity, the inherent characteristic of enzyme destabilise, and the capacity to stimulate lysis of cell walls of Micrococcus Luteus. The low-molecular weight fraction is divided into more than 20 main components as detected by Phase-Reversed HPLC-chromatography. In the report some mass-spectrometric characteristics of the leech saliva high- and low-molecular weight components will be introduced.

**Prothrombin variant and factor V Leiden do not explain the brain abnormalities detected by MRI in a Sicilian population with asymptomatic brain damage in thalassemic and sickle cell (β-thalassemia) patients**


*Division of Haematology with TMO and Institute of Radiology "P. Cigno-Liberti G*

**Objective** The purpose of this study was to evaluate the frequency and severity of silent brain damage (ischaemic lesions) an asymptomatic patient affected by β-thalassemia-major (TM), β-thalassemia intermedia (TI) and sickle cell β-thalassemia (S/β-Thal), correlating MRI findings to thrombotic genetic alterations for the detection of patients at risk of stroke. Design and Methods. The group included sixty asymptomatic patients younger than 50 y.o. with a diagnosis of sickle cell anemia in 5 patients, S/β-Thal in 20, TI in 19 and 13 with TM. All of them underwent brain MR imaging using axial and coronal T2-weighted Spin-Echo and FLAIR sequences. Furthermore we evaluated the prevalence of the prothrombin gene variant (G20210A) and the factor V Leiden (FVL) mutation using a multiplex PCR test, since it has been shown that these are the more prevalent defects in patients with ischaemic stroke and deep venous thrombosis. Results. Heterozygosity for the prothrombin gene variant was identified in three patients, one was a TI and two were sisters with S/β-Thal; none of these three patients showed any atrophy or ischaemic lesions on MRI findings. None of the patients studied carried the FVL mutation. As far as concerns silent brain damage, 35% of the TI group and S/β-Thal patients had multifocal lesions. In TM patients, although the group was smaller, ischaemic lesions were less prevalent than in the other two groups of patients. Conclusions. We observed a high rate of silent brain lesions as detected by MRI in TM, TI and S/β-Thal patients suggesting that MRI could play a role in identifying patients at risk of stroke. The alteration were not influenced by thrombophilia state. The prevalence of the prothrombin gene variant observed could reflect the prevalence of this defect in the normal Sicilian population.

**Prevalence of prothrombin G20210A in patients with venous thrombosis**

HadrerLeuth U, Hapich D, Kutschkow R, Compes M, Hanfland P

*Institute of Experimental Haematology and Transfusion Medicine, University of Bonn, Germany

**Objective.** Prothrombin G20210A is besides factor V Leiden (FVL) the most important inherited risk factor for venous thrombosis. Prevalence of this mutation in the general population of Europe is approximately 1-2% and the risk of thrombosis in heterozygotes is considered to be 2 to 3-fold. Here we investigated the frequency of prothrombin G20210A in patients (pts) with venous thrombosis. Design and Methods. A total of 278 consecutive pts (199F, 88M) admitted to our institute with a history of venous thrombosis were tested for prothrombin G20210A by genetic analysis using a modified method of Poir et al. (1). Pts were also investigated for presence of FVL either by APC resistance test (Chromogenix, Sweden) or, in case of an APE ratio below 2.4, by DNA-analysis. Results. Prothrombin G20210A was detected in heterozygous form in 7.2% (20/278) of all pts deriving from different families. Interestingly, 45% (9/20) of the pts with prothrombin G20210A were additionally positive for FVL (8 heterozygotes, 1 homozygote). Deep leg vein thrombosis was present in all pts, furthermore, pulmonary embolism, portal vein thrombosis, sinus sagittal thrombosis, arm vein thrombosis and eye vein thrombosis were registered. Mean age at the first thrombotic event was 35±12y in pts with prothrombin G20210A only, compared to 36±12y in pts with combined mutations of prothrombin and FV. Among the pts with prothrombin G20210A 50% (10/20) suffered from more than one thrombotic event. 4/10 of them were additionally APC resistant. Circumstantial risk factors, most frequently previous surgery and oral contraceptives, were present in 53% (11/20) of pts at the time of thrombosis. Conclusions. Our results support the view of a multifactorial etiology of venous thrombosis. In patients carrying prothrombin G20210A, other genetic or circumstantial risk factors can be observed frequently. Thus, previous reports about the relative risk of thrombosis for the two major genetic risk factors (i.e. FVL and prothrombin G20210A, each independently) might have been overestimated. 1. Poir et al. Blood 1996; 88:3698-703.
Among the acquired risk factors of DVT, hormonal treatment was commonly found in women (52%), surgery (31%) or prolonged immobilization (21%) in men. In our patients, the presence of the factor II gene G20210A mutation is frequently associated with severe thrombotic events and a high rate of recurrence. Venous thrombosis occurs usually in a period of increased thrombotic risk.

**PO-0757** Silent myocardial ischaemia coincides with elevated plasma fibrinogen and plasminogen activator inhibitor (PAI-1) levels

Gruniewicz A, Psiaj P, Zozulińska M, Eltkowski W, Zawilka K* Dept of Internal Medicine, J. Strus Hospital, *Dept of Haematology, K. Marcinkowski University of Medical Sciences, Poznan, Poland

Objective. The aim of the study was to analyse plasma fibrinogen and PAI-1 levels in young survivors of myocardial infarction, free of coronary risk factors but presenting with silent myocardial ischaemia. Design and Methods. The patients were examined at the age of 44 years, who were in a stable condition at least six months after the acute event. They were divided into two subgroups: group A (n=14) with- and group B (n=15) without ischaemic changes in a 24-hour Holter electrocardiogram. The two subgroups were similar in the number of involved vessels visible on the coronary angiography picture. Results. In the group A pts., we found higher mean levels of fibrinogen (3.92 vs 3.23 g/L, p<0.05), PAI-1 antigen (58.1 vs 34.8 ng/mL p<0.01) and PAI-1 activity (4.90 vs 3.40 IU mL, p<0.05) as compared to the controls as well as higher PAI-1 antigen level (58.1 vs 41.6 ng/mL p<0.05) as compared to the group without silent ischaemia. There were no differences between group A and the controls in any of the parameters measured. Conclusions. Our results indicate that elevated levels of plasma fibrinogen and PAI-1 are present only in patients with more severe disease, as revealed by silent myocardial ischaemia. An elevated plasma PAI-1 antigen level might be also considered as predictor of ischaemic events.

**PO-0758** The prevalence of antiphospholipid antibodies in young survivors of myocardial infarction

Lewandowski K, Eltkowski W, Goleciewska M, Turowiecka Z, Zawilka K* Dept of Haematology, Karol Marcinkowski University of Medical Sciences and Dept of Internal Medicine, J. Strus Hospital*, Poznan, Poland

Background. The paradox of prolongation of phospholipid dependent coagulation tests (PFCT) in vitro and the occurrence of thrombosis in vivo has not been completely elucidated. Recently, in patients with antiphospholipid antibodies (APLA) evidence of increased thrombin generation in vivo, with elevated plasma levels of prothrombin fragment F1+2 and fibrinopeptide A was found. Additionally, APA-induced inhibition of the protein C system and a diminished heparin dependent antithrombin III activation were confirmed. Objective. Determination of the frequency of antiphospholipid antibodies (Asserochrom APA, Diagnostica Stago, France) in young patients with myocardial infarction and its relation to the results of the PFCT (activated Partial Thromboplastin Time - aPTT, Kaolin Clotting Time - KCT and diluted Russel Viper Venom Time - dRVVT). Study group: A, consisted of 56 young survivors of myocardial infarction (56M), B, admitted to coronary heart unit with the symptoms of acute myocardial infarction (MI) and C consisted of 30 healthy persons of similar age. Results. In 33.3% subjects (1 case of IgG class) from the control group (C) the presence of antiphospholipid antibodies was confirmed (p<0.001). During the 21-day follow-up, the occurrence of antiphospholipid antibodies was not detected in the plasma of patients from group B. Additionally, in patients from A, B and C groups there was no correlation between APA concentration in the plasma and the results of aPTT, KCT and dRVVT. Conclusions. In young survivors of MI the determination of APA concentration in the plasma may help to choose the appropriate method of vascular disease prophylaxis.

**PO-0759** Urokinase and tissue plasminogen activators in some renal diseases

Polyantseva LR, Andreinkov GV, Podorokskaya LV, Bumbytyte ID Moscow Medical Setchenov's Academy Biological Department of Moscow State University, Moscow, Russia

The fibrinolytic system (FS) as an important system of limited proteolysis takes part in great variety of physiological processes. It acts using two types of plasminogen activators: t-PA and u-PA. It is considered that t-PA dissolves local thromb in systemic and microcirculation, while u-PA ensures different physiological phenomena (cell migration and proliferation, hemostasis, ovulation, etc.). The significance of FS in renal function is well known, but the investigation of the individual roles of u-PA and t-PA is at the beginning.
Background. Vascular damage is one of the major part in rejection episodes. It has recently been shown that pentoxifylline (PTX) treatment improves the outcome of rejection crises in renal transplantation. The aim of this study was to compare biological markers of endothelium lesion and monocyte activation in renal graft recipients with or without PTX Methods. One hundred and forty consecutive patients receiving cadaveric kidney grafts were registered in a randomised double-blind study comparing PTX treatment versus placebo during the first 6 months after transplantation. We prospectively examined before and each month after transplantation the levels of procoagulant activity (tissue factor amicydolic assay) of cultured mononuclear cells with or without endotoxin stimulation (PCA), plasma antigenic levels of thrombomodulin (TM), von Willebrand Factor (VWF), tissue plasminogen activator (tPA) and tumour necrosis factor (TNFα).

Conclusions. This parameter was studied regarding the occurrence of clinical complications. Results. All studied parameters were increased after transplantation and returned to pretransplantation levels within six months. TM was strongly correlated to creatinine levels and TM creait levels were not different in the placebo and PTX groups in patients with and without complications. VWF and IPA were significantly increased in the PTX group in patients with complications without difference between the placebo and PTX groups. PCA evolution showed a significant peak at the first month in patients with complications in the placebo group but not in the patients with complications with PTX treatment and not in patients without complications. In patients with complications, TNFα was significantly lower in the PTX group than in the placebo group. In the PTX group with methyl-prednisone treated rejection crises TNFα was significantly lower during treatment and PCA was significantly lower during the month following rejection than in the placebo group. Conclusions. PTX treatment had no significant effect on markers of endothelium damage which were significantly modified by general complications. PTX treatment was associated with a significant decrease of TNFα and monocyte TF expression in patients with complications including rejection. The protective effect of PTX on graft survival could be related to this biologic effects thus limiting thrombosis and early graft atherosclerosis.

**PO-0764 Effects of pentoxifylline on markers of microvascular damage in patients with renal transplantation**

Susen S Hazzan M, Labalette M, Dessaint JP, Lelièvre G, Jude B, Noel C Laboratoire d’Hématologie, Service de Néphrologie-Hémodialyse-Transplantation, Service d’ Immunologie, Centre Hospitalier-Régional Universitaire, Lille, France

Background. Vascular damage is one of the major part in rejection episodes. It has recently been shown that pentoxifylline (PTX) treatment improves the outcome of rejection crises in renal transplantation. The aim of this study was to compare biological markers of endothelium lesion and monocyte activation in renal graft recipients with or without PTX Methods. One hundred and forty consecutive patients receiving cadaveric kidney grafts were registered in a randomised double-blind study comparing PTX treatment versus placebo during the first 6 months after transplantation. We prospectively examined before and each month after transplantation the levels of procoagulant activity (tissue factor amicydolic assay) of cultured mononuclear cells with or without endotoxin stimulation (PCA), plasma antigenic levels of thrombomodulin (TM), von Willebrand Factor (VWF), tissue plasminogen activator (tPA) and tumour necrosis factor (TNFα). Each parameter was studied regarding the occurrence of clinical complications. Results. All studied parameters were increased after transplantation and returned to pretransplantation levels within six months. TM was strongly correlated to creatinine levels and TM creait levels were not different in the placebo and PTX groups in patients with and without complications. VWF and IPA were significantly increased in the PTX group in patients with complications without difference between the placebo and PTX groups. PCA evolution showed a significant peak at the first month in patients with complications in the placebo group but not in the patients with complications with PTX treatment and not in patients without complications. In patients with complications, TNFα was significantly lower in the PTX group than in the placebo group. In the PTX group with methyl-prednisone treated rejection crises TNFα was significantly lower during treatment and PCA was significantly lower during the month following rejection than in the placebo group. Conclusions. PTX treatment had no significant effect on markers of endothelium damage which were significantly modified by general complications. PTX treatment was associated with a significant decrease of TNFα and monocyte TF expression in patients with complications including rejection. The protective effect of PTX on graft survival could be related to this biologic effects thus limiting thrombosis and early graft atherosclerosis.

**PO-0765 Management of clinical manifestations in inherited thrombophilia: prospective analysis and follow-up of 219 patients**

Trilot N, Gavériaux-Garbez V, Preudhomme C, Watel A, Zawadzki C, Bauters A, Jude B Laboratoire d’hémostase, Hôpital Cardiologue, Lille, France

The clinical management of inherited thrombophilia remains controversial. We present the preliminary results of a non-randomised prospective study of patients with venous thromboembolism (VTE) and identified biological thrombophilia. Methods. From 1993 to 1998, 477 consecutive patients with venous thrombotic disease were eligible for recruitment. The population study consisted of 219 patients (men 42%, women 58%, mean age±SD: 45±16 years); 152 patients (70%) with identified biological thrombophilia. Results. Seventy-five patients experienced pulmonary embolism (34%). Heparin prophylaxis in high risk situations with no life-long oral anticoagulant treatment and without complications (9.1±4.3 vs 11±2.8 ng/mL, p<0.05). Women taking OC had even lower levels of free TFPI than women not taking OC (3.7±1.7 ng/mL, p<0.0001). Conclusions. This study consisted of 219 patients (men 42%, women 58%, mean age±SD: 45±16 years) with identified biological thrombophilia. The age at first thrombosis was 33±14 years. In 92 patients (42%), the first episode was spontaneous. Recurrent VTE was observed in 108 patients (49%), with 2±1.6 VTE episodes. The localisation of the first VTE event was superficial in 144 patients (66%), in the legs in 127 patients (59%), and mesenteric, intestinal or intracranial in 20 patients (9%). Seventy-five patients experienced pulmonary embolism (34%). Heparin prophylaxis in high risk situations with no life-long oral anticoagulant treatment (OA) was proposed in 3 situations: 1) when the thrombotic events clearly occurred in a risk situation (except for AT deficiencies), 2) when the last thrombotic episode was more than 5 years previously or 3) in patients with only AT, high risk situations with no life-long oral anticoagulant (OA) (INR: 2-3) were given in all the other situations. Results. Seventeen patients (8%) were lost to follow-up. Sixty-three patients (31%) were assigned no OA treatment. Recurrence of VTE was observed in 2 HE FVL carriers aged 31 and 69. OA were prescribed to 142 patients (69%). A major haemorrhagic complication occurred in a patient aged 82 (gastrointestinal bleeding) and minor bleeding complications occurred in 3 patients aged 38, 38
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and 65. A total of 33 surgical interventions or pregnancies were carried out using subcutaneous heparin prophylaxis, without thrombotic or haemorrhagic complications. Conclusions. These preliminary results indicate that the risk of recurrence of thrombotic disease in thrombophilic patients is higher than the haemorrhagic risk of OA. These results should be confirmed in larger and randomised prospective studies.

PO-0766 Prothrombotic risk factors in inflammatory bowel disease

Division of Clinical Science, University of Wolverhampton, England

Previous studies have shown that subjects with inflammatory bowel disease (IBD) are at increased risk of thromboembolic events and that the prevalence of this condition in haemophilia is reduced compared to matched controls. The aim of this study was to identify acquired and inherited factors associated with thrombophilic risk in a group of patients with stable chronic IBD. A group of 152 outpatients was investigated. The following parameters were measured: FBC, ESR, CRP, D-Dimer, Fibroingen, Prothrombin time and Partial Thromboplastin time. Additionally, Factor VIII and II were measured using one stage coagulation assays and activated protein C resistance determined using a commercial kit (Quadratett Ltd). Factor XII subsunits were assessed using an in-house ELISA method and thrombin and aPC resistance assays were used as screening tests for Factor II Leiden and V Leiden respectively. Results were compared to laboratory normal ranges. Three percent of patients had abnormal aPC resistance. Mean Factor II levels in the study population were 117 u/dL (s.d. 19.2) and 74% of the values were above the upper limit of our normal range of 56-130 u/dL. Using a normal test and assuming a median value of 100 u/dL for the normal population p<0.001 for both Factor VIII and II. Factor VIII and II d subsunits were assayed with values of 64.0 (s.d. 30-6)u and 71.0u (s.d. 34.0), respectively. There was a significant reduction in Factor VIII in the study group. Using a binomial test again p<0.001 compared to normal controls. Although clinically stable the patient group as a whole has evidence of an ongoing inflammatory response with ESR, CRP, and 71.0u (s.d. 34.0)u, respectively. There was a significant reduction in Factor II Leiden prevalence in the same as in our general population and does not predispose to IBD. The elevated Factor II levels were unexpected. This may be part of an acquired response or be due to over representation of Factor II Leiden in the study population.

PO-0767 The mechanism of effect of desmopressin in haemophilia: the role of plasma platelet activating factor

Karabük Ç, Huseyinol A, Polat A, Aydınoğlu Y, Kantar M, Çetinölüm N, Kansu S, Nidji G
Ege University Hosp. Dept. Pediatric Haematology, Izmir, Turkey

Objective. Desmopressin has been used to treat the patients with haemophilia A (HA) and von Willebrand’s disease (vWD). Despite the intensive use of desmopressin, the exact mechanism of its action is not completely understood. Our aim was to investigate a potential relationship plasma platelet activating factor (PAF) and desmopressin response in these patients. Design and Methods. Twenty-eight desmopressin-treated children (mean age 11±4 years; range 3-24) were enrolled in the study. Twenty of them (10 severe, 5 moderate, 5 mild) had HA and 8 of them (three type 1, one type 2, four type 3) vWD. Desmopressin was given subcutaneously as a single dose (0.3 µg/kg). Citrated plasma samples were collected and studied for FVIII:C and plasma PAF levels for baseline values and one hour after desmopressin administration. An increase of FVIII to levels >50% was used as a response criterion for FVIII and a level of at least 20% was accepted for HA. Measurement of plasma PAF levels have assayed with RIA. Twenty age-matched children were taken as a control group for basal plasma PAF levels. Results. Basal plasma PAF levels were significantly higher in 28 patients with HA and vWD than in healthy children (2336±1815 vs 205±889 µg/ml; p<0.0001). The plasma PAF levels one hour after desmopressin treatment were significantly elevated (from 2609±1020 to 5955±1220 µg/ml; p=0.01) in responders. However, in 15 non-responders, PAF levels did not increase significantly (1932±2172 vs 2199±2587; p>0.05). PAF levels after desmopressin were significantly higher in responders than non-responders (5965±1220 vs 2109±167; p=0.002). Conclusions. The response for higher basal PAF activity might be the hyperactivity of the plasma PAF-acylhydrolase system. These findings strongly suggest a relationship between PAF and response to desmopressin in vWD and haemophilia. PAF may be a secondary mediator for the effect of desmopressin.

PO-0768 Antithrombotic effects of proline-containing peptide PGP

Lypagina LA, Pastorrova VE, Ashmarin IP
Moscow V. M. Lomonosov State University, Faculty of Biology, Department of Human and Animal Physiology, Moscow, Russia

Some proline-rich oligopeptides are effective thrombin inhibitors. They possess anticoagulant properties, can activate fibrinolysis and prevent formation of thrombin in blood circulation. PGP is the most interesting among this peptide group. The purpose of the present study was to investigate the influence of PGP on the blood coagulation system in vivo by various methods of its administration. Experimental rats received intravenous (into the jugular vein) injection of PGP at doses of 40, 200 and 300 µg/kg b.w. Another group received intranasal PGP 100 µg/g. During the first 10 min after the intravenous injection of PGP (40 µg) anticoagulant activity and fibrinolytic indices were increased significantly. Increasing doses (200 and 300 µg) resulted in a very significant increase of both fibrinolytic and anticoagulant potential of blood plasma and decrease of platelet aggregation. Intranasal chronic administration (for 3 days each day) of PGP increased the fibrinolytic parameters, decreased platelet aggregation and the level of factor XII. Thus, PGP-peptide after administration to rats has complex antithrombotic effect by increasing anticoagulant-fibrinolytic potential in blood plasma and decreasing platelet aggregation.

PO-0769 The natural anticoagulants (prot C, prot S and AT-III) in crises and steady state in sickle cell anemia

Killing V, Sayma Z, Karabay A, Arentne B, Tanyelci A, Çakırov University, Faculty of Medicine, Dept. of Pediatric Haematology, Adana, Turkey

Fourteen patients (10 females, 4 males) aged 5-14 yrs (mean 8 yrs 3 mos) are included in this study. Blood samples were taken during crises (5 patients with painful crisis, 2 with pulmonary crisis, 3 with stroke, 1 salmolena arthritis, 1 splenic access, 2 haemolytic crisis; in three patients both infection and stroke and the other factors were coincidental). Another 17 patients (10 F, 7 M) aged 5-14 yrs (mean 9 yrs 8 mos) had blood samples taken in a steady state period. Sixteen (9F, 7M) healthy children with Hb AA pattern was taken as a control group. Routine haematological examination, coagulation and natural anticoagulants (Prot C, Prot S, AT-III) and indirect bilirubin levels were determined in all the patients in crises or in steady state. On haematological evaluation of the crises group; means of parameters were: Hb 7.2±3.5 g/dL, MCV 95.6±6.2, retic counts 6.6±6.2%, ISC 6.4±3.7%. The natural anticoagulants were: Prot C 56.6±26.2%, Prot S 56.4±46.9%, AT-III 84.3±31.9%. The mean value of indirect bilirubin was 2.8±1.5 mg/dL. For the patients in steady state: means of parameters are: Hb 8.0±1.4 g/dL, MCV 90±13.4, retic counts 4.6±2.0%. The natural anticoagulants were: Prot C 80.2±20.9%, Prot S 92.2±11.7%, AT-III 77.1±10.3%. The indirect bilirubin level was 2.6±0.8 mg/dL. In crises group: one patient was found to be AT-III deficient. In the comparison of crises group and steady state group there was no statistical difference between the mean values of AT-III (p>0.05) but there was a statistical difference for Prot C (p<0.01) and Prot S (p<0.05). When the two patient groups were compared with healthy controls, the statistical difference was more significant and important for Prot C (p<0.001) and Prot S (p<0.001). SCA is a disorder which causes vasculopathy. Also, preceeding factors such as deficiencies of natural anticoagulants (Prot C, Prot S, AT-III) contribute in SCA. The purpose of the present study was to investigate the influence of PGP on the blood coagulation system in vivo by various methods of its administration. Experimental rats received intravenous (into the jugular vein) injection of PGP at doses of 40, 200 and 300 µg/kg b.w. Another group received intranasal PGP 100 µg/g. During the first 10 min after the intravenous injection of PGP (40 µg) anticoagulant activity and fibrinolytic indices were increased significantly. Increasing doses (200 and 300 µg) resulted in a very significant increase of both fibrinolytic and anticoagulant potential of blood plasma and decrease of platelet aggregation. Intranasal chronic administration (for 3 days each day) of PGP increased the fibrinolytic parameters, decreased platelet aggregation and the level of factor XII. Thus, PGP-peptide after administration to rats has complex antithrombotic effect by increasing anticoagulant-fibrinolytic potential in blood plasma and decreasing platelet aggregation.

PO-0770 Assessment of homocysteine blood levels in patients with acute coronary artery disease

Bogiraskou I, Triantafillidi H, Vavuranakis M, Kouvorou E, Palatza Z, Stefanidis Ch, Toutzas P
Haematology Laboratory and University Cardiology Department, Hippokratio Hospital, Athens, Greece

Objective. Determination of increased homocysteine blood levels as a new independent risk factor in acute coronary artery disease. Design. Our study population consists of 133 patients (86 male, 47 female) who were divided into 2 groups. Group A (high risk) 84 patients (61 male - 23 female) with acute coronary event (unstable angina, acute myocardial infarction) who underwent coronary arteriography. Group B (low risk) 49 patients (25 males - 24 females) without a history of cardiac or vascular disease. Group A patients were divided into 2 subgroups. Group B (low risk) 49 patients (25 males - 24 females) without a history of cardiac or vascular disease. Methods. In both groups homocysteine levels were measured by a Fluorescence Polarisation Immunoassay method. Results. Homocysteine levels were higher in patients with a history of cardiac or vascular disease. The level of homocysteine was higher in patients who were male and female. The level of homocysteine was higher in patients who were male and female. The level of homocysteine was higher in patients who were male and female. The level of homocysteine was higher in patients who were male and female.
in group A women had higher levels than men, but the difference was not statistically significant. In group B homocysteine levels were higher (p=0.001) in the elderly patients (men >50, women >60 years old). No correlation was found between homocysteine and cholesterol levels in group A. Conclusions. Elevated homocysteine levels were found in a significant percentage of high-risk patients with coronary artery disease. A larger scale study is under way in order to acquire statistically significant evidence concerning the other parameters.

P0-0771 Differences in haemostatic disorders in patients with metastatic malignant melanoma and patients with metastatic testicular carcinoma
Filipovic-Ljekic V, Jelic S, Tormaicic Z, Stamatovic L
Institute of Oncology and Radiology of Serbia, Yugoslavia

Haemostatic disorders are frequent in cancer patients (pts) being detectable in about 50% of pts with localized tumour and in more than 90% pts with metastatic disease. There is an association between the type of malignant disease and the type and grade of haemostatic disorders. In this study pts with disseminated malignant melanoma (dMM) and pts with disseminated testicular carcinoma (dTC) were tested with the aim of determining type intensity of haemostatic disorders in those patients. The same parameters were done in control groups of platelet and blood donors. Results were presented in z-score and z-tailed probability. In both groups of pts with dMM, there was a significant discrepancy between fibrinogen concentration determined by immunological (rid) and functional methods (there is an unusual concentration biologically inactive molecules). In pts with dMM, plasminogen concentration, tested by a functional method (chromogenic method) was significantly increased compared with the normal concentration products (latex technique) were detected in 37.3% of cases, implying that there is an activation of the fibrinolytic process. In both groups investigated thrombin-antithrombin III (TAT) complexes were significantly increased (dTC, \( x \approx 15.3 \mu\text{g/L}, \text{dMM} \approx 43.6 \mu\text{g/L}, \text{healthy donors,} \approx 3.4 \mu\text{g/L} \text{implying the activation of the coagulation system, particularly in pts with dMM. C1 inhibitor concentration was significantly increased in both groups of pts (dTC < 0.361 \mu\text{g/L}, \text{dMM} < 0.436 \mu\text{g/L}, \text{healthy donors,} < 0.228 \mu\text{g/L}; this protein behaves as a parameter of biological evolution of disease. Other investigated parameters: antithrombin III, factor VIII, \text{a2 antiplasmin} were not significantly changed when compared with the control group. Our results reveal that there is a pathological activation of blood coagulation, in both group of pts, but particularly in those with dMM. In this group pathological haemostatic activation is significantly increased and it is followed by activation of the fibrinolytic process.

P0-0772 Thrombotic thrombocytopenic purpura is associated with platelet activation
Allford SL, Harrison P, Mackie IJ, Cohen H, Machin SJ
Haemostasis Research Unit, University College Hospital, London, UK

Objective. Thrombotic thrombocytopenic purpura (TTP) is characterised by endothelial perturbation and platelet aggregation. Since platelet aggregation initiates platelet activation we sought to study this phenomenon in both acute and chronic TTP. Methods. A whole blood flow cytometric method was used, platelets being identified by expression of CD61 whilst activation was measured by surface expression of CD62p (P-selectin) and CD63. Samples were collected under basal conditions and in the presence of 10 \mu\text{M ADP and 80 \mu\text{M TRAP}. In addition, quantification of reticulated platelet. Samples were analysed under basal conditions and in the presence of 10 \mu\text{M ADP and 80 \mu\text{M TRAP}. In addition, quantification of reticulated platelet number was performed by a dual labelling technique. Significant number was obtained over a period of three to five months from two patients with testicular carcinoma (dTC) were tested with the aim of determining type intensity of haemostatic disorders in those patients. The same parameters were done in control groups of platelet and blood donors. Results were presented in z-score and z-tailed probability. In both groups of pts with dMM, there was a significant discrepancy between fibrinogen concentration determined by immunological (rid) and functional methods (there is an unusual concentration biologically inactive molecules). In pts with dMM, plasminogen concentration, tested by a functional method (chromogenic method) was significantly increased compared with the normal concentration products (latex technique) were detected in 37.3% of cases, implying that there is an activation of the fibrinolytic process. In both groups investigated thrombin-antithrombin III (TAT) complexes were significantly increased (dTC, \( x \approx 15.3 \mu\text{g/L}, \text{dMM} \approx 43.6 \mu\text{g/L}, \text{healthy donors,} \approx 3.4 \mu\text{g/L} \text{implying the activation of the coagulation system, particularly in pts with dMM. C1 inhibitor concentration was significantly increased in both groups of pts (dTC < 0.361 \mu\text{g/L}, \text{dMM} < 0.436 \mu\text{g/L}, \text{healthy donors,} < 0.228 \mu\text{g/L}; this protein behaves as a parameter of biological evolution of disease. Other investigated parameters: antithrombin III, factor VIII, \text{a2 antiplasmin} were not significantly changed when compared with the control group. Our results reveal that there is a pathological activation of blood coagulation, in both group of pts, but particularly in those with dMM. In this group pathological haemostatic activation is significantly increased and it is followed by activation of the fibrinolytic process.

P0-0774 Thrombotic thrombocytopenic purpura in pregnancy: two new cases
Alvarez MT, del Pozo I, Rodriguez de la Rua A, Fernández MC, Morado M, Sevilla J, Hernández MC, Ojeda E, de la Cámara C, Hernández Navarro F
Haematology Department, Hospital “La Paz”, Madrid, Spain

Objective. Thrombotic thrombocytopenic purpura (TTP) complicating pregnancy is associated with maternal morbidity and mortality. Intrauterine foetal death is also a common complication of these pregnancies. We report two new cases of severe TTP, according to the Rose and Eldor score, in whom a complete response was achieved following an elevated number of courses of plasmapheresis. Case #1: A 25-year-old woman, at 27 weeks of gestation, with clinical and laboratory criteria for TTP came to our centre after 9 courses of plasmapheresis. The concomitant treatment included glucocorticoids and dipiridamole. Blood count showed haemoglobin 7.2 g/dL, platelets 11×10^11/L and LDH 3100 UI/L; the reticulocyte studies were normal. The patient began treatment with daily plasmaphereses but renal function worsened and night hemiparesis, dysphagia and hypertension were observed. At that time dMM was suspected and confirmed by flow cytometry.

Conclusions.

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PO-0775 Study of venous thromboembolic disease in readmitted patients

Cuevas B, Ruiz ML, Cuevas MV, Polo A, Lasierra J
Haematology Service, San Millán Hospital, Logroño; *D'Unino Valles Hospital, Burgos, Spain

Introduction. Venous thromboembolic disease (deep venous thrombosis and pulmonary embolism) can be caused by readmission after discharge from hospital. Design and Methods. The study was carried out on 68 patients who were readmitted during the two months after discharge with symptoms of venous thromboembolic disease. The analysis of the clinical histories enabled us to know which service the patients were previously treated in, the extension of the thromboembolic disease, whether the patients had received prophylaxis with low molecular weight heparin, the number of days in hospital after readmission and the number of days between discharge and readmission. Results. The 68 patients (32 women and 36 men) were aged 24-92 years (average 69 years). The originating services were: Traumatology (31), Surgery (8), Gynaecology (5), Pneumology (5), Ophthalmology (5), Internal Medicine (4), Urology (2), and Cardiology, Haematology, Thoracic Surgery, Plastic Surgery, Endocrinology, Otolaryngology, Neurosurgery and Neurology one case each. Forty-eight patients had deep venous thrombosis and 20 patients had pulmonary embolism. Two patients died from pulmonary embolism. Thirty-seven of the patients had no prophylaxis and 22 % had prophylaxis with low doses. The duration of stay of the readmitted patients was between 1 and 68 days, average 15.9 days. The frequencies of the time the readmission occurred after discharge were the following: 1 week (12 (18 %), 2 week (22 (32 %), 3 week (17 (25 %), 4 week (4 (6 %) and, after more than 4 weeks, 13 patients (19 %). Conclusions. These results suggest extending the use of prophylaxis use in hospital and continuing it in the patient’s home for 30 days in order to reduce the incidence of thromboembolic disease after discharge from hospital.

PO-0776 Haemocoagulative alterations in patients with acute myocardial infarction: genetic transmission

Ruiz ML, Cuevas B, Polo A, Lasierra J
Haematology Service, San Millán Hospital, Logroño, Spain

Objective. The aim was to be statistical analysis of several haemocoagulative parameters as possible risk factors in patients with acute myocardial infarction (AMI) and in close relatives (brothers, sisters and children) and compare the results with those found in the control group (Co). Design and Methods. The following haemocoagulative parameters were determined: intact fibrinogen (Int Fg), functional fibrinogen (F Fg), the ratio F Fg/Int Fg, tissue plasminogen activator inhibitor (t-PA Ag), plasminogen activator inhibitor agonist (PAI Ag), in 51 patients (Pat) affected by acute myocardial infarction, 39 brothers and sisters (BS) and 63 children (CH) of the patients and 42 people belonging to a control group. Results. After the statistical study we found these results:

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<th>Int Fg</th>
<th>F Fg</th>
<th>F Fg/Int Fg</th>
<th>t-PA Ag</th>
<th>PAI Ag</th>
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Conclusions. 1) Significant differences were found between the patients with AMI and the control group in all parameters studied. 2) Significant statistical variations were found between brothers and sisters of patients and the control group in all parameters determined. 3) We observed significant differences between patients’ children and the control group in all parameters studied except intact fibrinogen.

PO-0777 Thrombin induction of ICAM-1 on monocytes and the relationship between monocyte ICAM-1 and plasma ICAM-1 in pregnancy

Clark P, Boswell F, Pearson C, Walker ID, Greer IA
Department of Haematology and *O batie and Gynaeology, Royal Infirmary, Glasgow, Scotland, UK

Objective. Thrombin influences the inflammatory response via a trans-membrane receptor which is present on a variety of cells including monocytes. The effect of thrombin on monocyte ICAM-1 expression (mICAM-1) is unknown. A soluble form of ICAM-1 in plasma (sICAM-1) is widely used as a marker of inflammation, although its relation to cellular expression in vivo is unclear. Design and Methods. Using flow cytometric analysis, the in vitro dose-response relationship between mICAM-1 and thrombin was examined (n=8), mICAM-1 and sICAM-1 were also examined in pregnancy (n=149), and in normal non oral contraceptive pill-using women (n=18). Results. In vitro, a dose-response relationship between human alpha thrombin and mICAM-1 was observed after 24 hours incubation, with a maximal increase in expression of 184%. This response was confirmed with the thrombin receptor agonist SFLLRN and was inhibited by hirudin. These observations were confirmed in the monocyte cell line THP-1. In pregnancy (which is associated with elevated thrombin generation) a significantly higher level of mICAM-1 was observed (mean 18.361 Antibody Binding Capacity by ABC, SD 4.499) than in non-oral contraceptive using females (mean 15.554 ABC, SD 16.825, p=0.003). No difference in sICAM-1 levels was observed. No relationship between mICAM-1 and gestation was observed, however a relationship between mICAM-1 and sICAM-1 was noted (R=0.12 %, p=0.0001). A higher level of sICAM-1 was observed in smokers. Conclusions. These results indicate a specific interaction whereby thrombin may influence the antigen presenting function of the monocyte and also suggest that, in pregnancy, sICAM-1 may reflect cellular expression.

PO-0778 Treatment of life-threatening bleeding in haemophiliacs

Pašić D, Spasojević J, Stavić I, Popović P, Mihajlović M, Uzurov V, Savči A
Clinical Centre Novi Sad, Clinic of Haematology, Yugoslavia

Life-threatening bleeding in patients with haemophilia includes haemorrhages in critical locations (intracranial bleeding into the brain), bleeding resulting from trauma or surgical procedures with significant blood loss. Intracranial haemorrhages in haemophiliacs with severe deficiency of FVIII are life-threatening complications with high mortality. In addition to long-term replacement therapy patients very often require a risky surgical procedure. Management of an acute bleeding episode in haemophiliacs with antibodies demands high doses of FVIII concentrate in patients with low titer, or factor VIIIIC by-passing agents (prothrombin complex concentrates, Fxa, Fvila, tissue factor) in patients with high titre. In our institution ten haemophiliacs with severe or moderate FVIIIC deficiency and one woman with previously unrecognized antibodies to FVIIIc were treated due to intracranial haemorrhage (subdural 4, intraventricular 1, intracerebral 2) and abdominal bleeding (from gastric ulcer 1, into omentum 1, retroperitoneal with secondary infection 1, and into abdominal wall with intestinal obstruction 1). Cerebral and abdominal haemorrhages were caused by minimal trauma in ten patients. Two patients had high titre of antibodies to FVIIIc. Replacement therapy by huge doses of factor VIII concentrates was used in nine patients, and activated prothrombin complex concentrate (FEIBA) given to two patients. Six patients were successfully treated surgically: four patients with intracranial bleeding and coma by craniotomy and two patients with a picture of acute abdominal emergencies and haemorrhages was performed laparotomy. Local therapy by Fibrin was useful in one patient with high titre of antibodies and persistent bleeding from a gastric ulcer. Our experience confirms the feasibility for successful treatment of life-threatening bleeding in haemophiliacs, which depends on the possibility of sufficient replacement therapy, monitoring of hemostasis and team work.

PO-0779 Correction of over-anticoagulation: does oral vitamin K help?

Voke JM ,* Allain S, Thompson DS*
*Departments of Haematology and *Pharmacy, Luton & Dunstable Hospital, S. Beds, UK

Over-anticoagulation without bleeding is a worrying occurrence in all anti-coagulant clinics. If correctly prompted it is usually of no clinical consequence, but the risk of major haemorrhage is known to increase as the INR rises above the therapeutic range. A simple method of lowering the INR within 24hr in patients with high INRs would improve the safety of anticoagulation. Although iv vitamin K reduces the INR within a few hours this is not often used for non-bleeding outpatients due to the risk of reactions and the logistics of modern anticoagulant clinics with many patients receiving their results and dosage advice when already back at home. Oral vitamin K has generally been dismissed as an unreliable treatment but recent studies suggest it may be effective. Methods: We compared 5 mg and 10 mg doses of oral vitamin K (menadione) to correct over-anticoagulation (INR >6.9) in 58 uncomplicated outpatients on long-term warfarin. In addition 9 control patients were not given vitamin K. All patients stopped warfarin for 1-2 days before restarting at a lower dose. Patients were only included if the INR was 7.0 or above and there was no bleeding reported on specific questioning, no complicating acute illness requiring admission or treatment, no hepatic or renal dysfunction and they were able to return for repeat INR 24 hours and 7 days later. Capillary INRs (Manchester thromboplastin manual method) were used for prompt results. Results. Eight of the 9 patients without vitamin K had INR >4.5 after 24hr in spite of stop-
ping warfarin. Of the 27 patients given 10 mg menadion, 15% (4/27) had INR >4.5 after 24 h, 37% (10/27) had INR 2-4.5 and 48% (13/27) were below 1.5. Twenty-four hours after a 5 mg dose, 42% (13/31) had INR >4.5, 42% were within the INR range 2.4–4.5 and 16% (5/31) had INR values below 2.0. The mean INR in the 10mg group fell from 9.1 to 2.6 and in the 5mg group from 9.9 to 4.2 after 24 hours. Conclusions. Oral menadion is effective in partially correcting or anticoagulation when the INR is 7 or above in non-bleeding warfarinised outpatients without complicating illness. The study suggests that a 5 mg dose is preferable for patients in whom a high therapeutic range is desirable, with a 10 mg oral dose for patients requiring a lower range.

PO-0780 Once daily treatment with low molecular weight heparin (LMWH) for the treatment of extensive deep vein thrombosis (DVT) in pregnancy

Velang M, Lincoln K, Hutchison RS, Hudson J

Middlesex General and South Cleveland Hospitals, Middlesbrough, UK

Concerns regarding the use of unfractionated heparin (UFH) in pregnancy and difficulties in monitoring during the third trimester have stimulated interest in LMWH. Whilst thromboprophylaxis with LMWH is well described, there are few published data on its use in pregnancy. Our aim was to evaluate the efficacy and safety of a single daily injection of a LMWH (Deltaparin) for the once daily outpatient treatment of established DVT in two patients. A 19 year old woman in her second pregnancy presented at 35 weeks gestation with non-congestive iliofemoral DVT, made worse with intake of intravenous (IV) UFH. Subsequently she was commenced on Deltaparin 10,000 IU once daily, the last injection being 17 hours prior to uncomplicated spontaneous vaginal delivery (mean anti-Xa 0.36 IU/mL (range 0.3-0.41) with the dose increased after one week to 15,000 IU (0.64 IU/mL). The second 28 year old primiparous woman presented at 32 weeks of gestation with a large deep vein DVT. Following one week on UFH, she was commenced on 10,000 IU Deltaparin once daily for 2 days (mean anti Xa levels 0.35 IU/mL [range 0.32-0.34] measured 4 hours after injection and 0.13 IU/mL [0.1-0.15] prior to each injection). Further leg pain necessitated 5 days further IV UFH followed by 15,000 IU Deltaparin (peak anti-Xa: 0.38 IU/mL) until 24 hours prior to elective Caesarean section for a footling breech presentation. A single daily injection facilitates outpatient management. Peak anti-Xa levels achieved in our patients were proportionally low given concerns regarding the long half-life of LMWH, the potential for reversal and risk of unplanned labour and delivery. However, APTT and thrombin time were both normal at peak heparin concentrations. Despite this conservative dosing regime, we have established the presence of heparin at 24 hours. Further LMWH dosing studies with anti-Xa measurement are required to establish the optimal schedule for the successful outpatient management of thromboembolic disease in pregnancy.

PO-0781 FV Leiden, prothrombin G20210A and MTHFR C677T mutations in Portuguese patients with arterial and deep venous thromboses

Fidalgo T, Ribeiro ML,* Pinto CS, Abade A, Tamagnini A*

*Unidade Haematologia Molecular, Centro Hospitalar de Coimbra, Coimbra, Portugal; °Departamento de Antropologia da Universidade de Coimbra, Coimbra, Portugal

Thromboembolism is common in Europeans and causes significant morbidity and mortality. The pathogenesis is complex, and associates several genetic predispositions with environmental risk factors. The deficit of natural inhibitors of coagulation antithrombin III, protein C and protein S is implicated in only 5-10% of cases of thrombophilia. Other genetic anomalies have been described - FV Leiden, Methylene Tetrahydrofolate Reductase (MTHFR) C677T and Prothrombin variants 20210A and 20210G which predispose to a hypercoagulable state. FV Leiden has been reported as the most common known genetic risk for thrombosis (20-40%). The aim of the study was: 1) to evaluate the prevalence of these mutations in Portuguese patients with stroke, myocardial infarction and deep venous thrombosis; 2) to analyse the influence of circumstantial risk factors and cumulative effect of the genetic markers in four families with a deficit of protein S and variable expression. Design and Methods. Blood was collected from sixty-one individuals (316 patients and 145 controls) were studied. Genomic DNA was isolated from white blood cells by standard methods and studied by Polymerase Chain Reaction (PCR), Single Strand Conformation Analysis (SSCA), Restriction Fragment Length Polymorphisms (RFLP). Functional and antigenic Protein S was quantified by coagulation methods and ELISA, respectively. Results and Discussion. The prevalence of FV Leiden was 3.4% in the control group and 20.5% in individuals with venous thrombosis (p= 0.001). In this group homozygosity for the MTHFR 677CT T was also higher than in the controls (12% vs 5%) and significant. The prothrombin variant 20210G/A had a similar prevalence in both groups (3%). In those families with protein S deficiency 9 of 18 individuals also carry the MTHFR or the FV Leiden mutations. These individuals have a more severe phenotype with an earlier presentation and more frequent thrombotic events.

PO-0782 Prothrombin 20210A and prothrombin activity in young patients with cerebral ischaemia

Gónorz Garcia EB, van Goor MP,* Leebek FWG, van der Poel SCPAM, van Vliet HDHM, Jansen JH, Dippel DWJ*

Dpts. of Haematology and *Neurology, University Hospital D/znikt, Rotterdam, The Netherlands

The Go to A transition in the 3'-UT region of the prothrombin gene, has been reported to be a risk factor for venous thrombosis (VTE) and is associated with high prothrombin levels. Whether this prothrombin 20210A variant is also a relevant genetic risk for arterial thrombosis in general, and for cerebral ischaemia (C.I.) in particular, is unclear. In addition, the role of hyperprothrombinaemia, a risk factor for VTE by itself, in this patient population is unknown. The aim of the study was to investigate the prevalence of 20210A prothrombin and the prothrombin levels in young (45 years or less) patients with C.I. Design and Methods. We retrospectively studied 56 Dutch males and females, mean age: 35.9±6.9 years, diagnosed as having C.I., confirmed by CT-scan, in the previous three months. Twenty patients presented with TIA and 36 patients with ischaemic stroke (IS). Prothrombin concentration was determined by a chromogenic assay using Ecarin (Pentapharm, Kordia) as activator and expressed in U/mL. Detection of prothrombin 20210A was done simultaneously with FV Leiden by multiplex PCR of the involved regions of FII and FV performed in whole blood, as previously described by us. Results. Five patients (9.8%) were found to be heterozygous for prothrombin 20210A (OR=4.1, 95% CI: 1.4-12.3, when compared to a 2.3% population prevalence, published by Poort et al. Blood 1996; 88: 3698-703). Their mean prothrombin value was 1.80 U/mL (95% CI: 1.4-2.2). Among the 48 patients without the prothrombin variant who were not anticoagulated at the time of study, the mean prothrombin activity was 1.31 U/mL (95% CI: 1.2-1.4) and 36% had prothrombin levels above 1.32 IU/mL, our upper normal level. Among the patients with hyperprothrombinemia 71% were women, of whom 64% used oral contraceptives (OAC), as opposed to 25% use of OAC among the women with prothrombin levels <1.32 U/mL (OR: 5.4, 95% CI: 1.03-27.8). Conclusions. Our results suggest that the prothrombin 20210A allele is a risk factor for cerebral ischaemia in young patients. Discrepancies from other series could be related to patient selection criteria and/or geographic origin. In addition, the finding of hyperprothrombinemia in the absence of a known genetic mutation is a common finding among these patients, which seems to be associated with the use of OAC and might also be of pathogenetic importance.

PO-0783 Early initiation of vincristine in the management of thrombotic thrombocytopenic purpura

Retomaz F,* Durand JM, Poulillot P,* LeFevre P,* Soubyrand J

*Département de Internal Medicine, °Département de Haematophasie, Marseille, France

Objective. The current established treatment of thrombotic thrombocytopenic purpura is plasma exchange (PE) with fresh frozen plasma. When patients fail to respond to PE the prognosis is poor. Few patients have been successfully treated with Vincristine. To decrease the mortality and the morbidity and avoid the refractory phase, we used combined treatment with VCR and PE as first line therapy. Design and Methods. Ten patients with idiopathic TIP were treated during the last ten years. There was 5 men and 5 women with a median age of 40.4 years (range 22 to 66). They received combined treatment with vincristine (1 mg over 2 or 3 week) and PE with fresh frozen plasma continued until patients achieved remission. Response was evaluated at day 8, at the end of the first episode and after six months. Results. After the first cycle of treatment (Day 8), 4 patients (40%) had a complete response, 5 patients (50%) had a response, and one patient (10%) had no response. At the end of the first episode nine patients (90%) were in complete remission. One patient died during the first period procedure. The survival rate at one year was 100%. The survival rate at two years was 86%. Four patients had a relapse within 2 months to 2 years. Three patients died. The survival rate is 70%. Conclusions. These results are better than those previously published and confirm that early initiation of Vincristine therapy in conjunction with PE is beneficial in TIP. This association does not, however, prevent the relapse of this disease. We believe that combined treatment with Vincristine in addition to plasmapheresis must be done on a larger basis.
Endothelial cells (ECs) in culture synthesise and secrete uricinase-type plasminogen activator (u-PA), but the normal vascular endothelium is believed to synthesise only tissue plasminogen activator (t-PA), which is thought to be responsible for intravascular fibrinolysis. More recently, studies have shown that the biological role of u-PA in fibrinolysis has been underestimated, prompting a re-examination of its synthesis by the endothelium. In this study, we investigated whether u-PA was synthesised by non-atherosclerotic endothelial cells in vivo by testing venous ECs lodged by punctures from 12 normal volunteers and 17 patients admitted for plasmapheresis. The ECs were isolated with an anti-endothelial monoclonal antibody coupled to immunomagnetic beads and characterised morphologically and by labelling for vWF, CD31 and UEA-1 binding. U-PA antigen was found in 50% of the normal and in 60% of those from patients. U-PA enzymatic activity on zymogens was detected in 50% of the normal and 60% of the patient samples, with the latter also being more frequent and strongly positive. U-PA mRNA was found in all the normal and patient samples tested. The results indicate that u-PA is synthesised by the venous endothelium in vivo but that its expression is highly variable.

Thromboembolic events, protein C (PC) and protein S (PS) deficiencies, several platelet function anomalies and procoagulant activity of red blood cells have been reported in p-thalassaemia major (TM). Double heterozygosity for factor V R506Q and prothrombin G20210A mutations have been reported in p-thalassaemia major (TM). Double heterozygosity for factor V R506Q and factor II G20210A mutations seems to be linked with unprovoked thrombosis in a patient with TM bearing double heterozygosity for both factor V R506Q and factor II G20210A. A 21-year-old patient with TM was admitted with deep vein thrombosis in 1996 and 1997. Among his 13 sisters and brothers, 5 had TM and died before the age of 7. This patient has received red cell pack transfusions since the age of 4. Hydroxyurea since 1994 and underwent splenectomy in 1996. He was treated with unfractionated heparin followed by long term oral anticoagulants with good recovery. Investigations of thromboembolic risk factors were performed and revealed antithrombin, PS and atypical protein C (APC) resistance (8.8% vs 0%), PC deficiency (2.2% vs 0%) and APC Resistance (11.1% vs 2.8%). DNA analysis revealed that all the patients with APC Resistance phenotype were heterozygotes for FV Leiden. One more genetic thrombophilic factors were present in 22.2% of the patients, but in only 2.8% of the control group. Our findings suggest the existence of a genetic thrombophilic background in some patients with ON of the femoral head. Inherited coagulation disorders, such as APC resistance, suggest the existence of a genetic thrombophilic background in some patients with ON of the femoral head. Inherited coagulation disorders like APC resistance may lead to bone death. Various genetic defects of the coagulation mechanism have been established as risk factors for thromboembolic disease and in the present study their correlation to the development of ON was assessed. In 45 patients (34 males and 11 females, of a mean age of 34 years with ON of the femoral head, and in a control group of 70 healthy blood donors, a search for genetic coagulation disorders was performed. Protein C, ATIII and APC-Ratio were determined using platelet poor plasma. The G-α mutation of the nucleotide 1691 of the factor V Leiden was detected by PCR amplification of fragment 287 bp includin further modification of the restriction endonuclease Alu I analysis. Patients with ON, compared to the group of healthy controls, had an increased incidence of protein C deficiency (8.8% vs 0%), ATIII deficiency (2.2% vs 0%) and APC Resistance (11.1% vs 2.8%). DNA analysis revealed that all the patients with APC Resistance phenotype were heterozygotes for FV Leiden. One or more genetic thrombophilic factors were present in 22.2% of the patients, but in only 2.8% of the control group. Our findings suggest the existence of a genetic thrombophilic background in some patients with ON of the femoral head. Inherited coagulation disorders appear to be etiological factors, participating in the complex pathogenesis of ON. This observation may lead to early identification of patients at risk and development of new treatment approaches for ON of the femoral head.

Clinical importance of hyperfibrinogenemia in haematologic malignancies

Objective. Fibrinogen is one of the main plasma proteins and plays a very important role in coagulation and fibrinolytic processes as well as in primary haemostasis. In addition, fibrinogen is an acute phase proteins. The role of fibrinogen is different in blood coagulation pathophysiology and under the circumstances of fibrinogen reactive changes without haemostatic disturbances. It is not easy to say whether expression of hyperfibrinogenemia without consequences of thrombin generation must be the mark of hypercoagulability. However, the antithrombotic drugs can be effective in all cases of thrombotic events. The most useful drugs in these cases can be glycosaminoglycans - low molecular weight heparins (LMWH). Design and Methods. The clinical relevance of hyperfibrinogenemia (fibrinogen >4.0 g/L according to Clausus) was analysed in 109 patients suffering from haematologic malignancies with and without signs of coagulopathy and the response of hyperfibrinogenemia to antithrombotic therapy. The coagulation profile was evaluated by fibrinogen level, APTT, PT, ethanol-gefiction test (EGT), AT level and D-dimers (using standard methods). Coagulopathy was characterised as prolongation of coagulation times, decreasing of AT and D-dimers positivity. Thrombin generation was reflected by EGT positivity. Results. In our cohort of patients we found hyperfibrinogenemia in 30 patients (27.5%). In 8 hyperfibrinogemic patients there was throm-
Platelets and related disorders

**PO-0789** Acquired factor XII deficiency in liver disease

Kyriakou D,* Mavraki E,* Chakiadakis G,* Tsousiakis J,* Alexandrakis M,* Panoussi A,* Vastakis S *
Departments of Haematology, and *Surgery, University Hospital of Heraklion, Heraklion, Crete, Greece

Three patients with liver disease and prolonged activated partial thromboplastine time (APTT) on routine tests are presented. One woman had metastatic liver disease from gastric carcinoma, a second one had autonom-ne hepatitis, and one man had chronic hepatitis B. APTT was not corrected after mixing experiments with 25%, 50%, 75% of normal pooled plasma indicating the presence of an acquired inhibitor. In all three cases factor X/XII coagulant activity was reduced: <1%, <1%, and 3%, while all the other coagulation factors were normal. In all three cases no other auto-antibody was detected. In the first patient APTT was normalised after a left liver lobectomy while the primary lesion remained uninfected. In the second patient the FXII activity improved after corticosteroid therapy but never returned to normal values. In the third patient the APTT improved after hydroxychloroquine therapy. None of the patients had haemorrhagic or thrombotic phenomena.

**PO-0790** Detection of activated platelets by flow cytometric analysis of antigens CD62P and CD63

Kralj L
Department of Haematology, Clinical Centre Ljubljana, Slovenia

Background. Platelets may become activated in a number of disorders such as atherosclerosis, venous thrombosis and cancer. Therefore, the detection of activated platelets might facilitate the identification of certain thrombotic disorders and the evaluation of therapeutic strategies to prevent platelet activation. Previous methods for assessing platelet activity have been indirect, and quantification has been difficult, precluding their use in the clinical area. A promising new method is flow cytometry, which uses monoclonal antibodies specific for a glycoprotein marker of platelet activation expressed on the membrane surface following activation. Further evaluation of the technique is necessary before recommending its use out side the research setting. Methods. In the present study, cell surface expression of granule membrane antigens P-selectin (CD62P) and lysosome antigen CD63 were examined by flow cytometry in 30 healthy donors to determine their utility as markers of in vivo and in vitro platelet activation.

Results. Unstimulated platelets from 30 healthy donors had low levels of CD62P (5.2±1.2%) and CD63 (8.1±1.4%). There were differences in percentages of activated platelets upon stimulation with various agonists. Antigen CD62P were found upon stimulation with ADP in 4.1±0.8%, with ADP and ADR in 46.1±9.2%, with thrombin in 41.5±5.2% and with collagen in 40.5±4.8% human blood platelets. Antigen CD63 were found upon stimulation in ADP in 16.2±2.3%, with ADP and ADR in 16.5±6.0%, with thrombin in 26.0±5.6%, and with collagen in 19.2±6.2% human blood platelets. Conclusions. The results suggest that 1% solution of paraformaldehyde should be used for preventing of artifactual induction of platelet activation in blood samples. Platelets are likely to become activated by different agonists in different clinical situations. Therefore, it seems necessary to detect activated platelets by a panel of monoclonal antibodies.

**PO-0791** Platelet membrane glycoprotein analysis with flow cytometry in 8 cases with Glanzmann’s thrombasthenia

Yenerel MN, Aktan M, Nalçaci M, Keskin H, Peçekelen Y
Istanbul University, Istanbul Medical School, Department of Internal Medicine, Division of Haematology, Çapa, Istanbul, Turkey

Glanzmann's thrombasthenia is an autosomal recessive disorder of platelet aggregation characterised by a lifelong bleeding tendency due to quantitative or qualitative abnormalities of platelet membrane glycoproteins IIb-IIIa (GpIIb-IIIa). It is reported that methods using monoclonal antibodies (MoAb) are highly sensitive and specific as a means of demonstrating the thrombasthenic defect in the platelet membrane. Therefore we did flow cytometric studies on platelets of 8 patients diagnosed as having GT based on a prolonged bleeding time and absence of platelet aggregation in response to ADP, epinephrine and collagen but not ristocetin. Specific MoAbs for platelet GpIIb (CD41), GpIIa (CD42b), GpIIb (CD61) were used in the flow cytometric assays which were performed on the whole blood. The results showed that decreased levels of either GpIIb or GpIIa on platelets were less than control levels in 7 of 8 cases. Decrease in GpIIb levels was more pronounced than that of GpIIa in all except one case. Only in one patient was neither GpIIb nor GpIIa found to be diminished significantly (GpIIb 92%, GpIIa 62%). This patient was thought to have a variant form of GT due to a qualitative GP receptor defect. In conclusion, platelet analysis by flow cytometry provides an alternative rapid diagnostic procedure in most cases of GT.

**PO-0792** The antiplatelet activity of Escherichia coli lipopolysaccharide, is mediated through the nitric oxide/cyclic GMP pathway

Sheu JH, Hung WC
Graduate Institute of Medical Sciences, Taipei Medical College, Taipei, Taiwan

In this study, Escherichia coli LPS dose-dependently (100–500 µg/mL) and time-dependently (10–60 min) inhibited platelet aggregation in human and rabbit platelets stimulated by agonists. LPS also dose dependently inhibited intracellular Ca2+ mobilization in human platelets stimulated by collagen. In addition, LPS (200 and 500 µg/mL) significantly increased the formation of cyclic GMP but not cyclic AMP in platelets. LPS (200 µg/mL) significantly increased the production of nitrate within a 10-min incubation period. Furthermore, LPS also dose-dependently inhibited platelet aggregation induced by PDBU (30 µM), a platelet activator. These results indicate that the antiplatelet activity of Escherichia coli LPS may be involved in the activation of the nitric oxide/cyclic GMP pathway in platelets, resulting in inhibition of platelet aggregation. Therefore, LPS-mediated alteration of platelet function may contribute to bleeding diatheses in septicemic and endotoxicemia.

**PO-0793** CD10 positivity in children with acute immune thrombocytopenic purpura

Kocak U, Ozturk G, Günel T
Kırıkkale University Medical School, Dept of Pediatrics, Kırıkkale, Gazi University Medical School, Dept of Pediatric Haematology, Ankara, Turkey

Acute immune thrombocytopenic purpura (ITP) is a benign haematologic disorder affecting children during the first decade. It is characterised by thrombocytopenia due to immune-mediated platelet destruction. The aim of this study was to investigate the role of lymphocytes in the pathogenesis of acute childhood ITP and to evaluate the relation between immunophenotypes of the bone marrow cells and the prognosis. We studied lymphocyte immunophenotypes of the bone marrow in 11 children with acute ITP at the time of initial diagnosis. Three girls and eight boys were enrolled in the study and their mean age was 5.9 years. Eight children had previous upper respiratory tract infections and one child had a history of varicella infection. Viral serologic examinations (anti-immunoglobulin M for cytomegalovirus, Epstein-Barr virus and rubella virus) were negative in all children. CD10 positivity was observed in three children who had previous viral infections and in whom HLA-DR and CD19 were also positive. Only one of the 3 patients with positive CD10, was found to be positive for CD3, CD34 was negative in all of the 11 children whereas HLA-DR was positive in 9 of them. CD4 was positive in only one and CD8 was positive in 5 of these patients. None of the children with positive CD4 or CD8 was positive for CD10. Previous viral infections were associated with five patients who were positive for CD3 and four patients with CD6 and one patient with CD4 positivity. All of the patients were responsive to high dose methylprednison treatment. During the one year follow-up one patient had episodes of recurrences and three patients with positive CD10, CD4 or CD8 were diagnosed as having chronic disease. No evidence of acute lymphoproliferative disease was seen in any of the patients during
Srzenti results demonstrate that antiaggregatory activity of diquertin may be to
analogus. We studied the effect of diquertin on platelet aggregation and the release of
toxin B (TxB2) in human platelets after collagen stimulation. Platelet aggregation was determined using a "PICA" aggregator. The
agents used were ADP at a final concentration of 1.5 µM, collagen 4
aggregates reduction by 50% was found in all groups, but transfusion treatment with platelets was necessary only in patients with TMA (Group III).

PO-0794 Effect of diquertin on platelet function

Rudko I, Yadigarova Z, Kubatiev A, Tyukavkina N
Department of General Pathology and Pathophysiology, Medical Academy
of Postgraduate Education, Moscow, Russia

Flavonoids are a vast group of natural substances that possess anti-inflam-
atory, antioxygenant and anti-aggregatory activities. Diquertin is 3.3.4.5.7-
Flavonoids are a vast group of natural substances that possess anti-inflam-
atory, antioxygenant and anti-aggregatory activities. Diquertin is 3.3.4.5.7-

PO-0795 Effect of interferon on platelet functional activity

Yadigarova ZT, Kubatiev AA
Department of General Pathology and Pathophysiology Medical Academy
of Postgraduate Education, Moscow, Russia

The role of lymphocyte-platelet interactions and lymphokin ones produced by
lymphocytes in the pathogenesis of inflammatory processes and disorders of
haemostasis is of great interest. The effect of human interferon (INF) on
platelet functions was studied in platelet rich plasma (PRP) and suspension
of washed platelets (SWP) isolated from healthy controls. Platelet aggrega-
tion was determined by the standard turbidimetric technique using a
"PICA" aggregometer. Interferon induced stores (INF or DPH) that are specific for each patient. The ability of patients' sera to inhibit the
binding of monoclonal mouse antibodies to glycoproteins is measured to
detect human autoantibodies against GPIV, GPIb, GPIa/IIa and GP Ib/IX. The quantification of platelet-associated immunoglobulins are
assessed by comparison with values of glycoprotein availability thresholds of
normal platelets and free autoantibodies are estimated by comparison between
a negative sera. Sera of 40 normal subjects were analysed to give
the virtual number of normal platelets and free autoantibodies are estimated by comparison between
a negative sera. Sera of 40 normal subjects were analysed to give

PO-0796 Influence of cardiac surgery with extracorporeal circulation on

this period. As haemato poetic stem cells and progenitors could be found in the
bone marrow of children with previous viral infections without any evi-
dence of malignancy, the diagnosis of TMA should be made with clinical mani-
festations and bone marrow examination. Identification of mature lympho-
ocyte markers in the bone marrow suggests the lymphocyte effect in the
pathogenesis of this disorder. These various antigenic abnormalities in the bone
marrow may indicate the chronic progression of the disease. However
it may also be due to the previous viral infections.

PO-0797 Immune thrombocytopenic purpura: evaluation of the use of a
new enzyme linked immunosassay (thrombo-auto)

Courvilleaud C, Dine G, Komara B, Oudin M
IBT and Haematology Department, Troyes, France

Immune thrombocytopenic purpura is an autoimmune disorder caused by
autoantibodies against platelet membrane glycoproteins (GP), predomi-
nantly against GP Ib/IIa, GP IIb/IIIa and GP Ia/IIa. It becomes essen-
tial to detect the antiplatelet autoantibodies in order to propose a clinical
diagnosis and appropriate treatment. But, the screening of free autoanti-
bodies is a real problem because their low level in sera and in most cas-
es, only platelet-associated immunoglobulins can be detected. To eval-
uate the presence of free autoantibodies and glycoprotein-associated anti-
bodies, our laboratory developed a competitive enzyme linked immunoass-
orbent assay (Thrombo-auto) for the Damed company. The patient's platelets are coated on the wells of a microplate. This solid phase microassay are specific for each patient. The ability of patients' sera to inhibit the
binding of monoclonal mouse antibodies to glycoproteins is measured to
detect human autoantibodies against GP IV, GPIb, GPIa/IIa and GP Ib/IX. The quantification of platelet-associated immunoglobulins are
assessed by comparison with values of glycoprotein availability thresholds of
normal platelets and free autoantibodies are estimated by comparison between
a negative sera. Sera of 40 normal subjects were analysed to give
the virtual number of normal platelets and free autoantibodies are estimated by comparison between
a negative sera. Sera of 40 normal subjects were analysed to give

PO-0798 Effects of platelet aggregation, membrane fluidity, and free
radical scavenging activity of PMC, a potent α-tocopherol analogue

Lul HN, Sheu JR*
Department of Anesthesiology, Veterans General Hospital-Taipei, *Graduate
Institute of Medical Sciences, Taipei Medical College, Taipei,Taiwan

In this study, PMC, a potent antioxidant derived from
α-tocopherol was found to be over 5-10 times more potent than α-tocopherol in inhibiting
human platelet aggregation. Moreover, PMC was found to be over 5-10 times more potent than α-tocopherol in inhibiting
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human platelet aggregation. Moreover, PMC was found to be over 5-10 times more potent than α-tocopherol in inhibiting

PO-0799 The effect of anagrelide on platelet count and platelet
aggregation in patients with essential thrombocythaemia

*Department of Haematology, °Department of Clinical Biochemistry,
Medical University, Gdańsk, Poland

Essential thrombocythaemia (ET) is a clonal myeloproliferative disorder characterised by persistent thrombocytosis and an increased incidence of
Platelets and related disorders

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An analysis of the clinical course of treatment with anagrelide and thrombocytopenia 

PO-0801 Thrombocytopenia in HCV positive subjects

Gertli GC, Kosar A, Büyükkükü V, Öztati D, Hacendaroğlu I, Özçebeci O, Sayınlı N, Kiralı Ş, Dündar S, Hakettepe University Medical School, Department of Haematology, Ankara, Turkey

Thrombocytopenia (TM) is an integral endothelial glycoprotein, a factor present in the vascular and lymphatic endothelium in all organs except the brain. Serum concentrations of this molecule increase in diseases associated with endothelial injury. Reactive thromboctysis (RT) occurs in inflammatory diseases such as rheumatoid arthritis (RA), ulcerative colitis (UC) and iron deficiency anaemia (IDA). In this study plasma concentration of TM was evaluated by sandwich-type ELISA in different settings of RT, clonal thrombocytosis (CT) and autoimmune thrombocytopenic purpura (ATP). The study population consisted of 11 patients with CT (5 chronic myeloid leukemia, 3 polycythemia vera, 2 essential thrombocytopathy, 15 with RT (7 DFA, 7 RA, 1 UC), 8 with ATP and 12 healthy controls. TM was significantly increased in patients with CT compared to controls. Although TM was higher in the control group than in patients with RT and ATP, the difference was not statistically significant. There is an increase of TM in CT may indicate the presence of sub-clinical endothelial disturbance in these patients.

Table 1. Thrombomodulin level (mean [range]) in different groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Healthy Controls</th>
<th>CT</th>
<th>RT</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>37.2 ng/ml</td>
<td>110.6 ng/ml</td>
<td>57.8 ng/ml</td>
<td>48.5 ng/ml</td>
</tr>
<tr>
<td>Median</td>
<td>(30.7-44.5)</td>
<td>(45-163)</td>
<td>(28-105)</td>
<td>(18.5-105)</td>
</tr>
</tbody>
</table>

Objective. Thrombocytopenia was recently described to be associated with HCV positivity also in subjects without liver cirrhosis or portal hypertension. The aim of this study was: 1) to define the prevalence of HCV positivity in thrombocytopenic subjects; 2) to evaluate: the presence of anti-platelet antibodies: the relationship with other autoimmune phenomena; the immunologic mechanism putatively responsible for thrombocytopenia, and the distribution of HCV genotype in this subset of subjects. Materials and Methods. Eighty-eight consecutive thrombocytopenic patients is the Out Patients Clinic between 1996 and 1998 were seen and 31 out of 88 were HCV-RNA positive. Twenty-four out of these 31 (30.7%) without clinical signs of portal hypertension and as the control group 24 subjects with ATP (mean age 54±18), diagnosed according to the Guidelines of the ASH, were enrolled for the study. All subjects were negative for other viral markers (HBSAg and HIV included). Antiplatelet antibodies and thrombopoietin in 22 of these 31 patients and 22 of the control group in these subjects HCV genotype was also obtained. Results. The prevalence of HCV positivity in thrombocytopenic patients was 35%. Moderate or severe thrombocytopenia was observed in both groups; platelet count was statistically higher (97±41 vs 48±35×10^3/μL; p<0.0001) in HCV subjects. No statistical relationship between antiplatelet antibodies and thrombocytopenia in HCV subjects was observed. Thrombocytopenic studies showed a significant reduction of platelet life-span also in HCV positive patients, and genotype 1b was the prevalent one in this population. Conclusions. We conclude that an immunologic mechanism in HCV-associated thrombocytopenia seems to be ruled out, while it is recommended that HCV markers are monitored in all thrombocytopenic subjects.

PO-0802 Thrombopoietic cytokine levels in patients with platelet disorders

Baleanu E, Ghihranda P, Gârâs S, Măsău G, Cescâncio S, Ghio R, Department of Internal Medicine - University of Genova, Italy

Objective. In order to determine the relationship between endogenous thrombopoietic cytokine levels and circulating platelet counts in patients with different platelet's disorders. Design and methods. Serum concentrations of thrombopoietin (TPO), interleukin-3 (IL-3), interleukin-6 (IL-6) and interleukin-11 (IL-11) were evaluated by ELISA in 62 healthy volunteers and in patients with thrombocytopenia due to essential thrombocythemia (ET) (30) or with thrombocytopenia secondary to either immune thrombocytopenic purpura (ITP) (12) or marrow hypoplasia (12). Results. The serum concentrations (mean±SEM) form TPO in the patients with ET and the patients with ITP did not differ to TPO levels found in normal controls (34.9±11 pg/mL and 33.7±15.4 pg/mL vs 27.1±8.8 pg/mL respectively). On the other hand serum TPO levels of aplastic patients were significantly higher than controls (1080±285 pg/mL vs 27.1±8.8 pg/mL, p=0.0001), and inversely correlated with platelets counts. IL-6 concentrations were higher in secondary thrombocytopenic patients than in normal controls (63.6±21 vs 20±4 pg/mL, p=0.0024), while they did not differ from those in controls in the patients with ET (29.3±6 vs 20±4 pg/mL). IL-3 serum levels were significantly higher than those of normal controls in all the patients with platelet disorders, irrespective to their disease. Serum IL-11 levels were undetectable in most patients and normal controls. Conclusions. Our data indicate that TPO levels are not appropriately down-regulated in patients with ET, and that differential mechanisms seem to regulate endogenous TPO and IL-6 levels in thrombocytopenic patients, according to the different pathogenesis of platelet disorders.

PO-0803 Vincristine as salvage treatment for patients with thrombotic thrombocytopenic purpura refractory to plasma exchange or plasma infusion

Ferrara F, Coppia C, Spasiano A, Mele G, Antinolfi I, Prossomariti L, Ammuniti M, Division of Haematology, Cardarelli Hospital, Naples, Italy

Objective. To evaluate the efficacy and toxicity of vincristine (VCR) as salvage treatment for patients with thrombotic thrombocytopenic purpura (TTP) refractory to plasma manipulation including plasma exchange (PE) or plasma infusion (PI). Design and Methods. VCR (1.4 mg/m2 on day 1 followed by 1 mg on days 4 and 7) was administered to 8 patients with TTP. After an interval of one week, a second identical dose of VCR was administered to 7 patients. No patients not responding after the second course were considered as refractory. There were four males and four females, median age was 39 years (range 22-70). At diagnosis, 4 patients had been treated by PE (minimum 5 plasmaphereses), 4 by PI (15 mL/kg) due to poor clinical status. All had been concomitantly given corticosteroids and intravenous diprydamily. Three subjects had also received high dose immunoglobulin. All patients showed severe neurological signs; median LDH value at the beginning of treatment with VCR was 1550 IU/L (range 860-3250), median platelet count was 11×10^3/μL (range 1-191). Results. 7 out 8 patients (87%) achieved a complete response (CR) by VCR, 6 after the first week and one after the second week of treatment. CR included normalisation of platelet, Hb and serum LDH levels as well as disappearance of any subjective symptoms. Two patients received the treatment in intensive care unit after intubation because of coma and both did fully recover. One patient relapsed after 8 months and was successfully retreated with VCR. One patient was refractory to treatment and died within a few weeks. After a median follow-up 50 months, all responder are alive and well, carrying out their normal daily activities/work. Toxicity was mild, consisting of two episodes of leuкоpe-nia (WHO grade 2) and one of autonomic neuropathy leading to paralytic ileus which occurred in a patient aged 70 years. Conclusions. According to the schedule employed in this study VCR is highly effective in the treatment of patients suffering from TTP refractory to PE or PI. The toxicity of the treatment is negligible; in addition, as compared to other immunosuppressive approaches (i.e. high dose immunoglobulin or splenectomy), VCR offers substantial advantage in terms of cost/benefit ratio. Finally, our results raise new questions about the possibility of using VCR as initial treatment for TTP in combination with PE.
Refractory immune thrombocytopenic purpura combined with selective IgA deficiency: difficulties in their management.

Selective IgA deficiency (SIAD) is the most common primary immunodeficiency disorder that, like most other immunodeficiencies, is frequently associated with autoimmune phenomena. In patients with SIAD neither gammaglobulins nor blood products should be transfused because of the high risk of acute severe anaphylactic, immune-complex mediated reactions. Although previous reports describing a complete lack of IgA in patients with immune thrombocytopenic purpurs (ITP) have been published, this case demonstrates the difficulties involved in the management of such a patient when ITP is symptomatic and refractory. A 38-year-old man had been diagnosed as having ITP in October 1981. The initial episode and two recurrences in September 1982 and May 1985 were successfully treated with corticosteroids. The patient remained stable without treatment until October 1998, when he was admitted to hospital. Clinical manifestation included petechiae and haematomas involving skin and oral mucosa. The platelet count was $<$10 x 10^9/L and a complete lack of IgA was detected. IgA levels were normal in his sister and brother. No response was observed either with corticosteroids (conventional and high doses), or with vincristine. Spleectomy was performed one month after the last relapse with an improvement in the platelet number $>$150 x 10^9/L. Washed platelet pool from donors was transfused prior to surgical section and two packs of washed red blood cells were administered 24 h post surgery without complications. Generally, SIAD patients should be warned of the risk of severe transfusion reaction which may occur following transfusion of only a few milliliters of blood. The problem of transfusion blood products in patients with SIAD may be partially overcome by administering washed hemoderivatives, as in our case. Another possibility is transfusing units of red blood cells or platelets of donors also affected by SIAD. In this way, as SIAD can be transmitted by dominant or recessive inheritance, members of the same family may be studied. It would be interesting to keep a SIAD donors’ panel in the blood banks, taking into account that the prevalence of SIAD, is reported by some authors to range between 1:163-1:328 in the healthy population.

The efficacy and short term safety of IV Ig (Alphaglobin®) in the treatment of adults with ITP

M. Carr S.B., Duggan C., Crowley M., Martin M

Department of Haematology, St. James’s Hospital and Trinity College, Dublin, Ireland

Intravenous immunoglobulin (IV Ig) has a well established place in the treatment of ITP in adults. Alphaglobin® (Flebogamma) is a liquid intravenous immunoglobulin containing $>$85% intact (15) IgG. A specific viral inactivation step, pasteurisation at 60°C for 10 hours, is incorporated in the manufacturing process. A 5% solution was used in this study. This study was designed to ensure that the IV Ig treatment step did not compromise the efficacy or toxicity of Alphaglobin®. Adult patients diagnosed with ITP with a platelet count $<$10 x 10^9/L were included. Patients with autoantibodies indicating the presence of another syndrome responsible for the thrombocytopenia were excluded. Ethical approval and written informed consent were obtained prior to treatment. Eleven evaluable patients had been treated to date. Of these, 4.0 $\mu$g/kg Alphaglobin® for 5 days. Three patients who relapsed after 28 days were subsequently retreated with 1.0 $\mu$g/kg Alphaglobin® for one day. Five males and six females with an age range of 25-58 years were treated. Four had had a previous splenectomy and three had received steroids. Ten of the eleven patients had a complete or partial response to Alphaglobin® (complete response: platelets $>$10 x 10^9/L. Partial response: platelets $>$5 x 10^9/L) within 29 days from the time of administration of IV Ig. One patient failed to respond and subsequently underwent splenectomy. The three patients who were subsequently retreated for relapsed thrombocytopenia with Alphaglobin® 1.0 $\mu$g/kg for one day had a complete response. No serious adverse events were noted during the study period. Minor adverse events including headache, erythema and oedema were experienced by six of the patients, none of which required termination of the study. We conclude that Alphaglobin® at a dose of 0.4 $\mu$g/kg is an efficacious and safe product for use in the treatment of adult ITP.

Thrombopoietin and interleukin-11 response to thrombocytopenia in leukemic children at diagnosis and during treatment

Coraza F., Azzi N., Hemans C., Fusar A., Demulder A., Fondu P., Saribas E.

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Objective. We have previously reported that, in anemic cancer children, the erythropoietin response is adequate to the degree of anemia (Coraza, Blood 92:1793, 1998). In order to evaluate whether mediators of thrombopoiesis were dysregulated in leukemia, we measured serum thrombopoietin (TPO) and IL-11 levels in leukemic children at time of diagnosis (LD) or during chemotherapy (LCT). These results were compared to those obtained from normal controls, aplastic anemia patients (AA) and platelet destruclive disorder patients (PDD), including idiopathic thrombocytopenic purpura and hemorrhosis-uremia syndrome. Methods. Serum TPO and IL-11 levels were measured using ELISA (R&D System) with detection limits of 15 pg/ml and 8 pg/ml, respectively. Results. Although LD and LCT patients had similar low platelet counts, only LD patients had significantly elevated TPO levels. In addition, LCT patients had significantly lower TPO levels than to AA patients with a similar degree of thrombocytopenia. In all groups evaluated, IL-11 did not correlate with the degree of thrombocytopenia. However, in PDD patients, serum IL-11 levels were significantly increased.

Study of some immunological aspects of chronic idiopathic thrombocytopenia in Egyptian children

El Safy U.R.*, Zeinaw M.M.*, El Shenawi FA, Saada AA, Mahdy MH*

*Pediatric Department, *Clinical Pathology Department, Zagazig University and *Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt

Introduction. Being an autoimmune disease, the pathogenesis of idiopathic thrombocytopenic purpura is expected to be controlled by a genetic variance, because the human leukocyte antigen (HLA) system shows an extreme polymorphism and is an excellent set of genetic markers. Objective. This study is an attempt to demonstrate the presence of autoantibodies in the sera of the patients and to identify and characterize T lymphocyte immunoregulatory abnormalities and correlate these findings with different HLA antigen in a trial to clarify the pathophysiology of this disease. Design and Methods. The study included 31 chronic ITP children with and 10 age and sex matched healthy children as a control group. All patients and the control group were subjected to the following: full history taking and complete clinical examination, routine laboratory tests, bone marrow examination for patients only, special investigations including detection of serum antiplatelet antibodies by ELISA technique. Study of lymphocytic blastogenic response to phytomenaquin (PHA), Enzyma reaction of T cell subsets by immumne stim A-B monoclonal procedure. Determination of HLA-A, B, and -DR antigens for patients, only by microcytotoxicity technique. According to the results of antiplatelet antibodies, the patients group was divided into two subgroups: 1) Group A: comprised 20 patients associated with positive antiplatelet antibodies. 2) Group B: comprised 11 patients negative for antiplatelet antibodies. Results. Antiplatelet antibodies were detected in sera of 64.52% of patients, which was highly significant when compared to the controls. No statistically significant difference was detected in the CD3+% and CD4+4% when compared to controls. The CD8+ was significantly high in ITP patients when compared to controls with a consequent significant reduction in the CD4+ /CD8+ ratio. The blastogenic response of T lymphocytes to PHA was significantly depressed in patients. No statistically significant difference was detected in the T cell subsets and blastogenic response to positive PAG when compared to those with negative PAG. Correlation study revealed no significant positive or negative correlation between phenotypic and functional properties of T lymphocytes. Also no significant correla-
tions between platelet count and T cell abnormalities were found. The results of the present study revealed that only HLA-B21 and DR2 antigens were significantly frequent in patients than controls. Also the relative risks for both antigens were high which means a significant strength of association. The DR2/X haplotype was the most frequent one in the present study (32.2%) followed by the B21/DR2 XX haplotype (19.35%) and lastly, the B21/X haplotype (12.3%). The platelet count was significantly decreased in the DR2/X haplotype compared to X X haplotype. Also, the blastogenic response of Tlymphocytes to PHA was markedly depressed in the DR2/X and B21/DR2 XX haplotypes as compared to in the X X haplotype. Conclusions. These data suggest that abnormalities of T lymphoneocyte function and subsets may play an important role in the pathogenesis of ITP. The increased frequency of HLA-B21 and DR2 antigens in our patients, reflects an association or linkage between the HLA-antigens and the genet- ic control of ITP. Furthermore, the relative risk is significantly high and indi- cates that persons having these antigens are several times more suscepti- ble than those lacking these antigens.

PO-0808 The response to IV immunoglobulins in children with chronic thrombocytopaenia and the response to splenectomy

Bermond S, Barone P, Cerchio R, Pinzaianni R, Giambartolo C, Bosa E, Farina L, Fassio U. Haeomatology Unit, Paediatric Department, University of Turin, Italy

The suggestion that the increase in peripheral platelets (plts) after intra- venous immunoglobulins (IVIG) may predict the response to subsequent splenectomy in patients with chronic thrombocytopenia (1) seemed to be unfounded (2, 3). Identification of possible predictive factors is certainly of clinical importance, especially in paucisymptomatic patients. Our data are of interest in this connection, since they indicate that the response to IVig is indeed predictive. We have followed 7 boys and 10 girls aged 3 to 14 or 15 years who underwent splenectomy after IVIg courses of 2 to 5 g/kg IV in 2 or 5 days, and >20 mg/kg prednisolone for 3 or more days. Five patients also received 50 µg anti-D i.v. Ten patients have >150 to 10000 plts/L from 7 to 132 months after splenectomy, four have 50-150 to 10000 plts/L but no haematological changes, and two are still thrombocytopenic. Two poor responders to splenectomy also displayed a poor response to IVig. Fourteen patients with a good or intermediate response to IVig showed a good or complete splenectomy. Only one patient with an intermediate response to IVig showed a poor response to splenectomy. These results indicate that a failure to respond to IVig predicts a poor or nil response to splenectomy and can even be viewed as a contraindication. It is clear that this debated question deserves extensive investigation in a large series of patients from several centres.


PO-0809 Long-term follow-up of 167 splenectomised patients with idio- pathic thrombocytopenic purpura


Splenectomy is definitive treatment for refractory idiopathic thrombocytopenic purpura (ITP) because it removes both the sites of autoantibody producing cells and also the major site of platelet destruction. Several factors have been purged (ITP) because it removes both the sites of autoantibody producing cells and also the major site of platelet destruction. Several factors have been identified as possible predictive factors of good response. Significant differences were: 6 weeks of steroid therapy with platelet count < 10 10^9/L or 3 months with platelet count < 30×10^10/L; prednisone > 30 mg for more than 6 months to increase platelet count > 30×10^10/L repeated relapses, and contraindication to steroid therapy. Results. Postoperative complications developed in 16 pts (9.5%), 3 of them died (1.8%) due to thromboem- bolism and 17 pts discontinued later treatments. During follow up from 1 to 170 months (median 60) 111/147 splenectomised pts were in remission (75.5%), 99 in complete (>100×10^10/L, 12 in partial (50-100×10^10/L) and 36 pts (24.5%) had relapsed (<50×10^10/L). Response to splenecto- my decreased to 30% during the follow-up longer than 10 years. Remis- sion was achieved in 79/88 pts (89.8%) with a good response to predni- sone before splenectomy; and in 30/62 pts (51.6%) with a poor response to prednisone (<0.01). Remission was obtained in 9/11 pts (81.8%) who responded well to intravenous immune globulin (0.4 g/kg i.v) and only in 1/8 who did not (p<0.05). Higher response rate was achieved in pts under 40 years of age (81.6%) than in older ones (64.5%) (p<0.05). No difference was shown between sex and time intervals (3, 6, 12, 24 or >36 months) from diagnosis to splenectomy. Conclusions. Splenectomy is an effective form of refractory ITP with response rate of 75.5% after a median follow up of 60 months. In our patients better results from splenectomy were associated with age under 40 years, pre- operative good responses to steroid and intravenous immune globulin.

PO-0810 Desmopressin normalizes the bleeding time and defective serum prothrombin consumption in patients with isolated deficiency of platelet microvesicle generation

Cadtaman G, Rodeghiero F.
Department of Haematology, San Bartolo Hospital, Vicenza, Italy

We recently described 3 unrelated families with deficiency of platelet microvesicle generation associated with prolonged bleeding time and mod- erate bleeding diathesis (Br J Haematol 96, 458, 1997). All the patients were detected on the basis of abnormal serum prothrombin consumption. We evaluated the effect of desmopressin infusion on these abnormalities and its clinical usefulness in two of the formerly investigated propositions. These patients had been transfused in the past with whole blood and/or platelet concentrates for surgical bleeding. Both the patients underwent a test-infusion with desmopressin (0.5 µg in 0.9% NaCl 20 ml w) to plan adequate pro- phyaxis for hysterecctomy and tooth extraction. The bleeding time was nor- malised in both patients 1 hour after infusion (10.5 min → 6 min and 9 min → 7 min, respectively; N.V.< 7.5 min.). Similarly, defective pro- thrombin consumption was normalized (71% → 6% and 46% → 3%, respectively, N.V.< 10%). The compound was used for the prevention of bleeding during surgery, which were successfully carried out. In conclusion, desmopressin is clinically efficacious for the prevention of bleeding in patients with isolated deficiency of platelet microvesicle generation by shortening the prolonged bleeding time and by normalising the defective serum prothrombin consumption. It remains to be determined whether this effect is achieved through an increase of FVIII von Willebrand factor levels or of microvesicle generation caused by a as yet unknown mechanism.

PO-0811 The effect of normalisation of platelet count on platelet func- tion in patients with primary thrombocythosis

Milijić P, Janković G, Bošković D, Colović M.
Institute of Haeomatology, Clinical Centre of Serbia, Belgrade, Yugoslavia

Thrombocytosis and various abnormalities of platelet function are common in patients with chronic myeloproliferative disorders and may play an important role in pathogenesis of thrombo-haemorrhagic complications. Cytoadhesive therapy is usually effective in lowering platelet count but lit- tle is known about its effect on platelet function in pts with primary (clonal) thrombocythaemia (PT). In order to answer this question we investigated platelet function before treatment and after normalisation of platelet count (PC) in 34 consecutive patients with myeloproliferative disorders who pre- sented with PC above 10^10/L. Essential thrombocythaemia was diag- nosed in 21 (62%), polycythaemia vera in 9 (26%), primary myelofibrosis in 2 (6%) and chronic myelogenous leukaemia in 2 (6%). Normalisa- tion of PC was achieved by the use of anagrelide in 12 (36%), busulphan in 11 (32%) and hydroxyurea in 11 (32%) pts. The mean PC on presenta- tion was 990±283×10^9/L and after treatment 354±118×10^9/L. Bleed- ing time was prolonged in 2 pts before treatment and only in one patient after achievement of complete remission. There were no significant differ- ences between mean values of platelet adhesiveness before and after treatment (26±21% vs. 27±21%). Abnormal platelet aggregation response (absent or reversible aggregation) was observed in higher percent of patients before than after the treatment, when induced by ADP 5 µM (43% vs. 0%), ADP 10 µM (27% vs. 0%), collagen 5 µg/mL (25% vs. 7%), col- lagen 10 µg/mL (6% vs. 0%), adrenaline 10 µM (71% vs. 59%) and ris- tocetin 1.25 mg/mL (12% vs. 0%). Significantly higher mean values on pre- treatment than after were observed for fTG (91.5±45 µg/L vs. 42.6±12 µg/L) and FIX (24.8±70.1 µg/L vs. 10.0±9.9 µg/L). Although our study is limited in size, the results suggest incomplete correction of platelet function after achievement of normal PC in patients with PT. This may be a consequence of residual disease and persistence of a small propor- tion of clonal platelets in circulation despite normal PC.

PO-0812 Aspirin and platelet reduction in the treatment of vascular complica- tions in thrombocythaemia vera

Michalski J, von dem Borne AEGK
Goodheart Institute, Haematology, Haemostasis and Thrombosis Research Centre, Rotterdam and Department of Clinical Haematology, Academic Medical Centre, Amsterdam, The Netherlands

Clear indications for aspirin 500 mg in ET-patients are the presence of microvascular circulation disturbances including erythromelalgia, digital
ischaemia, cerebral ischaemic attacks, visual disturbances and superficial thrombophlebitis. Because a large starting dose of aspirin (300 mg per day) induces a platelet cyclooxygenase activity that completely inhibits platelet cyclooxygenase activity and to relieve the erythromelalgic pain within a few hours followed by reversal of the inflammatory and ischaemic circulation disturbances of the toes. With a low starting dose of aspirin (50 mg 100 mg per day) in symptomatic ET patients, it takes a few days to one week for aspirin to completely inhibit platelet cyclooxygenase activity and to alleviate the long-term prevention of platelet-mediated microvascular complications in ET-patients. The indication for aspirin in asymptomatic ET-patients is uncertain and it should be used with great caution or not at all for patients whose diagnosis of ET was made by routine blood investigation or by chance. Despite lack of evidence clinicians tend to prescribe platelet lowering agents when the platelet counts exceed 1000×10^9/L. Clear indications for cytoreductive treatment in ET-patients are: 1) a history of the presence of major thrombosis or ET-related bleeding, 2) platelet complications in ET patients inadequately treated with platelet lowering agents is easily achieved by the addition of low dose aspirin (50 mg day). ET-patients below the age 65 and a clear indication for platelet complications, 5) side effects of aspirin. There is good evidence that inadequate reduction of platelet count (>400×10^9/L) is associated with a continuous risk of vascular complications. Effective prevention of vascular complications in ET patients adequately treated with platelet lowering agents is easily achieved by the addition of low dose aspirin (50 mg day) ET-patients below the age 65 and a clear indication for platelet complications. ET-patients below the age 65 and a clear indication for platelet complications. ET-patients below the age 65 and a clear indication for platelet complications. ET-patients below the age 65 and a clear indication for platelet complications.
nucleotide 202, producing the NalI RFLP) using Polymerase Chain Reaction. We found, for the G6PD A-202A genotype (associated with enzyme deficiency), a prevalence of 21% in the general population. 28% for the G6PD A-202A genotype (a variant with enzyme activity in the normal range) and 51% for the G6PD B genotype (normal genotype). In females, we found an allele frequency of 0.22, 0.26, and 0.51 GdA, GdA+, and GdB alleles, respectively. For the six genotypes arising from female chromosomes, the frequency we found was 19.4% for the genotype B/B, 2.7% for A/A, 2.1% for A/B, 27.7% for B/A, 36.1% for B/B and 11.1% for A/A. In the light of this study, we note that G6PD deficiency prevalence is high in the Ivory Coast and must be considered when haemolytic anaemia occurs. This concerns both males and females because of the equal allele frequencies which give rise to a theoretical genetic frequency of 4.8% of GdA/GdA in the female population.

**PO-0817** Mean corpuscular hemoglobin concentration as a discriminating tool between extra- and intra-vascular hemolysis

Kim HM, Lee KA, Cha BH, Lim BK, Park SM

*Department of Pediatrics, Clinical Pathology, Yonsei University; °Wonju College of Medicine, Wonju Department of Pediatrics, Yonsei University College of Medicine, Seoul, Korea*

**Purposes.** The purposes of this study were to elucidate (1) the diagnostic significance of mean corpuscular hemoglobin concentration (MCHC) for various kinds of hemolytic anaemia, and 2) the discriminating power of MCHC between extravascular (EH) and extravascular (IH). 3) The mechanism of elevated MCHC in hereditary spherocytosis (HS) in children. Subjects and methods. The subjects consisted of 39 cases of autoimmune hemolytic anaemia (AIHA) (Group 1), 31 cases of IH such as DIC, snake bite or hemolytic uraemic syndrome (Group 2), and sex and age matched controls for each group, Groups 4, 5, 6 respectively. The pre-op. and postop. MCHC values were compared in 32 splenectomised HS patients. The age of the subjects ranged from 1 to 18 years old. MCHC values were obtained with a H2 Technicon automated counter. Results. 1) The MCHC values of patients were: 34.49± 2.35 g/dL in Group 1, 34.82± 3.01 g/dL in Group 2, 32.24± 1.99 g/dL in Group 3, 32.96± 1.06 g/dL in Group 4, 32.49± 1.12 g/dL in Group 5, and 32.67± 1.01 g/dL in Group 6. The MCHC of Groups of HS patients are significantly higher than those of Groups 3, 4, 5, 6 (p<0.05). 2) ROC analysis showed that EH could be discriminated from IH when MCHC was above 35 g/dL with a sensitivity of 0.44 and a specificity of 0.97. There was a significant difference (p<0.05) between preop. (34.92± 1.98 g/dL) and postop. (32.63± 2.74 g/dL) MCHC values in splenectomised HS patients. Conclusions. The MCHC obtained with laser scattering cytometry was elevated in cases of EH such as HS or AIHA, and was a good diagnostic tool for discriminating EH from either IH or a normal control in children. The mechanism of elevated MHCs in HS seems to be the result of splenic processing or hemolysis itself.

**PO-0818** Iron deficiency anaemia in pregnancy: intravascular iron dextrin replenishes iron stores better than oral iron therapy

Singh K, *Fong YF, *Kuperan P

*Department of Obstetrics & Gynaecology; *Department of Haematology, National University Hospital, Singapore*

**Objective.** To compare the efficacy of Intravenous Iron Dextrin with oral ferrous fumarate 200 mg taken 3 times daily. Haemoglobin estimation was done weekly and at delivery. In addition to haemoglobin, the indices for iron stores were checked at 36 weeks gestation and at 6 weeks postpartum. Compliance and side effects were checked at each visit. Results. Treatment with intravenous iron dextrin resulted in a significantly better level and rate of increase of haemoglobin (p<0.001). Serum ferritin, the best indicator of iron stores was significantly higher (p<0.001) in the intravenous group. Serum iron, serum transferrin and zinc protoporphyrin also showed a significant improvement in the intravenous group compared to those given oral ferrous fumarate. All women in the intravenous group reported good tolerance in contrast to about half those on oral iron. There were also no reports of any adverse reactions with intravenous iron dextrin in our study. Conclusions. Intravenous iron dextrin as a total dose infusion is able to replenish iron stores and haemoglobin more efficiently and at a faster rate than oral iron therapy. It is a safe, suitable and effective alternative to oral iron therapy in the treatment of iron deficiency anaemia in pregnancy.

**PO-0819** Post-natal changes in erythropoietin levels in premature infants


*Kyiv Acad. Postgrad. Med. Education, Ukraine; °Institution of Molecular Biology and Genetic, Kyiv, Ukraine*

The purpose of this study was to determine whether an inappropriately low erythropoietin response in premature infants (PI) could be a basis for the anaemia of prematurity. Erythropoietin (Epo) was measured by immunonanzymosay in conjunction with haemoglobin (Hg), erythrocyte (RBC) and reticulocyte (RTC) count in untransfused PI between 15 and 35 days of age. The 19 infants had a mean gestational age of 31.2±1.5 weeks (29-34) and a mean birth weight of 1512±738 g (1250-1900).

Table. Epo, Hb, RBC, RTC counts in preterm infants (PI).

<table>
<thead>
<tr>
<th>PI</th>
<th>Epo (mU/mL)</th>
<th>Hb (g/dL)</th>
<th>RBC (10^12/L)</th>
<th>Ht (%)</th>
<th>RTC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-20 days</td>
<td>12.2±3.4 (4-21)</td>
<td>14.97±2.01</td>
<td>4.3±0.14</td>
<td>14.1±1.5</td>
<td>5.1±5.8</td>
</tr>
<tr>
<td>21-25 days</td>
<td>26.2±5.6 (9-40)</td>
<td>13.82±2.25</td>
<td>3.7±0.11</td>
<td>34.3±2.1</td>
<td>10.1±2.6</td>
</tr>
<tr>
<td>26-35 days</td>
<td>24.8±4.2 (14-40)</td>
<td>10.7±2.64</td>
<td>3.1±0.22</td>
<td>29.0±2.4</td>
<td>12.5±4.2</td>
</tr>
</tbody>
</table>

*Brown et al, 1983*

Our results show that Epo levels are relatively low during the anemia of prematurity for reasons that remain uncertain. In comparably anemic adults Epo levels measured were 10 to 100 times higher (Garcia et al). However, Epo was also correlated with increasing age during this period. There was a strong relationship between Epo and the RTC count obtained in the subsequent week. Thus, the RTC count was a good and clinically practical indicator of an earlier Epo stimulus to red blood cell production in healthy preterm infants.

**PO-0820** Serum ferritin levels in infants on exclusive breast feeding and milk formula feeding

Stojanovic B, Mehrenadovic A, Nikoletovska D, Stojanovic A

Health Institute, Skopje, Macedonia

The aim of this study was the comparative analysis of serum ferritin levels in a group of infants exclusively breast fed versus a group fed with different milk formulas (adapted, semi-adapted and cow-milk nutrition). The study included 26 children, 14 on breast feeding and 12 children with interruption of breast feeding in the first month. Ferritin levels and standard haematological parameters were determined in the third month. The group of children on milk formulas were treated for three months with an oral suspension of trivalent iron poly maltose complex (Ferrum-Lek) and serum ferritin was investigated on routine systemic examination at six months age. The ferritin and haematological results are summarised:

<table>
<thead>
<tr>
<th>Breast feeding</th>
<th>Milk formula</th>
<th>Milk formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin (mg/L)</td>
<td>Fe treatment</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>6 months</td>
<td>3 months</td>
</tr>
<tr>
<td>38 (14-118)</td>
<td>7.7 (5-28)</td>
<td>117 (92-131)</td>
</tr>
<tr>
<td>117 (92-131)</td>
<td>7.7 (5-28)</td>
<td>114 (96-163)</td>
</tr>
<tr>
<td>79 (71-86)</td>
<td>12 (11-14)</td>
<td>81 (71-104)</td>
</tr>
</tbody>
</table>

Infants on milk formula feeding showed severe iron deficiency in the first three month period. Treatment with oral iron resulted in correction of haemoglobin, MCV and ferritin levels in our patients in our social conditions with frequent (iron deficiency and anaemia in pregnancy, children being breast fed showed lower limit values of haematological parameters, so the dilemma of adjuvant iron therapy in this group of children remains open.

**PO-0821** The effect of natural feeding of newborns on concentration of haemoglobin in blood

Dorćic Čevljaković N, Andjelić M, Đurđović S, Debejaković T, Krasojević M, Milosavljević N

Medical Centre, Children’s Department, Krusevac, Yugoslavia

The first year of life is a period of intensive growth and development of a child’s organism when the newborn, after intrauterine life and biological uni-
by mother, adapts to new life conditions and ways of feeding. Aims. The effect of natural feeding on the concentration of haemoglobin in blood. Design and Methods. Retrospective study of the documentation of the Pediatric Health Centre at Kruševo concerning 1333 newborns in 1996. A method of item stratification was used to choose only those babies born alive with a body weight of over 2.5 kg with complete data on feeding in the first year of life and known concentrations of haemoglobin (Hb) in blood in the sixth and twelfth months of life. We analysed 403 test- ed cases or 30.23% of population. The data were statistically analysed and hypothesis tested by T-tests. Results. Of the total number of tested chil- dren, 14.14% were artificially fed from the moment of birth; 46.9% were naturally fed until the third month whereas 53.1% continued breast- feeding six or more months. The mean value of Hb concentration for the whole group in the sixth month of life was $H_b_{VI}=112.97\pm 8.4\ g/L$ and in the twelfth month $H_b_{XII}=114.155\pm 11.06\ g/L$. The increased Hb concen- tration in the twelfth month is not statistically significant. The T-test proved the hypothesis of better utilisation of iron from mother’s milk. The mean val- ue of Hb concentration in the same babies in the twelfth month was $H_b_{XII}=115.72\pm 8.11\ g/L$ than in the whole group of babies $H_b_{XII}=112.97\pm 8.4\ g/L$ (T=3.8935; p<0.001) and the difference was a highly statistically significant. The mean value of Hb concentra- tion in the babies with the twelfth month $H_b_{XII}=116.93\pm 8.39\ g/L$ was considerably higher than the Hib concentration of the whole group $H_b_{XII}=114.155\pm 11.06\ g/L$ (T=6.55; p<0.001) and the difference was highly significant. Conclusions. The use of nutrient factors of haemato- poiesis from mother’s milk is better and particularly the absorption of iron is made easier.

PO-0822 Automated erythropoiesis in hereditary hemochromatosis

Muncunill J, Vaquer P, Guerra JM, Bargay J, Morey M, Novo A, Besadre
Hospital Son Dureta, Palma de Mallorca, Spain

Introduction. Classical therapy for hereditary hemochromatosis (HH) con- sists of weekly manual phlebotomy (MP). Limiting factors are patient com- pliance, blood volume and hypertensine. The study aim is to ascertain the role of automated erythropoiesis (AE) in overcoming these limitations. Study Design and Methods. Eight patients with HH were apheresed every other weeks to reach ferritin levels <20 ng/mL and when then ferritin was higher than 150 ng/mL. Five patients had previous MP during a period of 3-24 months. AE was performed with a Haemonetics MCS+Pl. The red cell volume removed (RCVR), during procedures was selected to achieve a postapheresis haematocrit (Hct) of 30%. Ferritin was tested before every AE. Average follow up time was 20 months. Results. The process was well tolerated with clear relief of related symptoms (fatty liver, skin coloration and libido). Ferritin decreased fast and remained within normal levels between +4 and +18 months after stopping AE. Three patients needed new AE at 35, 9 and 12 months. Serum proteins, B12 vitamin and folic acid remained normal. Glucose and hepatic enzymes did not change. In 6 out 8 patients the elevated MCV and MCH became normal.

PO-0823 Clonal evolution in aplastic anemia (SAA) patients treated with immunosuppression therapy (IST)

DEMA Ospedale S. Martino Genova, *DIMI Università di Genova, Italy

Background. The pathogenetic relationship between acquired aplastic ane- mia, paroxysmal nocturnal hemoglobinuria (PNH) and myelo-dysplastic syn- dromes (MDS) is not completely understood. The appearance of clonal hemopoiesis is a common finding during the course of the disease and has been ascribed to the presence of an intrinsic stem cell defect or to changes due to the immuno- suppression therapy. Aim. End points were development of PNH or clonal abnormality (MDS) and correlation of clonality with hematopoietic recovery and survival. Patients. 97 patients were followed from the onset of the dis- ease with a median follow-up of 1598 days (range 2-6964). Ninety-two of the 97 patients are alive and were complete responders (CR), 30% were partial responders (PR) and 14% were not responders (NR). SAA/PNH. 16/97 developed a PNH phenotype at a median interval of 180 days from IST (range 0-2890) in 9 the PNH and monocytes were affected, in 7 pts only the monocytes. Out of 16 SAA/PNH pts. 13 were CR, 2 PR and 1 NR. SAA/MDS. 12/97 acquired chromosomal abnormalities at a median interval of 1145 days (range 150-3740): 1 patient developed du1q24S41, 1 patient trisomy 6, 4 pts trisomy 8, 1 patient trisomy 15, 1 patient del(t)q7, 1 patient monosity 7, 1 patient monosity 19, 2 pts -y and 1 patient multiple chromosomal abnormalities. All patients who developed cytogenetic abnormalities became transfusion independent, with one exception. Of the 12 SAA/MDS were CR, 2 PR and 2NR. SAA/MDS/PNH. MDS and PNH features were diagnosed in 3 patients, whereas the majority (n=21) had either SAA MDS or SAA/PNH. Survival. There was no significant effect of any other factors than age and sex and, also because of the small number of deaths. Conclusions. (1) as report- ed by others a proportion of 30% of patients with SAA developed PNH or MDS; (2) there is a trend for a shorter survival in patients with PNH; (3) there is little if any impact of PNH phenotype/ cytogenetic abnormality on hemopoietic recovery and survival at 10 years.

PO-0824 Congenital dyserythropoietic anemia: a first approach to epidemiology

Heimpel H, Mai er K
Medizinische, Klinik und. Politiklinik, University of U m, Germany.

The congenital dyserythropoietic anemias (CDA's) are a heterogeneous group of inborn errors characterised by ineffective red cell production as the pre- dominant mechanism of anemia. On the basis of distinct morphological abnormalities of the erythroblasts, we proposed a preliminary classification into 3 types in 1968, which was widely accepted. However, it has now been realized that this classification, whilst including cases from a national, survey and case reports from the litera- ture. CDA II is the most frequent type with 151, followed by Type I, variants and type III with 77, 54, and 27 children known, respectively. CDA's are reported from many ethnic groups in Europe, South and North America and the Far East. Since the diagnosis requires bone marrow aspiration, the ascertainment rate may depend on socioeconomic variables. However, some observations suggest a higher frequency of type II in Mediterranean countries and of type I in Arabsians. There is some evidence of genotypic heterogeneity within the three phenotypes, and of clustering of genotypes in different geographic regions.

PO-0825 Iron exchange indices in the whole blood and plasma of healthy neonates

Mykhaylyk O.M., Dudchenko NA, Pryazetskaya NM, *Orlova TA*
Inst. Appl. Problems. (Bio)physics NASU, *Hospital "OCHMADET", Neonatology Clinic, Kyiv, Ukraine

The aim of the study was quantitative determination of nonheme iron species in the blood and plasma of healthy neonates using an electron spin resonance technique (ESR). Samples were taken from two groups of babies: 5-6 days of age (n=9) and 2 days of age (n=11) born after a ges- tation ranging from 36 to 40 weeks. The blood was collected from the fin- ger, plasma was prepared immediately after collection of the blood, and samples of 20-70 mg were stored in liquid nitrogen. Nonheme iron indices (transferrin iron [Tf-Iron], transferrin protein, and ferritin iron [Ft-Iron] concen- trations) were determined using the procedure developed [1]. Per- centage of transferrin saturationTf was calculated from the ratio [Tf- Iron]/transferrin protein concentrations. Hemoglobin and haematocrit indices were determined with a Coulter Counter. Fisher's criterion F was used as normalised measure of difference between samplings. [Tf-Iron] decreased from 24.6±5.4 mMK at 2 days of age to a level of 18.6±4.8 mMK at 6 days of age in the blood (F=0.9745) and from 30.4±3 mMK to 24.8±5.6 mMK in plasma (F=0.8495), respectively. The median value of serum iron at 0.5 months of age was determined to be 22 mMK [2]. Tf% decreased from 43.1±19.7% at 2 days of age to 12.2±4.5% at 6 days of age in the blood (F=0.9994) and seems to increase from (8.3±6.5)% to (21.9±9.0)% (F=0.9893) in plasma. In the blood increased from 63.5±34.5 mMK at 2 days of age to the level of 14/9±19.7 mMK at 6 days of age (F=0.7929), [Ft-Iron] in plasma shows the same ten-
Iron deficiency and prematurity in Venezuelan pregnant women: does an association exist?


Introduction. Anemia during pregnancy is a world-wide problem. Iron deficiency (ID) is considered as the first cause of maternal nutritional anemia. Controversy exists regarding the effect of ID on pregnancy outcome. Many studies support the belief that there is increase in the frequency of prematurity delivery, when ID is present. Other studies have not demonstrated such an association. However, the studies have been carried out in developed countries. We, therefore, decided to carry out this study in a developing country, where a high prevalence of iron deficiency exists. Objective. To determine the association between iron deficiency in third trimester at labor with prematurity and its magnitude. Study design. Cases and controls (2 controls per case). Setting and population. Data was obtained in a general university hospital in Valencia, Venezuela. Almost all patients had a low or very low socioeconomic status. No patient had hemoglobinopathies, thalassemia or clinical infections. Methods. A sample of 543 women having delivered between May and December 1996 were included. Cases were 181 pregnant women with preterm delivery (<37 wk completed gestation). Two controls per case were taken from the same population of pregnant patients that went to hospital in labor. Anemia was defined according to WHO criteria (Hb < 11 g%). The complete blood count was measured and by using the Spectro Ferritin™ (Ramco Laboratories, Houston, TX, USA), the serum ferritin was evaluated. A serum ferritin of <12 ng/mL was considered as iron deficiency. Significance was set at 0.05 level. The power was set at .80 level. The data analyzed by using logistic regression to control for potential confounders. Main results. The global prevalence of ID was 34.44%. ID was not found to be associated with prematurity (Odds ratio: 0.89, 95%CI 0.61–1.30, p=0.41).

Conclusions. As in developed countries, the current results suggest that prematurity is not associated with iron deficiency.

Iron deficiency in Venezuelan pregnant women


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Conclusions. As in developed countries, the current results suggest that prematurity is not associated with iron deficiency.
gain less than 10 g/kg/day despite an optimal calorie intake were present. Fourteen of the infants were randomised to be supplemented with an iron dose of 3 mg/kg/day starting by the end of the 1st week for 6-7 weeks. They received erythrocyte transfusions with a haematocrit of less than 0.30 when signs and symptoms attributed to anaemia including persistent tachycardia, frequent apnoea with bradycardia and weight gain less than 10 g/kg/day despite an optimal calorie intake were present. Haematocrit, reticulocyte count and serum TfR concentration were evaluated for comparison of stimulation of erythropoiesis between the groups at the beginning, during and at the end of the therapy. There were no significant differences between the groups with regard to birth weight (112.5±15.2 vs 122.8±21.5 g), gestational age (39.6±1.3 vs 39.7±1.8 weeks) and gender distribution (p >0.05) at the end of the study period (25.6±2.3 vs 32.9±3.6 ng/ml) (p=0.0349). High risk very low birth weight preterms (group 1), while 9 of them started iron supplementation when their serum ferritin concentration fell below 150 ng/mL (group 2). There were no significant differences in patients with Fanconi anaemia during 1994-1997 at Istanbul University, Our-Children Leukemia Foundation Health Centre, were studied. The control group was composed of 42 males and 38 females, for a total of 80 cases. The aim of this study of finger and palm prints in patients with Fanconi anaemia was to investigate the importance of dermatoglyphic patterns in the diagnosis and etiology of the disease. The dermatoglyphic patterns from fingertips and the volar hand surface of patients with Fanconi anaemia and the control group were recorded using the ink method on quality paper. A magnifier and a stereo microscopic microscope were used for this purpose. Many clinical studies have shown the beneficial role of anaemia except sickle cell anaemia, on the appearance of coronary heart disease (CHD). Purpose of the study. Was to evaluate whether normocytic normochromic anaemia can influence the occurrence of acute myocardial infarction (AMI). Design and Methods. Over the last six months we admitted into our Coronary Care Unit 72 patients (65 men and 7 women mean age 64.8 ± 7.4 years old) with an AMI of different severity. Blood samples were taken from all the patients for the determination of haematocrit (Ht), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), and according to these parameters 3 pts (4.16%) had acute posthaemorrhagic anaemia, 5 pts (6.84%) had chronic aplastic anaemia, 2 (2.77%) had anaemia due to chronic renal failure and 1 (1.38%) had anaemia due to liver cirrhosis. Results.

**PO-0833** Dermatoglyphics features in Fanconi anaemia

Polat MH,° Ridolfo E,* Yaliman N,* Gedikoglu G³

Istanbul University, Istanbul School of Medicine, Department of Internal Diseases; Istanbul University, Istanbul School of Medicine, Oncology Foundation Health Centre, Istanbul, Turkey

With the aim of examining the dermatoglyphic patterns of fingers and palm, 18 patients (11 male and 7 female) who have been diagnosed as having Fanconi anaemia during 1994-1997 at Istanbul University, Our-Children Leukemia Foundation Health Centre, were studied. The control group was composed of 42 males and 38 females, for a total of 80 cases. The aim of this study of finger and palm prints in patients with Fanconi anaemia was to investigate the importance of dermatoglyphic patterns in the diagnosis and etiology of the disease. The dermatoglyphic patterns from fingertips and the volar hand surface of patients with Fanconi anaemia and the control group were recorded using the ink method on quality paper. A magnifier and a stereo microscopic microscope were used for this purpose.

Many clinical studies have shown the beneficial role of anaemia except sickle cell anaemia, on the appearance of coronary heart disease (CHD). Purpose of the study. Was to evaluate whether normocytic normochromic anaemia can influence the occurrence of acute myocardial infarction (AMI). Design and Methods. Over the last six months we admitted into our Coronary Care Unit 72 patients (65 men and 7 women mean age 64.8 ± 7.4 years old) with an AMI of different severity. Blood samples were taken from all the patients for the determination of haematocrit (Ht), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), and according to these parameters 3 pts (4.16%) had acute posthaemorrhagic anaemia, 5 pts (6.84%) had chronic aplastic anaemia, 2 (2.77%) had anaemia due to chronic renal failure and 1 (1.38%) had anaemia due to liver cirrhosis. Results.

**PO-0834** The role of normocytic normochromic anaemia on the development of acute myocardial infarction

Petrogianopoulos C,* Zacharof A,* Koutroulis G,* Ginis A,* Nikolaidis B,* Ursu M,* Delouis A,* Lambropoulos L*

Hellenic Red Cross Hospital, Athens, Greece

Many clinical studies have shown the beneficial role of anaemia, except sickle cell anaemia, on the appearance of coronary heart disease (CHD). Purpose of the study. Was to evaluate whether normocytic normochromic anaemia can influence the occurrence of acute myocardial infarction (AMI). Design and Methods. Over the last six months we admitted into our Coronary Care Unit 72 patients (65 men and 7 women mean age 64.8 ± 7.4 years old) with an AMI of different severity. Blood samples were taken from all the patients for the determination of haematocrit (Ht), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), and according to these parameters 3 pts (4.16%) had acute posthaemorrhagic anaemia, 5 pts (6.84%) had chronic aplastic anaemia, 2 (2.77%) had anaemia due to chronic renal failure and 1 (1.38%) had anaemia due to liver cirrhosis. Results.
According to the above table we see that anemic patients have a statistically lower occurrence of AMI compared to those with normal values of Ht and Hb; 11 pts (15.2%) vs 61 pts (84.8%). Conclusions. Normocytic, normochromic anaemia of different origin seems to play a favorable role in the development of AMI reducing its occurrence. Main causative mechanisms for this protection could be low cholesterol levels and low blood viscosity.

**PO-0835 Erythroid marrow activity in CDA II**
Premietis E, Papasotiriou I, Skarmoutsou C, Stamoulakatou A  
Haematology Laboratory “Aghia Sophia” Children’s Hospital, Athens, Greece

Congenital dyserythropoietic anaemia type II (CDAII) is the most frequent form of inherited dyserythropoiesis. It is characterised by a mild to moderate long-lasting anaemia, ineffective erythropoiesis and morphological abnormalities of mature red blood cells and their precursors. Spleenomegaly and jaundice represent clinical features of the disease. In the biochemical point-of-view, the disease is due mainly to the deficiency of two key enzymes responsible for glycosylation (N-glycan synthesis), N-acetylglucosaminyltransferase II and α-mannosidase II. We studied the erythroid marrow activity in 5 (2M/3F) patients with CDA II by measuring erythropoietin (Epo) (Nichols Institute Diagnostics, USA) and soluble transferrin receptors (sTfR) (Orion Diagnostica, Finland) levels and Reticulocyte Production Index (RPI). The following table summarises the main results of the study.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Hb (g/L)</th>
<th>Epo (IU/L)</th>
<th>sTfR (mg/L)</th>
<th>RPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>106</td>
<td>480.0</td>
<td>4.4</td>
<td>1.3</td>
</tr>
<tr>
<td>II</td>
<td>119</td>
<td>389.0</td>
<td>7.8</td>
<td>2.0</td>
</tr>
<tr>
<td>III</td>
<td>91</td>
<td>202.0</td>
<td>5.8</td>
<td>0.9</td>
</tr>
<tr>
<td>IV</td>
<td>82</td>
<td>350.0</td>
<td>6.2</td>
<td>0.9</td>
</tr>
<tr>
<td>V</td>
<td>129</td>
<td>22.0</td>
<td>3.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Controls</td>
<td>130-160</td>
<td>9.2±3.3</td>
<td>1.8±0.7</td>
<td>1.0</td>
</tr>
</tbody>
</table>

All patients had increased Epo and sTfR levels while their RPI values were low. In conditions associated with a normal proliferative response to anaemia, the RPI relative to sTfR levels (erythroid activity) can differentiate between ineffective erythropoiesis and peripheral haemolysis. A RPI approximately 3 times normal with raised sTfR indicates adequate RBC production (peripheral haemolysis), while a RPI less than 2 times normal with raised sTfR indicates impaired RBC production (ineffective erythropoiesis). According to this, our results show that erythron expansion was due to ineffective erythropoiesis and confirm the relative observations in the bone marrow of the patients, although the high Epo levels of these patients indicate that Epo is produced but is not used by erythroid precursors.

**PO-0836 The effect of vitamin E supplementation in iron deficiency anaemia**
Öztürk G, Simşek F, Kocak Ü, Hasanoğlu A, Gürsel T  
Gazi University Medical School, Department of Pediatrics, Ankara; Kırıkale University Medical School, Department of Pediatrics, Kırıkale, Turkey

Iron deficiency is the main cause of anaemia and is a major health problem in childhood. Increased lipid peroxidation and variable levels of antioxidants are shown to play an important role in the pathogenesis of this disorder. It has also been shown that lipid peroxidation is increased and vitamin E levels are decreased in thalassemics. During the treatment of iron deficiency anaemia, the effect of vitamin E, which is known to be a lipid soluble antioxidant, has not yet been well defined. We aimed to investigate the effect of vitamin E supplementation in iron therapy in iron deficiency anaemia. The study group consisted of 20 patients who were nine months old and 10 healthy children of the same age as the control group. Ten patients (Group A) had iron treatment alone, whereas the other 10 patients (Group B) had both iron and vitamin E supplementation for one month. In both groups basal levels of two malondialdehyde (MDA) and erythrocyte superoxide dismutase (ESOD) were not different from the control group (p > 0.05). Basal vitamin E levels were lower in the treatment groups (p < 0.05). During the therapy, when groups A and B were compared, group B was found to have earlier reticulocyte crisis and lower MDA levels (p < 0.01). At the end of the therapy, the median reticulocyte volume was higher in group B (p < 0.01). In conclusion, iron treatment leads to increased lipid peroxidation in iron deficiency anaemia similar to the iron overload in thalassemics. An important effect of vitamin E supplementation is the significant decrease in MDA levels. The haematological importance of these results is the rapid recovery of microcytosis and the early formation of reticulocyte response. We recommend vitamin E supplementation to iron treatment especially in children who receive cow’s milk. Vitamin E enhances early recovery in microcytosis without any effect on hemoglobin values.

**PO-0837 Is oxidative stress involved in the pathogenesis of congenital dyserythropoietic anaemia (CDA) type 1?**
Mazor D, Kaplunchik Y, Meyerstein N  
The D7), Kaufmann Haematology Laboratory, Physiology Department, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva. The Haematology/Oncology Unit, Pediatric Division, Soroka Medical Centre, Beer-Sheva, Israel

Congenital dyserythropoietic anaemia (CDA), type 1 is a rare macrocytic anaemia of unknown etiology, characterised by ineffective erythropoiesis. In the present study, the blood antioxidant status of 11 Bedouin patients aged between 1-19 years was evaluated to establish whether oxidative stress is involved in the pathogenesis of congenital dyserythropoietic anaemia. The study group consisted of 20 patients who were nine months old and 10 healthy children of the same age as the control group. Ten patients (Group A) had iron treatment alone, whereas the other 10 patients (Group B) had both iron and vitamin E supplementation for one month. Normal values were obtained for: superoxide dismutase (SOD) 2351±120 U/gHb, methemoglobin (MetHb) 1.92±0.21%, plasma thiols 0.43±0.03 mM, and total plasma antioxidants 0.99±0.3 mM. However, catalase (CAT) levels were slightly lower 10.9±10.4 U/gHb, reduced glutathione (GSH) levels were slightly higher 2.99±0.15 mM, and glycerol lysis time (GLT) was significantly prolonged 63.8±6.7 versus 26.4±0.9 sec. Erythrocyte morphology as studied by scanning electron microscopy, showed the characteristic features of a variety of bizarre erythrocyte shapes, macrocytes, poikilocytes, anisocytosis and elliptocytes, as well as normal shaped cells. The present study does not provide direct evidence for oxidative damage in the blood of CDA 1 patients. However, the lower catalase levels may render the cells more sensitive to peroxidative challenge. The prolonged GLT levels may be found in association with abnormal lipid organization, or in cells with a relative increase in membrane surface area. Changes in the lipid pattern of the membrane which affect the rate of hemolysis in glycerol, may be induced by oxidative damage.
The cytokine receptor encoded by c-mpl and its natural ligand thrombopoietin (TPO) have been isolated and cloned recently. It has been suggested that TPO levels may have clinical utility in the diagnosis and management of various disorders that cause thrombocytopenia. However, neither normal ranges in children nor levels in childhood thrombocytopenic states are well defined. It is known that the levels of other haematopoietic growth factor levels differ according to age. We investigated TPO levels in 20 healthy neonates, 12 healthy children and 18 healthy children in whom platelet counts were within normal ranges. Plasma was prepared from EDTA anticoagulated whole blood and a commercially available ELISA Kit was used for TPO analysis. Mean TPO levels (and ranges) were as follows: 156.0±71.1 pg/ml (86.6-272.7 pg/ml) in neonates, 150.3±33.1 pg/ml (88.2-257.0 pg/ml) in infants, 145.2±30.8 pg/ml (86.5-269.4 pg/ml) in children. There were no statistical differences between groups (p>0.01). Determination and definition of normal ranges are essential to evaluate the TPO levels in various disorders. The haematopoietic growth factor levels could be low, normal or high in various states such as aplasia, suppression or infiltration of the bone marrow. Determination of normal ranges are also important in determining the indications and utility of recombinant forms of TPO in the treatment of these disorders.

**Table 1. Normal volunteers (n=18).**

<table>
<thead>
<tr>
<th>IFN</th>
<th>IL-2</th>
<th>IL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO3 T-cells</td>
<td>25.2 (11.6)</td>
<td>24.4 (14.4)</td>
</tr>
<tr>
<td>CO4 T-cells</td>
<td>24.2 (11.2)</td>
<td>32.7 (16.5)</td>
</tr>
<tr>
<td>CO8 T-cells</td>
<td>32.2 (16.3)</td>
<td>9.4 (7.9)</td>
</tr>
</tbody>
</table>

**Table 2. Tumour patients (n=5).**

<table>
<thead>
<tr>
<th>IFN</th>
<th>IL-2</th>
<th>IL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO3 T-cells</td>
<td>57.6 (14.3)</td>
<td>45.8 (18.7)</td>
</tr>
<tr>
<td>CO4 T-cells</td>
<td>49.4 (15.7)</td>
<td>59.4 (10.9)</td>
</tr>
<tr>
<td>CO8 T-cells</td>
<td>73.4 (15.1)</td>
<td>33.0 (20.8)</td>
</tr>
</tbody>
</table>

**Table 3. T-cells in the treatment of a vision-threatening orbital haemangioma in an infant with interferon-α2a.**

<table>
<thead>
<tr>
<th>IFN</th>
<th>IL-2</th>
<th>IL-4</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>CO4 T-cells</td>
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<tr>
<td>CO8 T-cells</td>
<td>73.4 (15.1)</td>
<td>33.0 (20.8)</td>
</tr>
</tbody>
</table>

**Table 4. T-cell subsets in the treatment of a vision-threatening orbital haemangioma in an infant with interferon-α2a.**

<table>
<thead>
<tr>
<th>IFN</th>
<th>IL-2</th>
<th>IL-4</th>
</tr>
</thead>
<tbody>
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<td>59.4 (10.9)</td>
</tr>
<tr>
<td>CO8 T-cells</td>
<td>73.4 (15.1)</td>
<td>33.0 (20.8)</td>
</tr>
</tbody>
</table>

**Table 5. T-cell subsets in the treatment of a vision-threatening orbital haemangioma in an infant with interferon-α2a.**

<table>
<thead>
<tr>
<th>IFN</th>
<th>IL-2</th>
<th>IL-4</th>
</tr>
</thead>
<tbody>
<tr>
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<td>45.8 (18.7)</td>
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<tr>
<td>CO4 T-cells</td>
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<tr>
<td>CO8 T-cells</td>
<td>73.4 (15.1)</td>
<td>33.0 (20.8)</td>
</tr>
</tbody>
</table>

**Table 6. T-cell subsets in the treatment of a vision-threatening orbital haemangioma in an infant with interferon-α2a.**

<table>
<thead>
<tr>
<th>IFN</th>
<th>IL-2</th>
<th>IL-4</th>
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<tr>
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<tr>
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<td>49.4 (15.7)</td>
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</tr>
<tr>
<td>CO8 T-cells</td>
<td>73.4 (15.1)</td>
<td>33.0 (20.8)</td>
</tr>
</tbody>
</table>
Cytokines and growth factors: clinical

PO-0843 Similar granulocyte colony-stimulating factor (G-CSF) levels in healthy donors and in patients with sepsis

Zarco MA,* Urbano-Ispizua A,* Fillet X,* Collivign B,* Marin F,* Martínez C,* Menja J,* Molina R,* Ballestra AM,* Rozman C,* M orn errat E DEpartments of Clinical Biochemistry, Haematology- Haemotherapy and Infectious Diseases, Hospital Clinic, Barcelona, Spain

Objective. The use of G-CSF in normal individuals has increased substantially over the past 5 years. Although short-term toxicity of G-CSF after 10 mg/kg is acceptable, concerns remain about the long-term safety. The objective of this study was to compare serum concentrations after administration of filgrastim with those reached by endogenous G-CSF in human subjects with sepsis. Design and Methods. Twelve serum samples from patients in the acute phase of infection were examined: pneumonia 4, acute pyelonephritis 4, abdominal infection 1, bursitis 1, sepsis immediately after kidney transplant 2. Samples were obtained soon after the clinical onset of infection and before antibiotic treatment. Sixteen serum samples from healthy donors before filgrastim administration and 12 hours after the fourth dose at 10 mg/kg were also analyzed. Serum G-CSF levels were analyzed by a quantitative sandwich enzyme immunoassay technique, (R&D Systems). Results. Median (range) serum levels of G-CSF (pg/mL) were: healthy donors before and after G-CSF treatment 22 (2-69), and 434 (286-750), respectively; patients with sepsis 59 (58-2121). Of note, whereas the highest serum G-CSF levels among healthy donors was 753 pg/mL, in 33% of patients with sepsis this figure was higher than 1000 pg/mL.

<table>
<thead>
<tr>
<th>N. cases</th>
<th>Age</th>
<th>Serum G-CSF levels (pg/mL)</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy donors before G-CSF treatment</td>
<td>16</td>
<td>38±10</td>
<td>22±7*</td>
</tr>
<tr>
<td>Healthy donors after G-CSF treatment</td>
<td>16</td>
<td>3±10</td>
<td>488±56*</td>
</tr>
<tr>
<td>Patients with sepsis</td>
<td>12</td>
<td>65±19</td>
<td>599±57</td>
</tr>
</tbody>
</table>

*P < 0.001

Conclusions. This preliminary report seems to indicate that similar serum G-CSF levels are reached in human subjects with sepsis and in healthy donors after G-CSF administration. This result would favour a safe profile of G-CSF use for PBPC mobilisation.

PO-0844 Cytokines and soluble cell adhesion molecules in atherosclerosis due to low-level irradiation after the Chernobyl accident

Kuchinskiy NG, Teptyakov AI, Pryshchepova EV, Chegerova TI, Teptyakova DV, O tapenko VA. Research Institute for Ecopathology and Occupational Disease, Mogilev, Republic of Belarus

Prior investigations have shown that there is an increase of thrombosis risk in atherosclerosis in patients chronically affected by low-level irradiation. The goal of our research was the comparative analysis of cytokines, soluble cell adhesion molecules secretion within blood coagulation and high shear rate. This was done to study haemosorption involvement in the pathophysiology of atherosclerosis in patients chronically exposed to low-level radiation. The study involved 30 patients. Among them, 10 patients were liquidators of the Chernobyl accident in 1986, 8 inhabitants of area contaminated more than 5 Ci/km² (a main group). Another 12 patients without additional irradiation were a control. To research the mediatory processes the initial levels of cytokines and soluble cell adhesion molecules were detected. The changes in their concentrations during coagulation and fibrinolysis (incubation of blood clot within 6 hrs. at 37°C) and standardised viscosimetric flow using a rotational viscometer (shear rate 100 sec⁻¹, exposure 60 sec at 37°C, sample incubation 6 hrs also) were compared to initial levels. Concentrations of IL-1α, IL-1β, IL-6, IL-8, IL-10 ("Immunotech," France), endothelin-1, P- E-selectin, ICAM-1, VCAM-1 ("R&D," UK) in both tests were detected by ELISA kits (photometer "Bio- mek-1000," USA). Significant differences in both main groups in comparison to the controls were found using Wilcoxon-matched test. Comparative analysis showed statistically significant differences in IL-1α, IL-6 and E-selectin levels between both groups, concerning their increase most rheologic probes in the main group, whereas not detectable in controls. The statistically significant increase of E-1 level in both groups, chronically affected by low-level irradiation was registered. These phenomena may be explained by inflammatory and TNF response augmentation. However, the mechanisms of long term membrane changes (mainly, in patients liquidators) are still uncertain. It has been shown that one of the effects of low-level radiation exposure is increased endothelial functions, which reflect not only the increased inflammatory response of vessel wall in atherosclerosis, but also the cell coordinate communication changes influenced by low-level ionising radiation exposure.

PO-0845 Progression markers in multiple myeloma patients after chemotherapy or chemo + enzyme therapy

Desser L,* Sakalova A,* Herbeck I,* Zavadova E,* Holomanova D,* Mahr T*

"Institute of Tumour Biology / Cancer Research, Applied and Experimental Oncology Department, University of Vienna, Austria; "Clinical Center of Haematology and Transfusiology, University of Bratislava, Slovakia"

The remission times of multiple myeloma (MM) patients after chemotherapy and after enzyme- (chymotrypsin, trypsin, papain) + chemotherapy were compared retrospectively. The remission time was significantly longer in the group of enzyme-treated patients (stage II). We determined soluble TNF receptors p55 and p75, β2-microglobulin, IL-6 and TNF in the sera of 198 patients with MM stage I-I and in 87 age-matched healthy volunteers. The serum concentrations of sTNF-Rs and β2-M were significantly (p<0.05) elevated in stage II and in patients before therapy. The levels of these serum markers (sTNF-R p55, sTNF-R p75 and β2M) were lower after chemotherapy and significantly lower after chemo- + enzyme therapy. Over 17 months the β2-M, p55 and p75 levels in 52 stage II MM patients (chemo- or enzyme- + chemotherapy) were significantly reduced in MM patients treated with enzyme- + chemotherapy (p<0.001) in comparison to the levels in the chemotherapy group. Treatment with proteolytic enzymes in addition to conventional chemotherapy prolongs remission times in stage II MM patients and reduces the concentrations of progression markers.

PO-0846 Biological profile to detect erythropoietin use in healthy sport performers

Dine G,* Van Lierde F,* Rehn Y*

"Institut Biotechnologique et Service d’Hématologie de l’Hôpital des Hauts-Clos, Troyes, France; "Institut de Médecine du Sport de Troyes, France"

High level of blood hemoglobin is interesting for the practice of aerobic sports. O₂ binding to muscular tissues is a significant factor for performance in some sports, in particular cycling. For 10 years the illegal use of erythropoietin by top racing cyclists has been suspected. Doping controls by urinary tests are not efficient for finding the erythropoietin molecule. We have determined a specific biological profile in blood samples capable of detecting erythropoietin use. We analyze red cell parameters: RBC, HGB, HCT, MCV, MCH, MCHC, RDW, laser automatic retic and the measurements of serum erythropoietin, ferritin and soluble transferrin receptor. To define this specific profile, in our preliminary trial, we studied blood samples from 3 different groups: blood donors (30), renal insufficiency patients (9) and supposed erythropoietin users (16). The combination of the different parameters of our profile allowed us to determine a significant level of erythropoietin stimulation after use of erythropoietin. The factor of correlation is 90% over a time of 4 weeks. This method is not direct. It is not a good tool as a doping control but it could be a solution to preventing the risks of erythropoietin use, particularly iron overload, metabolic disorders and secondary erythropoietic disturbances, in healthy sportsmen-women.

PO-0847 Increased levels of TGF-β1 and IL-6 in the sera and supernatant fluids from long-term bone marrow cell cultures in patients with chronic idiopathic neutropenia of adults

Papadaki HA, Couloucheri S, Gioumou K, Koumaki V, Eleopoulos GD

Dept of Haematology of the University of Crete School of Medicine, Heraklion, Crete, Greece

Recent studies in our laboratory have shown that patients with chronic idiopathic neutropenia of adults (CINA) have increased serum concentrations of inflammatory cytokines and chemokines which may affect leukocyte trafficking by inducing endothelial cell activation and enhancement of neutrophil extravasation, and we have suggested that a low grade chronic inflammatory process has to take place in these patients [1]. The aim of the present study was to investigate possible changes in the levels of other inflammatory molecules which may be involved in the regulation of granulopoiesis. The study was carried out on 46 patients, 9 men and 37 women aged 15 to 77 years (median 53 yrs). All fulfilled the diagnostic criteria for CINA applied in our department as previously detailed [12]. Serum and supernatant fluids from long-term bone marrow cell cultures (3rd wk) were collected and stored at -70°C until use. Serum and supernatant IL-6 and...
Cytokines and growth factors: clinical

TGF-β1 levels were determined by an ELISA method using the commercially available high-sensitivity assay Quantikine kit (R&D Systems). Results are shown in the Table.

<table>
<thead>
<tr>
<th>Sera</th>
<th>IL-6 (pg/mL)</th>
<th>TGF-β1 (pg/mL)</th>
<th>Supernatant culture fluids</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>940.7±193.4</td>
<td>525±138</td>
<td>815±177</td>
</tr>
<tr>
<td>BM</td>
<td>747.7±217.7</td>
<td>533±299</td>
<td>253±129</td>
</tr>
</tbody>
</table>

PO-0848  

TNF-α, IL-1β and IL-2 production by peripheral and bone marrow mononuclear cells in patients with aplastic anaemia

Rozanova O, Glaizanova T, Bubnova L, Pavlova I, Abdulkaikurov K, Popova T

Research Institute of Haematology & Transplantology, St. Petersburg, Russia

Objective. Inbalance of immunocompetent cell proliferation and differentiation, known to play an important pathogenetic role in the development of aplastic anaemia (AA), is influenced by different cell cytokines, especially TNFα, IL-1β and IL-2. Thus it seemed of interest to study the production of these cytokines by mononuclear cells in AA. Methods. We examined the ability of mononuclear cells (1×10^5 per well) obtained from peripheral blood (PB) and bone marrow (BM) of 19 patients with AA to produce, spontaneously or under induction, TNFα, IL-1β (ELISA measurement) and IL-2 (biological assay). Results. The data obtained are presented in the table.

<table>
<thead>
<tr>
<th>Group</th>
<th>TNFα-S (pg/mL)</th>
<th>TNFα-LPS-S (pg/mL)</th>
<th>IL-1β-S (pg/mL)</th>
<th>IL-1β-LPS-S (pg/mL)</th>
<th>IL-2-S (pg/mL)</th>
<th>IL-2 PHA (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>525±138</td>
<td>815±177</td>
<td>312±49</td>
<td>737±232</td>
<td>0</td>
<td>4.2±0.7</td>
</tr>
<tr>
<td>BM</td>
<td>230±107</td>
<td>533±299</td>
<td>25±121</td>
<td>408±257</td>
<td>0</td>
<td>2.0±0.4</td>
</tr>
</tbody>
</table>

Healthy PB 44±211 1100±755 332±715 765±51 0 15.0-25.0

Abbreviations: -S: spontaneous; -I: induced.

PO-0849  

Serm thrombopoietin, interleukin-3 and interleukin-11 levels of patients undergoing autologous peripheral blood stem cell transplantation


Division of Haematology-Oncology, Department of Internal Medicine & Institute for Clinical Molecular Biology Research, Soonchunhyang University School of Medicine, Seoul, Korea

Objective. High-dose chemotherapy followed by peripheral blood stem cell transplantation (PBSCT) has become an option for the treatment of haematological malignancies and solid tumors. Following high-dose therapy there is a significant period of pancytopenia during which patients are at risk of infection and haemorrhage. Haematopoietic recovery after BM and PBSCST strongly depends upon several haematopoietic growth factors. Granulopoiesis has been shown to be in part dependent on the supply of G-CSF and its exogenous administration enhanced myeloid engraftment after both BM and PBST. Recently, the ligand for c-mpl has been cloned; in vitro and in vivo studies have shown that it stimulates both megakaryocy-

topoiesis and platelet production suggesting that it is the long sought platelet regulatory factor, thrombopoietin (TPO) itself. Design and Methods. In this study we serially measured serum TPO levels as well as interleukin-3 (IL-3) and IL-11 in 98 samples from 6 patients who underwent autologous PBSCT using an enzyme linked immunosorbent assay (ELISA) and compared these cytokine levels to peripheral blood platelet counts. Results. Serum TPO levels significantly correlated (r=0.72, p=0.011) with the degree of peripheral thrombocytopenia and there was a strong inverse relationship in all patients examined between serum TPO levels and platelet count. Serum TPO levels began to rise as the platelet count decreased after high dose chemotherapy. TPO levels peaked at 500 pg/mL between day 3 and day 7: TPO levels then decreased gradually as the platelet count began to rise. However, serum levels of IL-3 and IL-11 had no apparent correlation with platelet counts. Conclusions. These findings support the hypothesis that TPO is the key regulator for megakaryopoiesis and platelet production after PBSCT.

PO-0850  

Soluble adhesion molecules in allogeneic peripheral stem cell donors primed with mG-CFF


Ankara University Medical School, İbni Sina Hospital, Department of Haematology and Apheesis Unit, Ankara, Turkey

In this study the effects of mG-CSF on serum changes of four soluble adhesion molecules (SAM) (sICAM-1, sSE-Selectin, sE-Selectin and sCD44) in healthy peripheral allogeneic stem cell transplantation donors and their correlation with acute GVHD and engraftment time for their recipients were analysed. Serum SAM of 15 consecutive healthy donors were monitored by using a commercial ELISA Kit (Bender Med, Austria) prior to, on the day of first apheresis and 24 hours after the cessation of mG-CSF (10 µg/kg/day s.c./day 5) administration. They were the HLA-identical siblings of the patients. Median age was 30 (range: 22-45, M/F: 7/8). The results indicate a steady rise in the levels of sSE-Selectin, sSE-Selectin, and sCD44 but not of sICAM-1. Leukapheresis was started on the 5th day of mG-CSF administration by using a continuous flow blood separator (Cobe Spectra, COBE BCT, Inc., Lakewood, CO, USA). Apoptosis rates were confirmed daily until a target of 4.0×10^10 CD34+ cells/kg (RW) was collected. Median number of mononuclear cells (MNC) and CD34+ cells transfused were 7.1×10^10/kg and 6.0×10^10/kg, respectively. There was a near significant correlation between the levels of sICAM-1 and CD34+ cell yield (r=0.49, 0.06). Median granulocyte and platelet engraftment days were 11 [10-18] and 12 [9-33], respectively. There was a significant inverse correlation between the CD34+ cell dose and granulocytes (n=0.68, p=0.022) but not platelet engraftment. The only correlation between SAM levels and engraftment was for sICAM-1 levels. Increasing sICAM-1 levels were a sign of prolonged neutropenia (n=0.72, p=0.011). No correlation between the apheresis day serum levels of adhesion molecules and acute GVHD was documented.

PO-0851  

Thrombopoietic factors in patients with iron deficiency anaemia with or without thrombocytosis

Akar H, Güven N, Aydoğuş I, Arat M, Üstün C, Çelebi H, Saltan Y

Ankara University, Faculty of Medicine, İbni Sina Hospital, Department of Haematology, Ankara, Turkey

Objective. Iron deficiency anaemia is a frequent cause of reactive thrombocytosis. Although it is frequent to see moderate increases in platelet numbers sometimes counts exceeding 1,000,000/mm^3 can be seen. The mechanisms causing reactive thrombocytosis are unclear. High levels of erythropoietin (EPO) may cause thrombocytosis as demonstrated in vitro studies, also there is a close structural relationship between EPO and thrombopoietin (TPO). Design and Methods. In this study, we evaluated 11 women with iron deficiency anaemia and thrombocytosis and 16 women with iron deficiency anaemia with normal platelet counts. Patients with acute bleeding and malignancy were excluded. Serum samples were taken before oral iron replacement therapy, one month after and at the end of replacement therapy. TPO, EPO, Leukaemia inhibitor factor (LIF), interleukin-6 (IL-6) and interleukin-11 (IL-11) levels were determined by ELISA. All patients were monitored by weekly blood counts, serum iron, iron binding capacity and ferritin levels during and at the end of therapy. Results. The results demonstrated that oral iron replacement therapy restored iron stores and blood counts reached normal with restoration of platelet counts to normal. Besides EPO, there was no change in the levels of other thrombopoietic cytokines (p>0.01 and p>0.05 respectively). Conclusions. It is clear that the change in EPO levels is a result of anaemia and its treatment. The correlation between high EPO levels and high platelet counts may suggest that EPO increases platelet counts but the same EPO level changes can also be demonstrated in women with iron deficiency anaemia but normal initial platelet counts (p>0.01). The levels of other cytokines remained.
unchanged during treatment. This shows that either these cytokines have no effect on reactive thrombocytosis or the change in platelet counts in our patients is in a narrow range thus not affecting the cytokine levels.

**PO-0852** Soluble transferrin receptor/ ferritin ratio as control of recombinant erythropoietin misuse

Bonifichi M, Baldini A,*, Lorenzi A, Marsigeli C, Arcaini L, Malcovati L, Bernardi L,*, Bernasconi C, Institute of Haematology, Laboratory of Biotechnology, Institute of Internal Medicine, University of Pavia, IRCCS S. Matteo, Pavia, Italy

Erythropoietin is possibly misused by athletes in sports for the purpose of improving performance. Presently there is no discernible or specific method to identify erythropoietin administration for doping control. Gareau et al. (Nature 1996; 380:113) recently reported the possible correlation between soluble transferrin receptor (sTfr) and ferritin (fTn) serum levels with the acute exposure to high altitude in 24 western subjects, normally living at sea levels. The data were collected during a scientific expedition to the ‘Pyramid’, the CNR laboratory situated in the Kuom Village (Nepal) at 5050 m. The blood harvests were performed at standard condition (30 m) at the Pyramid, at the 6th day, after 9 days walking from 2800 m till 5050 m(B) and after 8 days stay at the Pyramid (C). The results are reported in the following table:

<table>
<thead>
<tr>
<th>A (Standard)</th>
<th>(Arrival at 5050 m)</th>
<th>(Departure from Pyramid)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hb g/dl</strong></td>
<td>14±1.5</td>
<td>14±1.3*</td>
</tr>
<tr>
<td><strong>Ht %</strong></td>
<td>43±3.9*</td>
<td>43±3.9</td>
</tr>
<tr>
<td><strong>sTfr/ fTn</strong></td>
<td>1.3±0.4</td>
<td>1.5±0.2*</td>
</tr>
</tbody>
</table>

*p<0.05 vs standard; **p<0.05 vs arrival to 5050 m.**

Significant statistical modifications were observed in Hb values between time A and C, and in Ht values between time C and A; the sTfr/fTn ratio increased significantly only in C control. These data correlate with Gareau’s results, where non rHuEpo treated subjects showed a low increase of sTfr/fTn ratio, while treated subjects showed a more than 10 folds increase of this ratio. So we believe that the application of the sTfr/fTn ratio may help to discovery possible misuse of HuEpo especially in those situations where screening data are difficult to evaluate. We have planned to confirm our hypothesis on a higher number of patients in the future, utilising both rat and mouse models and rHuEpo for autologous blood donation as rescue before bone marrow transplantation.

**PO-0853** rh-CSF-induced phenotypic changes of neutrophils from patients undergoing autologous PBSC transplantation

Carulli G, Azzara A, Papinchesi F, Benedetti E, Caraccio F, Cecconi N, Vanacore R,*, Peñihi M, Division of Haematology of University of Pisa, *AOP, Pisa, Italy

Recombinant human granulocyte colony-stimulating factor (rh-CSF) induces several changes in neutrophils, such as modification of functions (enhanced phagocytosis, ADCC, reduction of chemotaxis), and modulation of surface effector molecules (i.e. increase in CD10, CD14, CD64 expression, decrease in CD16 expression). rh-CSF is currently used to improve neutrophil recovery after PBSC transplantation. We studied six high grade non-Hodgkin’s lymphoma patients undergoing hemoglobin (Hb) levels in athletes during doping evaluation. The aim of this study is to discuss the modifications of the sTfr/fTn ratio associated with hemoglobin and haematocrit with the acute exposure to high altitude in 24 western subjects, normally living at sea levels. The data were collected during a scientific expedition to the ‘Pyramid’, the CNR laboratory situated in the Kuom Village (Nepal) at 5050 m. The blood harvests were performed at standard condition (30 m) at the Pyramid, at the 6th day, after 9 days walking from 2800 m till 5050 m(B) and after 8 days stay at the Pyramid (C). The results are reported in the following table:

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<th>(Arrival at 5050 m)</th>
<th>(Departure from Pyramid)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hb g/dl</strong></td>
<td>14±1.5</td>
<td>14±1.3*</td>
</tr>
<tr>
<td><strong>Ht %</strong></td>
<td>43±3.9*</td>
<td>43±3.9</td>
</tr>
<tr>
<td><strong>sTfr/ fTn</strong></td>
<td>1.3±0.4</td>
<td>1.5±0.2*</td>
</tr>
</tbody>
</table>

*p<0.05 vs standard; **p<0.05 vs arrival to 5050 m.**

Significant statistical modifications were observed in Hb values between time A and C, and in Ht values between time C and A; the sTfr/fTn ratio increased significantly only in C control. These data correlate with Gareau’s results, where non rHuEpo treated subjects showed a low increase of sTfr/fTn ratio, while treated subjects showed a more than 10 folds increase of this ratio. So we believe that the application of the sTfr/fTn ratio may help to discovery possible misuse of HuEpo especially in those situations where screening data are difficult to evaluate. We have planned to confirm our hypothesis on a higher number of patients in the future, utilising both rat and mouse models and rHuEpo for autologous blood donation as rescue before bone marrow transplantation.
The aim of this study was to assess immunologic reconstitution following allogeneic haematopoietic stem cell transplantation (HSCT), as identified by circulating levels of lymphocyte subpopulations, and to test for possible predictive variables. We therefore monitored 363 patients weekly after HSCT until day +100 for surface markers (CD3, CD4, CD8, CD56) by flow cytometry, and then at 6 month intervals until last follow up. Patients were stratified according to the type of donor and manipulation of the graft. (1) HLA identical siblings: (1a) unmanipulated bone marrow (BM) (n=209), (1b) unmanipulated peripheral blood (PB) (n=29), (1c) T cell depleted (TCDD) BM (n=38); (2) mismatched family donors: (2a) unmanipulated PB (n=11), (2b) CD34+ selected BM+PB (n=15), (3) unrelated donor: unmanipulated BM (n=51). Median CD4 absolute counts were highest in unmanipulated matched PB grafts until one year post graft: on day +30 it was 230/µL vs 133/µL for unmanipulated matched BM, 40/µL for unmanipulated mismatched PB grafts, 36/µL for MUD BM, 38/µL for TCD matched BM and 18/µL for CD34+ selected mismatched BM+PB. A median CD4 count of 500/µL was reached 2 years post transplant. In contrast CD56+ lymphocytes engrafted significantly later in the TCD group. Median CD8 counts reached 2 years post transplant. All patients who achieved a CD4+ count of 500/µL were engrafted, and no significant differences in age, interval DX-BMT and FAB subtype between patients who relapsed and those who did not were found. Ninety-nine patients are surviving with a median follow up of 34 months (2-74); median age= 34 years (10-52). Conditioning regimens were 9/13, 17/29, 10/35 myeloablative and 16/29 non myeloablative. A total of 153 patients were referred to IFN after relapse. These data suggest that a low tumour burden (TBI dose) and GVHD prophylaxis schedule is an important factor in the outcome of patients transplanted for CML, however it also shows that patients who do not achieve a complete molecular remission and who relapse may be rescued by either IFN or 2nd transplantation: in these pts. a new molecular remission could be obtained. 3) a possible role of HLA A2 in protecting from relapse was also suggested.

Further studies are necessary to confirm these preliminary results and to assess engraftment potential of PBSC harvested on day 4 of G-CSF mobilisation therapy.
PO-0860 Advanced phase at relapse in PH+CMML after allogeneic BMT: AN E.B.M.T. retrospective analysis.

On behalf of the Chronic Leukaemia Working Party of the European Blood and Marrow Transplantation (EBMT) group

Despite monitoring of disease after allogeneic BMT performed in first chronic phase, some patients (pts) may present a sudden haematological relapse (HR) in advanced phase (AP); a situation with worse survival after relapse compared to HR in chronic phase (CP). Our objective was to investigate whether the stage of disease (CP or AP) at HR is associated with a series of prognostic factors in PH+ chronic myelogenous leukaemia (CML) patients who had HR after a BMT from an HLA-identical sibling. Data from 202 pts with PH+CMML (457 yrs, median 35) transplanted in 1st CP from 7.81 to 7.93 at 41 EBMT centers were collected. The disease stage at HR was AP (18 accelerated, 44 blastic) in 62 pts, CP in 140 pts. Median time from BMT to HR was 21 mos in AP and 23 mos in CP. We evaluated, by the chi-square test, the following factors known to be associated with survival:

- Factor CP/AP CP/AP P-value
  - Sokal @ diagnosis yes 62/24 >.10
  - Age @ BMT (years) ≤35 70/32 >.10
  - Days from diagnosis to BMT ≤216 64/28 >.10
  - T-depleted BM yes 84/28 no 40/27 >.10
  - Acute and/or chronic GVHD yes 72/33 no 66/26 >.10
  - Previous cytogenetic relapse yes 56/12 no 84/40 >.10
  - Days from BMT to HR ≤156 26/20 >.10

This study suggests that sudden haematological relapse in advanced phase is not associated with these factors.

PO-0861 Idarubicin-busulfan-cyclophosphamide conditioning regimen for allogeneic BMT in high-risk haematological malignancies

Iori AP, Guglielmi C, Annunziata M, Romano A, Bernasconi S, Laurenti L, Gentile G, Arceci R
Istituto di Medicina dell’Allogeneo “Giuseppe Papa”, Dipartimento di Biotecnologie Cellulari ed Ematologia, Università “La Sapienza”, Rome, Italy

Since June 1996, we have used an idarubicin-containing regimen (IDA-BUCY) in 24 consecutive patients (pts) (3 children) with high-risk haematological malignancies who received an allogeneic BMT from their HLA-identical sibling. IDA-BUCY consisted of idarubicin 21 mg/m2 continuous infusion over 24 hours on days -2 and 1-1, busulfan 4 mg/kg/day from day -7 to -4, and cyclophosphamide 60 mg/m2/day on days -3 and -2. GVHD prophylaxis consisted of CSA alone in 5 cases and CSA-ATG in 19. Donor BM (n=18) or PB (n=6) cells were infused at day 0. Median age was 28 yrs (3-43). 3 CMV-ve recipients received CMV+ve donor cells, 1 patient died on day +21 of CMV reactivation. There were 3 transplant-related deaths on day +21, +26 and +5 months. A fourth patient died of grade IV steroid-resistant GVHD 11 months post-BMT following a DLIs infusion for relapsed mantle cell lymphoma. Eleven patients are alive with a follow-up of 1-14 months. 3 patients are in remission or progression free. One patient with AML is alive in relapse and currently receiving DLI infusions. In summary, in this high-risk group of allograft recipients, the protocol was associated with minimal toxicity and a high incidence of engraftment.

PO-0862 Preliminary results of a non-myeloablative conditioning regimen for allogeneic stem cell transplantation

*Departments of Haematology University College Hospital and #St George’s Hospital, London; &Heartlands Hospital, Birmingham; ¾Christie Hospital, Manchester; ¼Leeds General Infirmary and ½Sir William Dunn School of Pathology, University of Oxford, UK

We have investigated a novel low intensity conditioning regimen. The treatment was designed to suppress the recipient immune system enough to allow allogeneic engraftment without causing excessive mucositis but to allow for adequate tumour control to facilitate a graft versus leukaemia/lymphoma effect using DLI if necessary. Data from fifteen consecutive patients are reported. Patient diagnoses were as follows: NHL (n=7), Hodgkin’s disease (n=3), AML (n=3), myeloma (n=2). Several patients had high-risk features including previous high dose therapy (n=8); low LVEF (n=2); failure to mobilise autologous stem cells (n=2) and AML refractory to induction (n=2). Patients received the conditioning regimen which consisted of CAMPATH-1H 20 mg/day on days -8 to -4, fludarabine 30 mg/m2 on days -7 to -3 and melphalan 140 mg/m2 on day -2. The 13 sibling recipients received unmanipulated G-CSF mobilised peripheral blood stem cells on days 0 and +1 from their HLA identical siblings. Two patients received unmanipulated marrow from matched unrelated donors. Graft-versus-host disease prophylaxis was with cyclosporin A alone in all but 5 sibling recipients who received cyclosporin A plus methotrexate. One patient who died on day +21 of Gram negative neutropenic sepsis was not evaluable for engraftment. Of the other 14 patients, 13 had sustained donor engraftment with one patient rejecting donor cells after initial donor engraftment. This patient had autologous reconstitution on day +33. Preliminary results of chimaerism analysis using microsatellite PCR indicate that most patients are full donor chimaeras. There was minimal mucositis in patients who received cyclosporin A alone as GVHD prophylaxis. Only one patient developed grade I acute GVHD, no patients had chronic GVHD. There were 3 transplant-related deaths at days +21, +26 and 5 months. A fourth patient died of grade IV steroid-resistant GVHD 11 months post-BMT following a DLIs infusion for relapsed mantle cell lymphoma. Eleven patients are alive with a follow-up of 1-14 months. 3 patients are in remission or progression free. One patient with AML is alive in relapse and currently receiving DLI infusions. In summary, in this high-risk group of allograft recipients, the protocol was associated with minimal toxicity and a high incidence of engraftment. There was a low incidence of GVHD. However, the longer-term effects of this treatment on disease control and immune reconstitution is currently unknown.

PO-0863 Early infectious complications in 107 patients who underwent peripheral blood progenitor cell autotransplantation

Baray J, Novo A, Morey M, Guerra JM, Espejo M, Duran MA, Galmés A, Miróncini J, Besaluch J
Hospital Son Dureta, Palma Mallorca, Balearic Islands, Spain

From June 1993 to December 1997, 107 patients (M36, F71; median age=42 range 4-62) with haematologic malignancies (59) and solid tumors (48) who underwent suitable high dose chemotherapy and APBCT (median CD3+ recovery=2.86 x 10^6/kg range 0.7-19.3) were enrolled. During hospitalisation the patients were kept in rooms with reverse isolation and received antimicrobial chemoprophylaxis consisting of oral quinolone, fluconazole, nystatin oral solution and parenteral acyclovir; all patients had a central (subclavian) venous access placed prior to transplant and 52 patients received G-CSF at a dose of 300 µg/day from day +4 until neutrophil (PMN) count >5 x 10^9/µL. The median duration of the neutropenia (PMN count less than 500/µL) was 7 days. Ninety-seven patients (90%) developed fever >38°C with a median duration of 4 days and were treated with broad spectrum antibiotics for 9 days (range 7-23). In 29 patients (27%) the febrile episodes were classified as fever of unknown origin. Documented clinical infection occurred in 22 patients (20%), and microbiologically documented infections in 45 Gran-veccie pathogens were the most frequent cause of bacteremia and were isolated from 48 patients (46%). Gran-veccie organisms were detected in blood cultures of 12 patients (10%); 8 patients (6%) had polymicrobial sepsis; 2 patients had fungal infection. No bacterial infec-
tion related deaths occurred. No viral infection was observed in the early period of transplant. In conclusion, although the neutropenia in the APBCT was short, most of the patients suffered from infection. Gran-veccie cocci were the predominant pathogens producing not life-threatening infections.
**PO-0864 Complete cytogenetic response with interferon-α (IFNα) after donor lymphocyte infusions (DLI) failure in chronic myeloid leukemia (CML) relapse after allogeneic bone marrow transplantation (allo-BMT)**

Perales M, Urbano-Izquierdo A, Carreras E, Feliz P, Rovira M, Martinez C, Rozman C, Monserrat E. Hemaatology Department, Hospital Clinic Barcelona, IDIBAPS, Spain

DU is increasingly used for treatment of relapsed CML following allo-SCT. However, there is a significant percentage of patients (pts) unresponsive to DU. Although a second transplant is then considered the treatment of choice, the procedure related toxicity can be extremely high. We have treated three pts with IFNα after DU failure; in all of them a complete cytogenetic remission (CCR) was observed. The three pts (48, 34 and 23 years old; 2 males) were in first chronic phase when they received an allo-BMT from an HLA-identical sibling donor, one of them with T-cell depleted (TCD) graft. The conditioning regimen consisted of cyclophosphamide, 120 mg/kg, and TBI 12 Gy (13 Gy in the case of TCD) and GVHD prophylaxis was with CsA and MTX. All pts achieved a molecular remission following allo-BMT. Treatment with hydroxyurea and CCR was achieved four months later. Without response in the following six months, IFNα (3×10^6 IU/3 days weekly) and IFNα (9×10^6 IU/3 days weekly) for 11 months without response; DU was performed (1×10^10 CD3/kg) and IFNα was discontinued. No response was observed in the following six months, IFNα was restarted and six months later a CCR was observed which has lasted now for 13 months. Case #2: ten months after TCD transplantation a cytogenetic relapse was observed, the pt. received 10^8 CD3/kg, despite of which he evolved to hematological relapse in five months. He received a second DU (1.97×10^10 CD3/kg) without response in the following seven months. IFNα (3×10^6 IU/3 days weekly) was started. Major cytogenetic response was observed at five months and CCR was achieved four months later. Case #3: a hematological relapse appeared 24 months after allo-BMT. Treatment with hydroxyurea was started until the pt. received DU (2.03×10^10 CD3/kg). Five months later, 100% of metaphases remained Ph+. Three months after IFNα was introduced (3×10^6 IU/3 days weekly) a CCR was observed. In conclusion, treatment of CML in relapse after allogeneic allo-BMT which does not respond to DU is challenging. IFNα should be considered as treatment for these pts, particularly those in whom a second transplant would imply a high-risk.

**PO-0865 Successful antigenia-guided therapy to prevent allogeneic haematopoietic transplants from CMV disease**

Vallejo C, Perez E, Heras I, Sanchez I, Moraleda JM, Vicente V. General University Hospital, Murcia, Spain

Objective. We analysed the incidence of CMV disease in patients undergoing haematopoietic stem cell transplantation (HSCT) from fully HLA-matched sibling donors. Design and methods. Twenty-four patients at risk are included in this analysis. Pre-HSCT CMV serology was negative in both donors and patients in just two cases (8.3%). CMV antigenemia and shell vial cultures were performed weekly from day 0 to day +120 (or +180 if the patient was on steroid therapy). Gancyclovir (GCV) therapy was started at the time of detection of viremia (pre-emptive treatment). GCV was administered at 5 mg/kg 12 h x 7 days, followed by 5 mg/kg/day 5 days a week until day +120. Results: see table.

<table>
<thead>
<tr>
<th>Positive CMV virema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence 10/24 (41.6%)</td>
</tr>
<tr>
<td>Onset (median range) Day +33 (+31 to 102)</td>
</tr>
<tr>
<td>Resolution after GCV 9/10 (90%)</td>
</tr>
<tr>
<td>Weeks to resolution (median range) 3 (1 to 5)</td>
</tr>
</tbody>
</table>

**CMV disease**

<table>
<thead>
<tr>
<th>Incidence 2/24 (8.3%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset Day +4 (case #1) Day +15 (case #2)</td>
</tr>
<tr>
<td>Previous pre-emptive GCV No (case #1) Yes (case #2)</td>
</tr>
<tr>
<td>Organs involved Bone (case #1) Skin, G-I tract, mouth (case #2)</td>
</tr>
<tr>
<td>Clinical manifestations Cytopenias (case #1), severe G-I bleeding (case #2)</td>
</tr>
<tr>
<td>Resolution after treatment* 2/2 (100%)</td>
</tr>
</tbody>
</table>

Conclusions. These results support previous studies suggesting that pre-emptive therapy (viremia-guided) is a safe approach to prevent CMV disease in patients undergoing allogeneic HSCT.

**PO-0866 High incidence of clinical GvHD in allogeneic peripheral blood stem cell transplantation**

Vallejo C, Perez-Ceballos E, Arriba F, Iniesta P, Moraleda JM, Vicente V. General University Hospital, Murcia, Spain

Objective. We analysed the incidence of clinical graft-versus-host disease (cGVHD) in patients undergoing unmanipulated G-CSF-mobilised PBSC transplants from fully HLA-matched sibling donors. Design and methods. We present data from 22 patients at risk (13 men/9 women; age range: 13-53 years old) GVHD prophylaxis was with CsA and short MTX, as per the Seattle protocol. The median follow-up was 16 months (range: 6-36). Asymptomatic histologic findings were not considered in this analysis. Results. See table.

| Incidence of clinical GvHD 16/22 (72.7%) |
| Onset Before day +100 8/16 (50%) |
| After day +100 (range: +170 to +301) 8/16 (50%) |
| Presentation Quiescent 9/16 (56.25%) |
| De novo 5/16 (31.25%) |
| Progressive 2/16 (12.5%) |
| Affected organs Mouth 14/16 (87.5%) |
| Liver 1/16 (6.25%) |
| Skin 13/16 (81.25%) |
| Gv-tract 5/16 (31.25%) |
| Eyes 4/16 (25%) |
| Joints 2/16 (12.5%) |
| Valva 2/7 (women) (28.6%) |

Conclusions. Allogeneic PBSC transplantation may be associated with a high incidence of extensive clinical graft-versus-host disease.

**PO-0867 Transfusion requirements after haematopoietic stem cell transplantation**

Vallejo C, Cano H, Candela MJ, O’rufio F, Moraleda JM, Vicente V. General University Hospital, Murcia, Spain

Objective. We analysed the units of packed red blood cells (PRBC) and platelet concentrates (PC) required after autologous and allogeneic haematopoietic stem cell transplantation (HSCT). Design and methods. One hundred and forty-seven patients undergoing HSCT for haematological and, oncological diseases, at our institution between Jan/93 and July/98 were included. Stable patients received PRBC and PC transfusions to hemoglobin > 8.5 g/dL and platelets > 10,000/µL. Each PC consisted of 1 unit of single donor platelets or 1 unit/10 kg of random donor platelets. Results. See table.

<table>
<thead>
<tr>
<th>Within the first 3 weeks post-HSCT</th>
<th>After the first 3 weeks post-HSCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous</td>
<td>Allogeneic</td>
</tr>
<tr>
<td>PRBC 4/122 (3.3%)</td>
<td>0/25 (0%)</td>
</tr>
<tr>
<td>1-5 83/122 (68%)</td>
<td>14/25 (56%)</td>
</tr>
<tr>
<td>6-10 30/122 (24.6%)</td>
<td>9/25 (36%)</td>
</tr>
<tr>
<td>&gt;10 5/122 (4.1%)</td>
<td>2/25 (8%)</td>
</tr>
<tr>
<td>CP 0 2/122 (1.6%)</td>
<td>1/25 (4%)</td>
</tr>
<tr>
<td>1-5 77/122 (63.2%)</td>
<td>14/25 (56%)</td>
</tr>
<tr>
<td>6-10 31/122 (25.4%)</td>
<td>5/25 (20%)</td>
</tr>
<tr>
<td>&gt;10 12/122 (9.8%)</td>
<td>5/25 (20%)</td>
</tr>
</tbody>
</table>

Conclusions. Within the first 3 weeks, most of the patients needed to be transfused with 1-10 PRBC and 1-10 PC in both autologous and allogenic HSCT procedures. After the first 3 weeks, most of the patients undergoing autologous HSCT no longer needed PRBC or PC anymore, while the majority of the patients undergoing allogeneic HSCT continued to need PRBC and PC transfusions.

**PO-0868 Factors that influence collection and engrafment of autologous peripheral blood stem cells (PBSC), Experience in 168 cases**


Between May 1991 and November 1998, 168 pts (87 m, 81 f, median age 45y, range 17-68y) were autografted with autologous PBSC in our centre (56 lymphomas, 54 myelomas, 27 solid tumors, 12 CML, 12 AML, 7 ALL).

*Case 1: GCV/Case 2: Foscarnet

**Resolution after treatment** 2/2 (100%)

**Resolution after GCV** 9/10 (90%)

**Conclusions**
At the time of the transplantation, 52% of the pts were in CR. 40% in PR. 8% were refractory or had disease progression. Four pts received a 2nd transplantation because of relapse or progression. Fifty-four percent of the pts had received >2mg of prednisone. 78% of patients were older than 65 years. The median number of CD34+ cells was: in group I 1.4 \times 10^6/kg (n=8), Group II 9 (8-10) \times 10^6/kG (n=11), Group III 18.4 \times 10^6/kg (n=8). The median number of leukaphereses was 2. (1-7). The median number of MNCs >10^9/kg was: in Group I 0.6 (0.5-1.6), Group II 1.6 (1.0-1.8), Group III 4.2 (1.2-12) and 255 (99-576), respectively. GVHD prophylaxis consisted of Cy+Prednisone (Group A) or Cy+MTX (Group B). Median time to reach a neutrophil count >0.5/\mu L and >1/\mu L and a platelet count >50,000/L, >100,000/L was 95, 95 days for neutrophils and >20,000/L, >50,000/L, and >100,000/L was 13, 15, 11, 18, 95 days respectively. In Group A compared to 16, 19, 14, 26, 42 days in Group B. Group A required a median of 8 U of red-blood cells and 30 U of platelets infused. In Group B compared to 17 U and 57 U, respectively, in Group B (p<0.009). Medi-an neutrophil count xnl at 30, 90, 180, 270, and 360 days was 3, 3, 1, 0, 0, respectively, in Group A compared to 3, 2, 1, 0, 0, respectively, in Group B (p<0.009). Median neutrophil count xnl at 30, 90, 180, 270, and 360 days was 3, 3, 1, 0, 0, respectively, in Group A compared to 3, 2, 1, 0, 0, respectively, in Group B (p<0.009). Medi-an neutrophil count xnl at 30, 90, 180, 270, and 360 days was 3, 3, 1, 0, 0, respectively, in Group A compared to 3, 2, 1, 0, 0, respectively, in Group B (p<0.009). Median neutrophil count xnl at 30, 90, 180, 270, and 360 days was 3, 3, 1, 0, 0, respectively, in Group A compared to 3, 2, 1, 0, 0, respectively, in Group B (p<0.009). Median neutrophil count xnl at 30, 90, 180, 270, and 360 days was 3, 3, 1, 0, 0, respectively, in Group A compared to 3, 2, 1, 0, 0, respectively, in Group B (p<0.009). Median neutrophil count xnl at 30, 90, 180, 270, and 360 days was 3, 3, 1, 0, 0, respectively, in Group A compared to 3, 2, 1, 0, 0, respectively, in Group B (p<0.009). Median neutrophil count xnl at 30, 90, 180, 270, and 360 days was 3, 3, 1, 0, 0, respectively, in Group A compared to 3, 2, 1, 0, 0, respectively, in Group B (p<0.009).
PO-0873 Immunological recovery after UCB transplantation in congenital neutropenia

Viucci A, Pietraporta A, Campanale D, Capocasale M, Lucivero G*, Tannone T
Chair of Haematology II, University of Bari; *Internal Medical Department, II University of Naples, Italy

At present umbilical cord blood is considered as an alternative source of haemopoietic stem cells capable of restoring haemopoiesis. The first successful umbilical cord blood (UCB) transplant was reported in 1989 in a child with Fanconi anaemia. We studied immune reconstitution in 3 children affected by Thalassemia major transplanted with stem cells. 1 subject was transplanted with cord blood stem cells only, 2 subjects with bone marrow too. No significant differences were found in circulating distribution of the 3 main lymphocytic lines T,B and NK. Two months after transplantation CD4+, CD3+ and CD7+ 20-33% of circulating lymphoid cells were less than 1 because of increasing CD8+. Subsequently there was CD4+/CD8+ ratio was between 1 and 2. The CD56+/CD57+ ratio on NK cells. expression of chronic antigenic stimulation of immune system cells, was greater than 1 for 6-9 months after UCB transplantation; subsequently in 2 of 3 patients the ratio was less than 1. Expression examination of RA and RO isoforms of CD45 antigen in patients transplanted with UCB only showed a prevalence of CD45RO cells for 2 months after transplantation, perhaps in relation to their intense growth; subsequently there was a prevalence of CD45 RA cells with a RA/RO ratio between 2:1 and 3:1. In conclusion patients transplanted with UCB a late and adequate immune reconstitution was observed. Further clinical correlation between final outcome and clinical course can be carried out with a larger group of patients and a longer clinical and immunological follow-up.

PO-0874 Autologous BMT for acute lymphoblastic leukaemia in a single centre experience

Holowiecki J, Wojnar J, Krawczyk-Kulis M, Wojciechowska M, Kruziel T, Markiewicz M, Macko L
Univ. Department of Haematology & BMT, Katowice, Poland

From April 1991 to December 1998, 83 patients (median age 22 years) with acute lymphoblastic leukaemia (ALL) – 56 risk high in first (CR1) and 27 in second remission (CR2) – were treated with autologous BMT (ABMT). Patients were conditioned with CAV and bone marrow was stored for 72 hours at 4°C. The median follow-up period was 23 months. The survival was significantly better in CR1 (73% long-time survival) than in CR2 (19%), p<0.0001. The relapse rate was 80% for patients in CR2 and 47% in CR1, p=0.0007. The figures in next column presents Kaplan-Meier survival and relapse curves for both groups. Frequent relapse was associated with pre-ABMT dose/time reduced treatment schedule. Relapse was the main cause of death in both groups. These results indicate, that ABMT, when performed in first CR, may be an effective therapy in high risk ALL patients with no relative donors available.

PO-0875 Successful cord blood transplantation in a patient with severe congenital neutropenia (SCN)

Department of Paediatrics, University of Turin and O.I.R.M.-S. Anna Hospital, Turin, Italy

SCN (Kostmann’s disease) is a rare autosomal recessive disease primarily affecting neutrophil maturation. The chronic administration of G-CSF has markedly improved quality and expected survival of patients with SCN. However, the number and severity of infections. However G-CSF cannot cure the disease in these patients who are burdened by side effects of prolonged cytokine administration and the risk of developing myeloid leukemias. Thus alternative curative treatment must be considered. A few patients with SCN have been transplanted and in most of them the transplant did cure the disease. To our knowledge, only 2 patients have been given cord blood and one was cured. We report on a girl with SCN treated with more than 9 years with high dose G-CSF, who developed a severe thoracic granulomatous infection due to cat scratch disease and who was transplanted with cord blood from her HLA matched sibling. At the time of transplant the patient was 10 years old and her weight was 29 kg. Conditioning regimen included: cyclophosphamide (60 mg/kg die days -3,-2), thiotepa (3 mg/kg l.d. day -4) and busulfan (14 mg /kg days -3,-2). GVHD prophylaxis consisted in cyclosporine A (3 mg/kg die). Pneumocystis carinii prophylaxis (nebulised pentamidine 300 mg every 3 weeks) and CMV / HEV prophylaxis (Acyclovir) were started on the first day of the conditioning regimen. G-CSF was given at the dose of 5 µg/kg die i.v. from day +1 until day +30. After thawing 2.63·10^7/kg, 0.6·10^7/kg and 0.06·10^7/kg nucleated cells, CFU-GM and CD34+ cells respectively were infused. Neutrophil engraftment was achieved on day +25 and platelet on day +51. Neither GvHD or other complication was observed. At day +200 she is well with persisting full lineal full engraftment; her neutrophils show both normal count (more than 2000/mmc) and normal in vitro chemotactic function. Conclusions. This case confirms that cord blood transplant (CBT) from an identical sibling donor may provide full engraftment and complete correction of the disease in patients with SCN. Considering the low morbidity related to CBT, this option should be offered, if available, to young patients, even if responsive to G-CSF. Bone marrow transplantation, especially from unrelated donors, might be reserved to patients not responding to G-CSF and/or with mutations in G-CSF-receptor gene, the role of CB from unrelated CB banks should be carefully assessed.

PO-0876 Adjuvant single agent chemotherapy followed by modified high dose CTC in breast cancer patients

1. Int. Dept, KH der Elisabethinen, Linz, Austria

Thirty-three women with locally advanced breast cancer (stage II/III, G 2/3, >4 pos. nodes) were treated with single agent chemotherapy followed by a modified CTC regimen with CD 34 selected stem cell rescue. The age of the patients ranged from 25 to 59 years (median: 45). Fourteen patients were treated with single agent chemotherapy followed by single agent chemotherapy and a longer clinical and immunological follow-up.

PO-0877 Autoimmune BMT for acute lymphoblastic leukaemia in a single centre experience

Haematologica vol. 84 (EHA Abstract Book); June 1999

ASCT (from day +1) to patients with a haematological malignancy, a good performance status, a caregiver 24 hours per day, lodging near to the hospital and an appropriate cultural and social environment. Outpatients were monitored daily in the Hospital. Results. Sixty-four ASCT were performed during the study period (April, 57-6 June, 98). Fifteen of them were managed on an outpatient basis. Conditioning included cyclophosphamide + TBI in eight patients (2 high acute leukaemia, 2 NHL, 1 CLL) and combined chemotherapy (BEAC or BEAM) in seven (4 NHL, 2 HD, 1 ALL). TBI was administered on 4 consecutive days, the total dose being 12 Gy in 7 cases and 13 Gy in one. Median age was 47 years (range, 15-62) in TBI group and 40 years (15-60) in the non-TBI group. Both groups had a similar incidence of re-admissions: 3 (38%; 2 fever, 1 intractable nausea) and 4 patients (51% 2 fever, 1 nausea, 1 capillary-leak syndrome), respectively. No differences were observed in the haematological recovery, 500 neutrophil/ml by day 11.5 (10-28) and 11 (9-16), respectively. Although mucositis was more severe in patients receiving TBI, median hospital stay (from day +1) was not longer in these patients: 3.5 days (range, 0-22) in the TBI group versus 11 (0-17) in the non-TBI group. Conclusions. Outpatient ASCT seems to be a safe procedure even in patients receiving TBI as conditioning.

PO-0878 Successful cord blood transplantation in a patient with severe congenital neutropenia (SCN)

Department of Paediatrics, University of Turin and O.I.R.M.-S. Anna Hospital, Turin, Italy

SCN (Kostmann’s disease) is a rare autosomal recessive disease primarily affecting neutrophil maturation. The chronic administration of G-CSF has markedly improved quality and expected survival of patients with SCN. However, the number and severity of infections. However G-CSF cannot cure the disease in these patients who are burdened by side effects of prolonged cytokine administration and the risk of developing myeloid leukemias. Thus alternative curative treatment must be considered. A few patients with SCN have been transplanted and in most of them the transplant did cure the disease. To our knowledge, only 2 patients have been given cord blood and one was cured. We report on a girl with SCN treated with more than 9 years with high dose G-CSF, who developed a severe thoracic granulomatous infection due to cat scratch disease and who was transplanted with cord blood from her HLA matched sibling. At the time of transplant the patient was 10 years old and her weight was 29 kg. Conditioning regimen included: cyclophosphamide (60 mg/kg die days -3,-2), thiotepa (3 mg/kg l.d. day -4) and busulfan (14 mg /kg days -3,-2). GVHD prophylaxis consisted in cyclosporine A (3 mg/kg die). Pneumocystis carinii prophylaxis (nebulised pentamidine 300 mg every 3 weeks) and CMV / HEV prophylaxis (Acyclovir) were started on the first day of the conditioning regimen. G-CSF was given at the dose of 5 µg/kg die i.v. from day +1 until day +30. After thawing 2.63·10^7/kg, 0.6·10^7/kg and 0.06·10^7/kg nucleated cells, CFU-GM and CD34+ cells respectively were infused. Neutrophil engraftment was achieved on day +25 and platelet on day +51. Neither GvHD or other complication was observed. At day +200 she is well with persisting full lineal full engraftment; her neutrophils show both normal count (more than 2000/mmc) and normal in vitro chemotactic function. Conclusions. This case confirms that cord blood transplant (CBT) from an identical sibling donor may provide full engraftment and complete correction of the disease in patients with SCN. Considering the low morbidity related to CBT, this option should be offered, if available, to young patients, even if responsive to G-CSF. Bone marrow transplantation, especially from unrelated donors, might be reserved to patients not responding to G-CSF and/or with mutations in G-CSF-receptor gene, the role of CB from unrelated CB banks should be carefully assessed.
Portugal
Italy
Urine samples were tested and 26% were positive for polyomavirus. A patient had reached a steady engraftment with no GVHD. A total of 225 were tested weekly for the presence of polyomavirus particles using EM, obtained with cyclosporin A and either MTX or Methylpred. Urine samples in Institution. Most of them had acute leukaemia, and received a condition- ing regimen, but more commonly supervenes later and is then gone allogeneic bone marrow transplant for haematologic conditions at our B.M.T. Unit and E.M. Unit; *Portuguese Institute for Oncology, Lisboa, Guimarães A

To assess the long-term results of autologous transplantation and the prog- nosis influence of pre-transplant characteristics we analysed 290 patients (M=150, F=140; median age 52 yr, range 19-70) reported to the GITMO registry. Ig class was G in 171, A in 61, D in 3, BJ in 37. Non-secre- tor MHC class was found in 11. Thirty-four (11.6%) patients received 0.5 ± 10^9 L-granulocytes and 50 – 10^9 L/platelets on day 13 (6-72) and on day 21 (6-176), respectively. Following the autograft, 111 patients (40%) were in CR, 137 (50%) in PR and the remaining 24 (9%) did not respond or progressed. TRM was 3%. At a median FU of 23 months OS and EFS at 7 years are 47% and 33%, respectively. The EFS curve shows no plateau. In multivariate analysis, age, β2-microglobulin level and status at transplant emerged as significant both for OS and EFS, while time from diagnosis to transplant showed border-line significance. Double autograft showed no influence. We also analysed the various combination patterns of significant factors. Probabil- ity of OS appears highest in remission patients autografted within 6 months of diagnosis, but at the opposite end it becomes very poor in patients with >4 mg/L β2-microglobulin who do not respond to first line therapy. The sta- tistical model may be employed to predict the transplant outcome, though it needs to be validated in prospective studies.

Reduction in the rate and severity of haemorrhagic cystitis

Guimarães A, Moura-Nunes J*, Machado A, Ferreira I, Abecasis M B.M.T. Unit and E.M. Unit; *Portuguese Institute for Oncology, Lisboa, Portugal

Haemorrhagic cystitis due to polyomavirus is a well known clinical condi- tion that can follow bone marrow transplantation mainly in the allogeneic setting. It sometimes arises early in the course of the transplant, within the first 2 weeks, and correlates well with the use of cyclophosphamide in the conditioning regimen, but more commonly supervenes later and is then clearly associated with polyomavirus excretion in the urine. During 4 con- secutive years from 1992-96, 78 patients (children and adults) have under- gone allogeneic bone marrow transplant for haematologic conditions at our Institution. Most of them had acute leukemia, and received a condition- ing regimen consisting of BUCY (1pt). GVHD prophylaxis was taken with cyclosporin A and either MTX or Methylpred. Urine samples were tested weekly for the presence of polyomavirus particles using EM, starting on week 0 and continuing until 6 months after BMT or, until the patient achieved a steady engraftment with 10% GVHD. A total of 225 urine samples were tested and 26% were positive for polyomavirus. Accord- ing to the schedule of IVIG as part of the GvHD prophylaxis, the cohort of 78 patients was divided in two groups: A (n=25) had 4 weekly treat- ments with 500 mg/kg IVIG; B (n=37) had 3 treatments with 500 mg/kg IVIG, and C (n=16) had no IVIG included in the regimen for GvHD prophy- laxis. In an exploratory data analysis no correlation could be found between

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the detection of polyomavirus and the subsequent development of cystitis and variables such as age, sex, initial diagnosis, type of conditioning reg- imen, acute GvHD or the degree of MTX or prednisone used in the regimen for GvHD prophylaxis. On the other hand the presence and timing of IVIG in the GvHD prophylaxis regimen correlated well both with the detection of polyomav (p=0.019), but also with the resolution and the severity of infec- tion (p=0.012). Patients who received IVIG as part of their GvHD prophylax- ias had a lower rate of polyomavirus infection than patients who did not get immunoglobulin. Among patients in the IVG group, the ones that received 4 treatments of immunoglobulin fared better than the group that only received 3 treatments of IVIG. We conclude that inclusion of IVIG in the GvHD prophylaxis regimen in the allogeneic bone marrow transplant setting is important for the prevention of polyomavirus associated haem- orrhagic cystitis.

A single centre experience of unrelated cord blood transplantation

Picardi A, Dcntamoro T, Cudillo L, Postorino M, Del Principe M, Cox C, Venditti A, Bussciano F, Caravita T, Adorno G, Balitare Bruno A, Del Principe D,* Menichelli A,* Amadori S Dept. of Haematology and *Pediatrics, University Tor Vergata, St. Euge- nio Hospital, Rome, Italy

Umbilical cord blood (UCB) represents an alternative source of haematopoi- etic stem cells for allogeneic transplantation. We present our experience of 6 unrelated CBT performed between January 1997 and January 1999. All patients received a CBT from a mismatched donor (1 locus 4; 2 loci 2). The recipients were 4 children and 2 adults aged between 16 months and 41 years; the body weight ranged between 10 and 70 kg (median 21 kg). The CB units came from Milan (5), Barcelona (1) and New York Banks (2). Stat- us of disease at CBT was: 1st relapse of CML in 1 child, RABE-T in another child, RA in 2 children and 2nd relapse of Ph + ALL in the adult patients. Conditioningregimen consisted of TBI or BUS + CTX 1616 in 4 patients, TBI + Thiotepa in 1, and Fluodara + Ara-C + VP16 + Thiotepa in 1 child who had already failed a mismatched a/PSBCT from the mother after BUS + CTX conditioning. ATG was added in all cases and GvHD prophylaxis (CSA and 6-MP) was identical for all patients. G-CSF was used in the adult patients. Median number of NC, CD34 + and CFMU-infused, after thawing, were 3.85 × 10^6/kg, 1.14 × 10^10/kg and 1.93 × 10^10/kg, respectively. Results. One child is on day + 14 from CBT showing initial signs of haematopoietic recovery. The other three pediatric patients engrafted and achieved complete donor chimaerism without evidence of acute GvHD (grade II-VI). The medi- an time to neutrophil recovery was 24 days (range 23-29) and to PLTs >200000 was 35 days (range 28-39). Two children are alive and well with- out evidence of disease at 6 and 15 months from CBT; the other died on day +39 of heart failure. Both adult patients died: the first achieved a tran- sient engraftment documented by chimaera evaluation (donor = 100%) and developed grade II GvHD; secondary graft failure rapidly followed and she died in aplasia of cerebral bleeding on day +44. The second patient failed to achieve engraftment and died with grade 4 neurotoxicity on day +46, after autologous peripheral stem cell back up reinfusion. Conclusions. UCB constitutes a good alternative source of haematopoietic stem cells for allogeneic transplantation in children. The low stem cell content of the units represents the main problem of this transplant procedure limiting its exten- sion to pediatric patients because of the associated high risk of graft fail- ure in adults.

Idarubicin-intensified myeloablative therapy for allogeneic stem cell transplantation in haematologic malignancies

Cudillo L, Dentamoro T, Picardi A, Postorino M, Santinelli S, Tamburini A, Masi M, Amadori S Haematology, University Tor Vergata, St.Eugenio Hospital, Rome, Italy

Allogeneic stem cell transplantation (alloSCT) is an effective therapy for haematologic malignancies, but relapse continues to be a major problem. An approach to decrease the risk of disease recurrence is to use an HLA identical or mismatched donor to achieve a steady engraftment with 10% GVHD. A total of 225 urine samples were tested and 26% were positive for polyomavirus. Accord- ing to the schedule of IVIG as part of the GvHD prophylaxis, the cohort of 78 patients was divided in two groups: A (n=25) had 4 weekly treat- ments with 500 mg/kg IVIG; B (n=37) had 3 treatments with 500 mg/kg IVIG, and C (n=16) had no IVIG included in the regimen for GvHD prophy- laxis. In an exploratory data analysis no correlation could be found between

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time of peak concentration (t_{max}) and clearance (Cl). Our results showed no significant difference in t_{max}, C_{max} and AUC between the two oral formulations. Cmax and AUC were highest in the intravenous formulation. The interindividual variability was very high with Optoral in comparison to Sandimmun and the intravenous CsA formulation. We conclude that the high interindividual variability by Optoral with sometimes very high Cmax levels could be associated with an increase of renal-related adverse events observed in the past. These high C_{max} levels were not detected by our 12h trough level monitoring. An improvement in monitoring CsA levels under treatment could be possible with a limited sampling model.

**PO-0883** Autologous peripheral blood stem cell transplantation (PBSCT) in patients with acute leukaemia

Division of Haematology, IRCCS “Casa Sollievo della Sofferenza”, Hospital S. Giovanni Rotondo, Italy

Between May 1994 and December 1998, 16 patients (pts) with AML in first (n = 10) or later complete remission and 5 pts with ALL in first CR underwent autologous transplantation using PBSCT + BM (n = 3) or PBSCT alone (n = 18) at our Institution. There were 10 males and 6 females, with a median age of 44 years (range 3-66). Six pts had cytogenetic abnormalities in marrow cells examined before mobilisation, in detail 3 ALL Ph1+ and 2 AML M3 with t(8;21). Karyotype and hematopoietic phenotyping were possible in all pts in all collections. No cytogenetic abnormalities were found in repeated sampling and on bone marrow biopsy performed after full hematopoietic recovery. Conditioning regimen included TBI (ALL pts) and TBI + chemotherapy (10 AML pts). However, 6 AML pts (age >50 yrs) received only chemotherapy. A median of 7.5±10^9 kg CD34+ cells (range 2.1-23) and 9.2±10^9 kg CFU-GM (range 6.4-13) were infused. Recombinant G-CSF was given from day +1 at a dose of 5 mg/kg/day until the peripheral counts had recovered. The engraftment was fast with a mean of 13 days (range 9-28) for granulocyte level >500/µL and 14 days (range 9-14) for platelets >20,000/µL. Fourteen pts experienced infections complications, 7 bacterial, 7 fungal. No transplant related deaths were observed. Thirteen out of 21 pts relapsed and 11 died of progressive disease. In detail all pts affected by AML >60 yrs and all pts with ALL Ph1+ had early relapse. One AML pt in first CR post PBSCT relapsed at +9 months and in day 1 in second CR after a second autologous transplantation using BM + PBSCT at 12 months. Three pts with AML are alive in first CR at +30, +20, and +12 months. One pt with APL in third CR is alive and doing well, at +54 months, with t(15;17) and negative Philadelphia chromosome. Six pts are in first CR at +30 months (5 AML and 1 ALL), 5 pts are in CR at +30, +20, and +12 months. One pt with ALL are alive in first CR at +10, +9 and +1 months. In summary, our experience shows that pts receiving PBSCT have rapid engraftment and relatively low transplant-related mortality. However, PBSCT with or without bone marrow was associated with a high relapse rate resulting in an overall survival that was not superior to our historical experience.

**PO-0884** Overall results of a single centre autologous haematopoietic cell transplantation program

Azevedo AM, Vigoretti AC, Eid KAB, Aranha FJP, Oliveira GB, Gama P, Corrêa MEP, Miranda ECM, de Souza CA
Bone Marrow Transplantation Service, State University of Campinas (UNICAMP), Campinas, SP, Brazil

The autologous bone marrow transplantation program at UNICAMP was started in September of 1993. Up to May of 1998, 162 autologous procedures had been carried out in 143 patients: 105 bone marrow transplants, 40 peripheral blood progenitor cell transplants, 16 donor leucocyte infusions and 1 cord blood transplant. The most frequent diagnoses were chronic myelogenous leukaemia (56), aplastic anaemia (28), acute myelocytic leukaemia (23), acute lymphocytic leukaemia (12), aplastic syndrome (11). The median age of the patients was 29 years (3-56.9), 62% being male. The most frequently used preparative regimen was BU-Cy2 (50), Cy-Cy/7) and Bu+Cy1 (12) for chronic and acute leukaemias; and Bu (4 mg/kg)-Cy2 (24), Cy (6) and ALG-Cy (5) for aplastic anaemia. The prophylaxis of graft-versus-host disease was cyclosporin and methotrexate in 89% of cases, the remainder receiving cyclosporin and methylprednisolone. The median time for neutrophil (10^9/L) and platelet (>200,000/µL) engraftment were 19 and 18 days, respectively. The median day of hospital discharge was +27. The overall survival of all patients was 46%, at a median follow-up of 349 (8-1686) days. The most frequent causes of death were acute GvHD (17), relapse of leukaemia (15), chronic GVHD (13) and infection (6). According to diagnosis, the follow up and overall survival are:

**Clinical transplantation II**
Haematologic malignancies who received HDC (modified CEP protocol) with relapsed or refractory lymphoma and as adjuvant therapy in other secondly, to determine the efficacy and outcome of CEP protocol in patients dose chemotherapy (HDC) with a suitable stem cell collection regimen and is to find a simple, less expensive and more effective protocol for high are undoubtedly capable of restoring short term haematopoiesis when rein- It is now possible to conclude that PBSC collected in sufficient amounts PBSCT has been increasingly used in place of ABMT (HDC). We studied 24 patients (2 Hodgkin's disease, 11 non Hodgkin's lymphoma, undergoing introduction. We developed an alternative method to analyze the degree of haematopoietic chimaerism in male patients after bone marrow transplantation (bmt) with female donor cells. This method takes advantage of both a SRY-gene specific DNA sequence of Y-chromosomes and b) quantita- tive measurement of SRY-specific amplicons generated by a fluores- cence-based 5' nuclease assay (TaqMan technology, PE-ABI). After BMT only XX-related cells should by found in peripheral blood cells, therefore increase of Y-specific DNA sequences can be interpreted as chimaerism or relapse of the patients' disease. Methods. 1) DNA is extracted by stand- ard procedure from peripheral blood cells. (2) For evaluation of the method serial dilutions of X:Y-specific DNA in the background of female-DNA were prepared. (3) Real-Time-PCR was performed in 50 µL assays: Primer and MgCl2 concentrations were defined experimentally ([SRY-F = 200 nMol, Tm=60°C; SRY-R = 240 nMol, Tm=60°C; MgCl2 =5 mMol). (4) PCR-products were quantified by degradation of the internal binding hybridisation probe during PCR ([SRY-Fam: 5'-CGCCTCTCAGGGAATCACTT-3', SRY-quencher= TMRMA, Tm=74°C). For internal positive controls (IPC+) an Actin-gene specific Taq- man assay (Reporter-dye=FITC, Quencher-dye=6-FAM, Tm=76°C) was used. Results. Experiments were done in quintuplicate and were checked for pos- itivity of IPC+. With this method we found relative ratios as low as 0.1-0.2% (50-100 X cells in a background of 50,000 Y cells). This method is fast and reliable: the 5' nuclease-assay is done in optical 96-well plates (8-wells for controls; 4-wells each probe, up to 22 individual samples in paral- lel). The procedure takes approx. 110 minutes and eliminates the need for aquamarine gel-electrophoresis.

Haematopoietic reconstitution with autologous peripheral stem transplantation (APBST) after high dose chemotherapy (HDC)

Salamo O.S.*, Mahmoud LA.*, El-Wahidi GF*, Marouf S*.
*Haematology Dept. and "Clin. Oncology & Nuclear Medicine Dept.
Faculty of Medicine, Mansoura University, Mansoura, Egypt

PBSC has been increasingly used in place of ABMT (Gottwhal et al, 1996). It is now possible to conclude that PBSC collected in sufficient amounts are undoubtedly capable of restoring short term haematopoiesis when rein- fused after myeloablative chemotherapy. This study has two aims; the first is to find a simple, less expensive and more effective protocol for high dose chemotherapy (HDC) with a suitable stem cell collection regimen and secondly, to determine the efficacy and outcome of CEP protocol in patients with relapsed or refractory lymphoma and as adjuvant therapy in other malignancies. This study included 11 patients with haematologic and non hematologic malignancies who received HDC (modified CEP protocol) with stem cell support (4 BMT and 7 PBSC). PBSC were mobilised either with low (1.5 ng/ml) or high (4 ng/ml) dose etanercept with delayed addition of either G-CSF or GM-CSF (10–12 µg/kg/day) to day 5 or day 10 respec- tively. Single leukopheresis was done when WBCs exceeded 15 x 10^9/L with target cell dose of MNC 2-4 x 10^11/kg, CD34+ve cells > 10^6/kg PBSC were preserved at 4°C-80°C or -180°C while BM was preserved at 4°C. All patients received modified CEP protocol (etanercept 60 µg, etoposide 1 g/m² and platinol 160 mg/m²). We concluded that all cases showed engraftment without mortality and haematopoietis had been satisfactorily restored with- out any need for post transplantation haematopoietic growth factors.

Human herpesvirus-8 infection in autologous peripheral blood stem cell transplant patients from Italy

Department of Medical Sciences. Section of Haematology, Modena, Italy: *Department of Medical Microbiology, Liverpool, UK

We studied 24 patients (2 Hodgkin's disease, 11 non Hodgkin's lymphoma, 4 multiple myeloma, 1 chronic myeloid leukemia, 3 acute myeloid leukemia, 1 acute lymphoid leukemia, 2 breast cancer), undergoing autologous peripheral blood stem cell (PBSC) transplantation, for human herpesvirus-8 (HHV-8) DNA (ORF 26 and KI) in serum by polymerase chain reaction (PCR). All patients received acyclovir and immunoglobulin pro- phyaxis. Fourteen of 24 patients had at least one clinical event during the post-transplant period, in absence of either bacterial or fungal infections. We detected HHV-8 DNA by one PCR in the sera collected immedi- ately before and/or concomitant with clinical events in 3 of these 14 patients, at days 8, 9, 12, respectively, after PBSC reinfusion. HHV-8 was variant A in two patients and variant C in one patient, based on the sequencing of hypervariable KI gene. The presence of antibodies to HHV- 8 was detected by two assays (ELISA for ORF 65 and lytic IFA) in one patient, and by a single assay (either ELISA for ORF73c or lytic IFA) in the other two patients, respectively. HHV-8 viremia was no longer detectable after the resolution of the associated clinical events (fever in three, cuta- neous rash and elevated aminotransferases in one, bone marrow aplasia with plasmacytosis in one cases, respectively). HHV-8 DNA was absent in the sera from the 19 autotransplanted patients without clinical events. Epstein-Barr virus, human herpesvirus-6 and -7 DNA were absent in the 24 patients' sera examined. Cytomegalovirus antigenemia was detected, con- comitant with intestinal pneumonitis and with fever with cutaneous rash, in two further patients, respectively. HHV-8 DNA was not detected by one step PCR in the purified CD34 + cell fraction from the reinfused apheresis products of the 3 patients who developed HHV-8 viremia. Our study shows, for the first time, that HHV-8 viremia may occur in the setting of autolo- gous PBSCT transplantation in association with clinical events, at least in our area of Italy (the lower Po river valley), where HHV-8 seroprevalence in blood donors is relatively high.

Allogeneic bone marrow transplantation using G-CSF primed bone marrow

Li Gioi F, Peluso RD, Inghirerra G, Indelicato F, Guido G, Stagno F, Fraioli N, Mancini G, Giustolisi A.* Dipartimento di Haematologia e Bone Marrow Transplantation Unit, University of Catania, Italy

Possible advantages of G-CSF-primed bone marrow harvest in allogeneic BMT are fast engraftment and low GvHD risk, likely due to G-CSF induced cytokine modulation (Th2 polarisation). However published data on such a type of transplant are very limited. We describe the outcome of four cases of allogeneic bone marrow transplantation in which we used, as inocu- lum, bone marrow cells harvested after G-CSF priming of the donor. Glyco- sylated G-CSF (Myelostim-Italfarmaco) at the dosage of 5 mg/kg/day was administered to the donor for 3 days. S.C. and bone marrow harvests were done on the 4th day using multiple punctures in ilium bones, harvests were done during spinal analgesia. In one case the harvest was infused with no further manipulation whereas in 3 cases, due to major ABO incompatibili- ty between donor and recipient, the harvest was depleted of red cells and mature myeloid cells using a CS-3000 cell separator. Underlying disease were: 1 CML, 2 AML, 1 ALL; in all cases donor/recipient pairs were HLA iden- tity between donor and recipient, the harvest was depleted of red cells and white blood cells, and was then infused with G-CSF (myelostim-italfarma) at 10 microg/kg/day for 5 days. In 4 cases we found negligible donor contribution in the T and B cell lineages, therefore we considered the graft as totally donor-derived.

Herpes virus KI gene in patients with aplastic anaemia and idiopathic haematopoietic failure

Baccaglini A, Orlandi A, Torelli G.
Department of Medical Sciences. Section of Haematology, Modena, Italy.

In our preliminary experience G-CSF primed bone marrow seems to lead, indeed, to a fast haematopoietic recovery.

Bone marrow transplantation for paroxysmal nocturnal haemoglobinuria (PNH)

Raiola AM, Van Lint MT, Lamparelli T, Guidali F, O’cchinii D, Mordini N, Berisso G, Bregante S, Frazzoni F, Bacigalupo A

Departments of Ematologia Ospedale San Martino, Genova, Italy

PNH is an acquired clonal disease of the haematopoietic stem cell (HSC) characterised by intravascular hemolysis, panhypertension, versus thrombosis and rare leukemic transformation. Between January ’91 and July ’98 six patients with PNH (4 hematol, 2 hypoplastic), aged 23 to 37, were transplanted with unmanipulated bone marrow from an HLA identical sibling. Median time from diagnosis to BMT was 2.5 years (range: 1–16). All patients were trans- fusion dependent and received other therapy before BMT: steroids, vitamins, cyclosporin (CyA), growth factors (one patient ATG). Three patients were
HbsAg positive and one antiHCV positive. At time of the BMT the median val-
ue of HB was 9g/ dl, range (5.6-11 dl), WBC 4.9 <10 ⁿ⁹/L (range: 2.9-7.7),
plt 220 <10⁶/L (range: 51-355), LDH 3102 U/L. The condition regimen was
cyclophosphamide (range 100-160 mg/kg), busulfan (range 10-14 mg/kg),
followed by unmanipulated bone marrow (median of 4.7 <10⁹ cells/kg) and
CyA for GvHD prophylaxis (+MTX in one patient). Time to achieve a PMN count
followed by unmanipulated bone marrow (median of 4.7 <10⁹ cells/kg) and
CyA for GvHD prophylaxis (+MTX in one patient). Time to achieve a PMN count
of >0.5 <10⁹/L, platelets >30 <10⁹/L and HB >10 g/dl was respective-
ly 14.5, 17.5 and 19.5 days. Acute GvHD was limited or mild, chronic GvHD
was limited. This was also achieved in one patient (Rh factor negative) who
had a Rh factor positive donor: she had received a large number of transfu-
sions (>60) and had an intermediate/high grade titer (>1:20000) of anti-
D antibodies. All six patients are alive and full chimaeras, with complete
engraftment, with one PNH patient, also in the haemolytic phase of the disease, and may be con-
considered before complications of the disease and of transfusion therapy
become clinically evident.

PO-0890 A multiple-dose phase I pharmacokinetic study of intravenous
busulfan as myeloablation prior to bone marrow
transplantation
Olavarria E, Craddock C, Cwynarski K, Timmins A, Eades A, Kanfer E,
Apperley J, Goldman JM
ICSM, Hammersmith, Hospital, London, UK
Busulfan is an alkylating agent only available in oral form. High variability
in pharmacokinetics (PK) has been documented. Spartajet™ technology
encapsulates busulfan in phospholipids forming an aqueous suspension.
We present the preliminary results of a phase I study of multiple iv doses of
1 mg/kg. Six patients with chronic myeloid leukaemia underwent autol-
ogous peripheral blood stem cell transplantation (5 patients) or allogene-
ic sibling bone marrow transplantation (1 patient). Conditioning was with
busulfan alone (16 mg/kg). Two patients (1 AML, 1 ALL) underwent autologous
peripheral blood stem cell transplantation in first complete
remission after busulfan (16 mg/kg) and cyclophosphamide (120 mg/kg).
Median age was 41.3 years (21-53). Each patient received a total of 4 to
16 iv doses without short-term side effects. Toxicity was minimal. There were
no cases of veno-occlusive disease of the liver or toxic deaths. Pharmaico-
kinetics showed little variation between the first and last doses. The results
of the mean area under the curve (AUC), maximum concentration (Cmax),
half life (T1/2) and plasma clearance (CL) are shown below and suggest
that the PK values are more predictable than with an oral form.

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<th>AUC (ng.h/mL) mean (sd)</th>
<th>Cmax (ng/mL) mean (sd)</th>
<th>T1/2 (hr) mean (sd)</th>
<th>CL (mL/min/kg) mean (sd)</th>
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We conclude that Spartajet™ iv busulfan is safe and easy to administer.
It may have a future role in myeloablative regimens in both autologous and
allogeneic stem cell transplantation.

PO-0891 CD34-positive cell selection and progenitor cell expansion
in thawed cord blood grafts
Querol S, Capmany G, Gabarró M, Azqueta C, Martín-Heano G, García J,
Barcelona Cord Blood Bank and Cell Therapy Centre, Institut de Recerca
Oncològica, L’Hospital de Lle負egat Barcelona, Spain
Objective. The aim of this work is to optimise CD34 + cell selection of
thawed CB grafts and define a static, serum-free culture method for ex-vivo
expansion of CB progenitor cells, done in clinical grade condition. Design
and Methods. CB was collected after delivery while the placenta is still in
utero in a closed system. Within 24 hours was cryopreserved after red sed-
imentation process using HES in 10% DMSO and 1% Dextran as cryopro-
tectants. Twelve samples were thawed and washed using Dextran, Albumin
and DNAase to avoid clamping. CD34 + cell was selected using a direct
immunomagnetic method. Briefly, 2-10⁵ SAM-Dynabeads were pre-sen-
tised with 200 micrograms of 9C5 monoclonal-antibody and incubated for
30 minutes with the cells. A buffer containing DNAase, citrate, albumin
and immunoglobulin in PBS were used along the procedure. CD34 + cells
were eluted and detached using Isolex-300-SA kit. Cells from the enriched
fraction were adjusted at 25,000 cells/mL and cultured at 37°C and 5%
CO₂ for 6 days in a serum-free medium (CellGro, Genet cell supplement-
ed with hRSCF, hRIL-3, hRFLT-L and hRTPD (50 ng/mL each; CellGenix)).
Cells were expanded either in 1 mL wells or in cell culture semi-permeable
bags (Teflon or PL2417 bags). Results: The percentage of CD34 + cells in
the enriched fraction was 69% ±12 and the total of CD34 + cells = 1.94 <10⁹
± (1.55 ±10⁹), yielding a CD34 + cell recovery of 52% ±12.
 Colony forming unit assay shows a preserved functional state. CFU-GM
recovered were 33% ±15 and BFU/E-CFU-Mix group 27% ±12. Cell viabil-
ity of the expanded fractions on day 6 was 93% ±4. When comparing the
enriched progenitor cell generated, CD34 + cells fold-increase was 33 ±15
for expansion in wells, and 17 ±9 and ±20 ±12 for PL2417 and Teflon bags,
respectively. CFC assessed showed an increase of 24 ±13 for CFU-GM,
and 84 ±43 for BFU/E-CFU-Mix group. CFC expansion using bags were half
that observed using wells and there were no statistical differences between
type of plastic used. Conclusions. Using this protocol, we generated a 12-
fold increase of CD34 + cells, 6-fold more CFU-GM and 13-fold more BFU-
E/Mix colonies than that contained in fresh.
Supported by grants of Fundación Internacional José Carreras para la
Lucha contra la Leucemia.

PO-0892 Blood stem cell transplantation in 107 patients with cryo-
preservation of haematopoietic progenitor cells with 5-10%
dimethyl sulfoxide at 80°C without rate-controlled freezing
Bargay J, Galmes A, Guerra JM, Nova A, Morey M, Durán MA, Espeso
Hospital Son Dureta, Palma Mallorca, Balearic Island, Spain
Between June 1993 to December 1997, we performed blood cell trans-
plantation in 107 patients with solid and haematological malignancies (44
Breast cancer, 23 NHL, 11 Hodgkin’s disease, 12 acute leukaemia, 2 CLL,
11 myeloma multiple and 4 other solid tumour). The haematopoietic cells
were cryopreserved with a simple method of 5-10% dimethyl sulfoxide
(DMSO) as the sole cryoprotectant without rate-controlled freezing and
stored in a -80°C mechanical freezer. Results. The median age of the
patients was 42 (4-61), 71 were women and 36 men. The median num-
ber of transfused mononuclear cells and CD34 + cells was 4,377 (2.26-
9.3) <10⁶/kg and 2.86 (0.7-19.3) <10⁶/kg respectively. The medi-
an number of transfused colony-forming units-granulocyte-macrophage
was 12,4 (3.4-55.5) <10⁶/kg. All patients, except one, showed rapid
and sustained engraftment. The median times to reach a neutrophil count
of >0.5 <10⁹/L per L and a platelet count of >20 <10⁹/L were 15 and 12.2
days respectively. Only one patient fail to maintain engraftment. Conclu-
sions. This experience demonstrated that engraftment is long lasting and
that a simplified cryopreservation technique will be useful for Institu-
tions without rate-controlled freezing facilities.

Haematologica vol. 84 (EHA4 Abstract Book); June 1999
PO-0893 Ex vivo purging using MCS40 photodestruction therapy enhanced by amifostine (WR 2721)

Danelatu V, Lydakis E, Dimitriou E, Kalmanti M
Dept of Pediatric Haematology/Oncology, University Hospital of Heraklion, Crete, Greece

Photodynamic treatment using MCS40 as a photosensitising dye has a potential use in the purging of neoplastic cells from autologous bone marrow grafts. Aminoethoxyacetonaphthoquinone on normal cells is blocked by systemic chemotherapy. This study was designed in order to investigate the effect of Amifostine (WR-2721) on leukemic and normal bone marrow cells after MCS40 photodestruction therapy. Bone marrow cells from children with acute leukaemias (AL) at initial diagnosis and in remission under maintenance chemotherapy as well as HL-60 leukemic cell line were incubated with Amifostine (1.5 mg/mL) for 15 min and then with MCS40 (20 mg/mL) for 1 hour. Afterwards, they were exposed to different Argon Laser 514 nm doses. Cell suspensions which were not incubated with Amifostine were used as controls.

Cell survival was estimated by trypan blue supravital staining following a 24 hour incubation and leukocyte cell line was studied in continuous cell cultures of 4 weeks duration. The survival of normal bone marrow progenitors has been estimated by colony formation assay in semisolid cultures. Our results showed that Amifostine: 1) enhanced the photokilling effect of MCS40 on both HL-60 cell line and fresh bone marrow leukemic cells; 2) significantly protected bone marrow precursors from children with AL under chemotherapy from cytotoxicity induced by photodynamic treatment (39.05+11.1% vs 62.9±9.9%, p=0.008); 3) improved the survival of bone marrow committed progenitors (24.17±8.8% vs 5.67±7%, p=0.08 for CFU-E, 76.33±39.14% vs 48.23±21.14%, p=0.003 for CFU-GEMM and 44.69±11.2% vs 29.15±9.6%, p=0.15 for CFU-GM). These differences were found to be statistically significant only for BFU-E (60.27±15.37% vs 18.82±6.4%, p=0.017) colony formation. In conclusion, Amifostine (WR-2721) seems to enhance the photokilling effect of MCS40 photodestruction on leukemic cells and in addition to the above action exerts cytotoxicity upon normal bone marrow cells; thus this agent could play a significant role in clinical use of MCS40 mediated phototherpay.

PO-0894 Mobilisation with G-CSF single or divided dose versus G-CSF plus cyclophosphamide in breast cancer

Arbona C, Prosper F, Benet I, Solano C, Garcia-Clavel B, Luch A, Garcia-Conde J
Haematology Oncology. Hospital Clinico. Valencia, Spain

The purpose of our study was to compare mobilisation with G-CSF versus chemotherapy plus G-CSF and to determine predictive factors of PBPC mobilisation in an homogeneous group of patients. G-CSF [10 (µg/kg) day 4 days] were given to 144 patients, 19 patients received G-CSF (5 µg/kg) 12 hours 4 days) and 51 patients were mobilised with cyclophosphamide based chemotherapy followed by G-CSF (5 µg/kg) day) with apheresis starting when WBC x 10^9/L. The total WBC count, ANC, CD34+ cells/µL and CFU-GM/mL were determined in PB before and after mobilisation as well as in the apheresis product. More than 2.5 x 10^6 CD34+ cells were obtained in 83% and 95% of HRBC mobilised with G-CSF (single or fractionated dose respectively) and 93% of HRBC patients mobilised with chemotherapy plus G-CSF (p<0.001). The total number of CD34+ cells collected was 4.2, 4.8 and 7.3 x 10^6/kg respectively (p<0.001) with a median of 2 apheresis in each group. More than 2.5 CD34+ cells were collected in the first apheresis in 44%, 64% and 65% of patients mobilised with single, fractionated or cytoxan+G-CSF respectively (p<0.05). We then analysed metastatic patients mobilised with G-CSF (single dose or fractionated dose). More than 2.5 CD34+ kg cells were obtained in 70% and 100% of patients mobilised with G-CSF single dose or fractionated dose while 21% of patients receiving single dose and 80% receiving fractionated dose obtained more than 2.5 CD34+/kg cells/kg with a single apheresis (p<0.01). In conclusion, 10 mg/kg of G-CSF administered in divided doses mobilises significantly more CD34+ cells than 10 mcg/kg in one dose in patients with HRBC and metastatic breast cancer.

PO-0895 Long-term cultured peripheral blood stem cells may show different maturation capabilities: a multivariate analysis

Haematology Section, University of Ferrara, Ferrara, Italy

The analysis of long term cultures (LT) from peripheral blood stem cell (PBSC) collections could be a useful tool for the enrichment of cell populations that are usually misunderstood or not detected in the study of fresh samples. Seventeen patients with haematologic malignancies (13 malignantlymphoma, 1 AML, 2 ALL, 1 MM) and 1 soft tissue sarcoma, were treated with different mobilisation regimens to collect PBSC. Cells were collected in T25 flasks and in collagenated Petri dishes suspended in long term culture medium. After 7 days (range: 6-13 days), in 10/18 samples (55.5%) mainly in the collagenated Petri, spindle-shaped cells (SSC) were observed, which were in close proximity to scattered macrophages. Though morphologically similar to bone marrow thrombocytes, SSC were collagen I, III, IV-v, factor VIII, HLA-DR+ve, CD14+ve, CD68+ve, CD11c+ve, and acid phosphatase +ve, thus showing a monocyte origin. However, no CFU-F were detected from LTC, and cell proliferation occurred in a contact inhibition-way. Starting from 25th day of culture (range: 20-38), in both collagenated petri and non-collagenated flasks derived from 6 out of 18 samples (33.3%), the presence of a mononucleated cell clusters with a large flattened cytoblast was noticed. They progressively gave rise to giant mul- ti-nucleated cells, containing up to 15 nuclei. Although at first thought it would be fusion macrophages, cytochemical and immunochemical data gave evidence of the osteoclast (OC) nature of these cells, since they resulted strongly positive for tartrate resistant acid phosphatase (TRAP) reaction, while lacking CD14 antigen. OC are now considered haematopoetic cells derived from a common progenitor cell (or in some cases one that is not usually need vitamin D, ascorbic acid or the presence of other cell types (stromal cells) to support their growth and proliferation. In our culture conditions neither stromal cells were present nor vitamins were added, so it could be argued that OC growth obtained from transfusions might represent a spontaneous phenomenon. Moreover, we analysed the possible correlation between OC frequency and the apheresis content of CD34+ cells, CD34+ subsets, CFU-GM, monocytes. lymphocytes (which can stimulate in vitro OC through L-3) as well as the speed of engraftment after autologous transplantation. Based on these data, positive correlations between CFU-GM number and OC frequency were observed.

PO-0896 Peripheral blood progenitor cell (PBPC) collection in patients previously exposed to fludarabine (FAMP)

Dept of Haematology Niguarda Hospital Milan, *Dept of Haematology Cardarelli Hospital Naples and +University Dept of Haematology, Torrette Hospital, Ancona, Italy

Objective. Some subjects suggested that FAMP exposure may influence negatively the yield of PBPC. We studied retrospectively the results of CD34+ cells collection in pts with acute leukaemia (AL) or lymphopo- liferative disorders (LPD) previously treated with FAMP alone or in combination chemotherapy. Design and Methods. Thirty-six pts received a chemotherapy program including FAMP. Collection of PBPC was performed in AL pts after CR and at the moment of major response in the LPD group. Time from last FAMP administration and CD34+ collection was calculated (Dt) and total dose of FAMP received by each pt was considered. Results. Twenty-three pts with LPD (5 CLL, 18 LG-NHL median age 54 (range 39-65) were treated. FAMP was administered either alone (5 pts) or in combination therapy with dexamethasone plus mitoxantrone (FND=14 pts) or cyclophosphamide (FLUCYD=4 pts). AL pts were 13 (7 AML, 6 ALL) median age 28 (range 20-56); all pts have been pretreated with regimes including antirreticelines and received FAMP+ara-C and G-CSF as salvage therapy. A successfully collection of CD34+ > 2.5 x 10^6/kg was obtained in 15 LPD pts (62%) and in 10 AL pts (77%). Four pts that received FAMP as only treatment collected successfully. No difference in terms of regimens used (n° and D) and at pts when were grouped according to the success of PBPC collection. The two groups of pts with AL received the same median dose/sqm of FAMP while the dose of drug administered in the LPD group of pts collecting ≥2.5 x 10^6/kg CD34 cells was superior. Data are shown in the table below:

<table>
<thead>
<tr>
<th>LPD</th>
<th>CD34+ cells pts n° nX (range)</th>
<th>FAMP mg/sqm (range)</th>
<th>Δ months (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥3 x 10^6/kg=15</td>
<td>2 (0-6)</td>
<td>270 (150-750)</td>
<td>4 (2.5-19)</td>
</tr>
<tr>
<td>&lt;2.5 x 10^6/kg=8</td>
<td>2 (2-4)</td>
<td>150 (150-750)</td>
<td>4 (2.5-17)</td>
</tr>
<tr>
<td>AL: CD34+ cells pts n° nX (range)</td>
<td>FAMP mg/sqm (range)</td>
<td>Δ months (range)</td>
<td></td>
</tr>
<tr>
<td>≥2.5 x 10^6/kg=10</td>
<td>1 (0-4)</td>
<td>300 (150-500)</td>
<td>1 (2-3)</td>
</tr>
<tr>
<td>&lt;2.5 x 10^6/kg=3</td>
<td>2 (2)</td>
<td>300 (150-450)</td>
<td>1 (2-3)</td>
</tr>
</tbody>
</table>

Conclusions. FAMP dosage did not adversely influence stem cell yield in a population represented mainly by heavily pretreated pts with haematologic malignancies.

Haematologica vol. 84 (EHA-4 Abstract Book); June 1999
PO-0897 Semi-automated flow cytometric analyses of CD34 positive cells in blood stem cell concentrates

Carrero J, Gutensohn K, Krueger W, Magens M, Kuehnel P* Transfusion Medicine, Bone Marrow TX, Univ. Hamburg, Germany

Objective. The purpose of this study was to evaluate a new analysis kit and semi-automated software for the measurement of CD34-positive cells. Design and Methods. The flow cytometric test kit (ProCOUNT®) (PC, Becton Dickinson (Becton), San Jose, USA) in combination with software-sup- port for data acquisition and analysis was applied for analyses of 90 samples obtained from PBSC apheresis concentrates from 39 patients with haematological-oncological diseases (e.g. NHL, breast cancer). Aliquots were pipetted into ready-to-use tubes (TruCOUNT™, BDIS), and incubated with antibodies (CD34, CD45) and a nucleic acid dye (FL-1). Hereafter, a lysis-wash procedure and the data acquisition and analysis, Pro- COUNT™ software was used. For data comparison, parallel, a second measurement was performed using the German reference protocol (GRP) for CD34-analysis (Inffusions/Transfusionsmed 1996; 23:1-24). Results. By use of the PC kit a purity of 82% (range 65-89). The relative proportion of CD34+ cells was obtained, but a PCR-detectable contamination was still evident. To increase purity of the PC kit, a warning of the PC software occurred. Following the recommendation for manual re-evaluation with CellQUEST™ software (BDIS), a correlation of r=0.97 compared to the GRP was obtained. Conclusions. We conclude that the kit and semi-auto- mated software, for CD34-cell data acquisition and analysis, represents a promising approach and progress for CD34-cell measurements. However, the occurrence of software warnings has still to be reduced, and reviewing the plots and analysis by experienced staff is still mandatory.

PO-0898 Clinical safety of immunoadjuvancy CD34+ cell selection: results in low grade NHL patients

Donelli A, Pietramaggiore A, Narni F, Paneselliti T, Savarino M, Chiodino C, Marasca R, Ferrara L, Longo G, Torelli G. Dpt. Medical Science Section Haematology and Internal Medicine, University of Modena and Reggio Emilia, Italy

The efficacy of immunoadjuvancy CD34+ cell selection from PBMC using the Cepreate® PC device (Cell-Pro Bothell WA, USA) and its clinical safety as a graft for autologous transplantation were evaluated in 13 patients affected by low grade NHL (12) and 8B-ALL (1). All patients in CR or good partial response at first line chemotherapy were mobilized with CIT 4g/ml (bolus) and Filgrastim 300 µg (bolus). The relative proportion of progenitor cells prior to selection was 2.4±10%/kg (range 0.3-7.1) corresponding to 16.54±10 CFU-GM/kg (range 4.3-37). After positive selection the median yield was 49% (range 30-87) with a CD34+ cells median purity of 85.8%. The relative proportion of CD34+ cells was 3±10/kg (range 1.4-4.8) and CFU-GM content 6±10/kg (range 3.5-10.3). The median B and T cell depletion obtained after CD34+ selection was 2 and 2 log respectively. One out 5 patients with follicular lymphoma had a T (14.38) detectable, by nested-PCR in stem-cell harvests. After positive selection CD34+ purity was 83% and 2 log depletion of B cells was obtained, but a PCR-detected contamination was still evident. To increase lymphoma cell depletion, a negative selection with CD19/20-biotin antibo-odies (Cell-Pro Biotech) was applied after the positive selection using a Cepreate® TCD device like a regular TCD procedure. The yield of CD34+ cells after negative selection was 2.2±10/kg with a purity of 87% and a viability of 98%, the CFU-GM content was 7.4×10³/kg and the final recovery was 18%. A log total B cell depletion was achieved and PCR became negative (sensitivity 10-5). After conditioning regimen (BEAM) the double selected CD34+ cells were infused. Haematopoietic engraftment was prompt with PMN recovery to 0.5×10⁹/L on days +9 and platelets recovery to 20×10⁹/L on days +12 post-transplant. Comparable time to PMN and platelets engraftment was obtained when selected CD34+ cells were infused. Our data show that immunoadjuvancy-purging procedure is safe and reproducible in heavily pretreated patients.

PO-0899 The role of Oncostatin M (OSM) in the ex vivo expansion of haematopoietic progenitor cells from umbilical cord blood: preliminary results

Forte L, Caravita T, Maniccia L, Bataglia A, Modello A, Tambrunni A, Adorno G, Rossi P*, Caniglia M, DeRossi G, Amadori S. Haematology, University "Tor Vergata", Rome; *Haematology and *Immunology "Bambino Gesù" Hospital IRCCS, Rome, Italy

Oncostatin M (OSM), a member of the gp 130 cytokine family, has a demonstrated action in early stage haematopoiesis. To further investigate the potential effect of OSM in amplifying progenitor cells of umbilical cord blood (UCB), CD34+ cells immunoselected by MiniMACS from 17 UCB samples were established in serum free liquid culture with or without OSM (10 ng/mL) in combination with SCF (10 ng/mL) and IL3 (10 ng/mL). In order to evaluate the action of OSM we performed two sets of experiments: in group A OSM was added to liquid culture at day 0 for seven days and in group B at day 7 for four days. Proliferative response to OSM was assessed at day 0, +7, +11 evaluating nucleated cells, clonogenic progenitors CFU-GM, BFU-E, CFU-GMML in methylcellullosa medium supplied by Stem Cell Technologies in presence of IL3, G-CSF, GM-CSF, IL6 and EPO. The cells were analyzed by FACS at day 0, +7, +11 for the presence of myeloid and lymphoid differentiation markers (CD34+, CD3, CD2, CD19, CD7, CD14, CD38, CD15). BFU-E, CFU-GMML and CFU-GEMM were assessed at day 0, +7, +11 evaluating: nucleated cells, clonogenic progenitors BFU-E, CFU-GMML, CFU-GEMM and IL3/SCF, GM-CSF, GM-CSF, IL6 and EPO. The increase of nucleated cells and CFU-GM colonies after seven days of exposure to OSM was 10 fold and 1.72 fold respectively in comparison with control groups (p<0.01), while after 4 days this effect was not significant. FACS analysis showed a significant increase of the cell population that expressed the marker CD38 and a decrease of CD34+CD7 cells only after seven days of exposure to OSM. In conclusion, OSM may be useful for enhancing the action of the early acting cytokines SCF and IL3; in particular this preliminary study shows that its action seems to allow the amplification of the myelomonocytic compartment in vitro.

PO-0900 Mobilisation and collection of PBPC in healthy donors: comparison of two G-CSF schedules

Martinez C, Urbano-Ispizua A, Marín P, Rovira M, Merino A, Carreras E, Montserrat E. Haematology Department, IDIBAPS, Hospital Clinic, University of Barcelona, Spain

An important issue in autologous PBPC transplantation is the optimisation of the regimen of mobilisation of progenitor cells from normal donors. It has been shown that for G-CSF doses up to 10 µg/kg/day, a relationship exists between G-CSF dose and degree of progenitor cell mobilisation. Formal comparisons with doses higher than 10 µg/kg/day, however, have not been reported. The aim of this study was to compare the mobilisation and collection results of two different G-CSF (filgrastim) schedules: 10 µg/kg 24 hours (n=20; group A) vs 10 µg/kg 12 hours (n=17; group B). Median age of the donors was 36 years (range 21-61) and 47 years (range 21-60), respectively (p NS). Apheresis sessions were started on day 5 (after 4 days of G-CSF) and were performed with a Fenwall CS3000 plus blood-cell separator; processing 16-12 L of blood daily. Adverse events consisted of bone pain, headache and fatigue which required treatment with acetaminophen/paracetamol in both groups. One donor from group A developed fever after 4 days of G-CSF administration. Discontinuation of G-CSF administration for intolerable side effects was not necessary in any case. Increase in peripheral leucocyte and lymphocyte counts >10/L on day 5 was significantly higher in group B (3.3 (3.1-8.5) and 6.2 (2.1-7.5), respectively) than in group A [2.75 (1.32-5.3) and 2.6 (1.9-5.1), respectively] (p<0.005 and p=0.08). Platelet count >10/L did not change in group A whereas a decrease of platelet count to 100×10⁹/L was observed in group B (214 [161-283] before G-CSF administration to 161 [155-288] on day 5) (p=0.063). Hemoglo- bin levels were not modified. Following the first apheresis, a significant decrease in platelet count was observed with both G-CSF schedules without differences between groups. The number (>10/kg) of nucleated cells and CD34+ cells collected after one apheresis session was higher in group B (632 [398-914] and 6.1 [3.1-9.5], respectively) than in group A [484 [270-827] and 3.2 (0.8-9.9), respectively] (p=0.004 and p=0.001). Median num- ber of CD3+ cells collected was similar with both G-CSF schedules. In conclusion, the schedule of 10 µg/kg 12 hours was well tolerated and resulted in the collection of a higher quantity of progenitor cells than 10 µg/kg 24 h without increasing T cells content. This approach could avoid a second apheresis to the donor and facilitate further graft manipulation.

PO-0901 Absence of cancer cell contamination as detected by maspin assay in vitro selected CD34+ peripheral blood progenitor cells in patients with high risk stage II/III breast cancer

Pietramaggiore A, Donelli A, Morselli M, Nani F, Sabbatini R, Cagossi K, Longo G, Torelli G Department of Medical Sciences, Section of Internal Medicine. Oncology and Haematology, University of Modena, Modena, Italy

Preliminary studies suggested a high rate of tumour cells contamination in peripheral blood progenitor cells (PBPC) related to a higher risk of relapse in patients subjected to high dose chemotherapy followed by rescue with

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PBPCs. Maspin is a protein related to the family of protease inhibitors which has been suggested as possible marker in the detection of breast cancer (22). Allion et al. (1996) suggested that maspin negatively affects cell proliferation in breast cancer cell lines. The peripheral blood of 26 patients with high risk primary breast cancer (stage II-III A with axillary node involvement > 10) was evaluated for the presence of cancer cells by maspin RNA assay before and after mobilization of PBPCs with cyclophosphamide and G-CSF. All samples collected before mobilization resulted maspin negative, while 11 out of 26 patients had maspin positive cells in cellular harvests. PBPCs from 5 of these contaminated patients were processed with an immunomagnetic separation using a mouse monoclonal antimouse CD34 antibody (Isotype: mouse IgG(x)) to obtain purified hematopoietic stem cells (positive selection). One of these 5 was subjected to a double in vitro selection using a second mouse monoclonal antibody (HEA) specific for human epithelial cells. The maspin determination in these in vitro purged samples resulted negative in all 5 cases. After an average 12 months follow-up, 2 out of these 5 patients (n = 6) with diagnoses of breast cancer (8), non-Hodgkin lymphoma (11) or multiple myeloma (5). PB CD34 was determined on the day of apheresis and at hourly intervals during the 6 hr procedure. The stem cell product was collected into 3, 2 hr bags which were subsequently incubated for 24 hours to selectively kill the cells in S-phase, and then plated in methylcellulose for CFC evaluation and seeded in liquid culture for LTC-IC assessment. Only a small proportion of PB-derived CFC was found to be in S-phase, since the number of CFC grown after Ara-C incubation was not statistically different from that in control cultures (35±9 vs 29±8, respectively; p > 0.05). Similarly, very few LTC-IC were found to be in S-phase (22±9 vs 19±7 LTC-IC in control cultures and after Ara-C incubation, respectively; p > 0.05). These estimates were confirmed by flow cytometric DNA analysis, which showed that 96.2% of CD34+ cells were in G0/G1 and only 1.6% in S phase. Staining of CD34+ cells with an antistatin monoclonal antibody indicated that 68±7% of cells were in the G0 phase of the cell cycle, incubation of mononuclear cells with IL-3, SCF and G-CSF significantly increased the proportion of cells in S-phase for both CFC (37±4 vs 11±8 colonies grown in the absence or presence of Ara-C, respectively; p < 0.01) and LTC-IC (22±9 vs 4±3, respectively; p < 0.01) without inducing any loss in a mitotic index. Our findings indicate that: i) very few CB-derived CFC and LTC-IC are in S-phase; ii) a substantial amount (about 27%) of CD34+ cells in G0/G1 is cycling, i.e. in G1 phase; and iii) a 24-hour incubation with IL-3, SCF and G-CSF can drive a proportion of progenitors into S-phase without reducing their number. These data might be useful for both gene transfer protocols and the ex vivo expansion of CB-derived haematopoietic progenitor cells. PO-0910 Comparison between a byse-and-then-wash method and a byse-non-wash technique for the enumeration of CD34+ haematopoietic progenitor cells Menedez P,*, Redondo O,*, Rodriguez A,*, Lopez-Berges MC,*, Ercilla G,*, Lopez A,*, Duran A,*, Almeida J,*, Perez-Simon JA,*, San Miguel J,*, Menendez P,*, Redondo O,*, Rodriguez A,*, Lopez-Berges MC,*, Ercilla G,*, Lopez A,*, Duran A,*, Almeida J,*, Perez-Simon JA,*, San Miguel J,*, Grataua JW,*, O’Donnell PV,*, Noffsinger LE,*, Grayson C,*, Condon K,*, Scott S,*. Division of Clinical Haematology, Hosp. de la Santa Creu i Sant Pau and Institut de Recerca Oncolònica, Barcelona, Spain Adequate mobilisation of PBSC in pts with lymphoid malignancies depends on various factors, including the type of chemotherapy (CT) and growth factor used, the underlying disease, the type and amount of previous therapy and the presence of bone marrow involvement at mobilisation. In the current study we have analysed these and other factors in a group of pts who received a homogeneous mobilisation protocol. From 1993 to 1998, 49 consecutive pts with non-Hodgkin’s lymphoma (n=44), myeloma (n=3) and Hodgkin’s disease (n=2) received CT with the, IAP VP-1 6 protocol (ifosfamide 5 g/m² days 1-2; ara-C 7.5 g/m² days 1-3; prednisone 80 mg/m² days 1-5) followed by G-CSF (5 mg/kg/d) from day 6. Nine variables were analysed for their impact on an adequate (>2x10^6/kg) and optimal (>5x10^6/kg) harvesting of CD34+ cells, as well as the relationship between the number of CD34+ cells in PB pre-apheresis and the amount harvested. Fifty mobilizations were performed, of which 47 were effective, including 12 pts who had failed previous attempts with G-CSF + cyclophosphamide-G-CSF. The mean CD34+ cells harvested was 13.3±10^6/kg (SD 13.9). 68% of pts had an adequate harvest with 1 apheresis, while 32% required 2-3 procedures. Of the 47 successful mobilizations, 35 (70%) were an optimal PBSC harvest. Bone marrow involvement, the number of prior lines of CT (>2), prior mobilisation failures and the development of fever during neutropenia had a negative impact on the mobilisation and final harvest (p < 0.05). When the pre-aphereses PB CD34+ cell count was >3000/mL, an adequate PBSC harvest was always achieved with 1 aphereses. For an optimal PBSC harvest, however, a threshold of 15,000 cells/mL predictive in >95% of cases. In conclusion, the IAP-VP-1 6 regimen plus G-CSF obtains high yields of CD34+ cells, including pts who failed to mobilize with other regimens.
We confirmed the published high frequency of LTC-IC from cord blood and their high proliferative potential, as compared to PBPC from children and adults (p=0.045 ; p=0.036). The frequency in LTC-IC in PBPC from children and adults were similar (p=0.28). No significant difference in CFC-derived LTC-IC was observed between child and adult origin (p=0.11). These results suggest that two cord blood progenitors cells features, as the high frequency of CFC and LTC-IC and the general production of CFC from LTC-IC, are rapidly lost after the birth to persist at the same rate all the life. The quality of bone marrow stroma, immature haematopoietic cells homing, and the accessory cells could be important factors for haematopoietic reconstitution and post transplant clinical tolerance, and maybe differ between adults and children.

PO-0907 Mobilisation of peripheral blood stem cells (PBSC) from acute myeloid leukaemia patients in first CR: comparison with data obtained in non-Hodgkin lymphoma and volunteer donors


Istituto di Ematologia-Università di Pavia, IRCCS Policlinico S. Matteo, Pavia; °Serv. Immunoematologia e Tradusione IRCCS Policlinico S. Matteo Pavia, Italy

PBSC are considered a valid alternative to bone marrow for transplantation in many clinical conditions. We have analysed acute myeloid leukaemia (AML) patients (pts) in first CR the PBSC obtained after a consolidation course of HD-ARAC (3 g m²/12 h × 4 doses) followed by G-CSF 5 µg/kg sc. every day. With the aim to evaluate the quality of AML PBSC we have analysed leucapheresis numbers, CFU-GM growth, mononuclear (MC) cell number and CD34+ cell presence in collection from 12 AML patients. The data were compared to those obtained from 89 NHL pts and 11 volunteer donors (VD), the latter involved in a program of alloBMT for haematological malignancies. In NHL pts the mobilisation was reached with HD-cyclophosphamide 7 g/m² followed by G-CSF 5 µg/kg sc. every day (6-15 days) and in VD with 5 days administration of GCSF 5 µg/kg bid sc. The collections of PBSC were made with a CS3000 Plus (Baxter). The results (mean and SD) are:

\[\text{AML} \quad \text{NHL} \quad \text{VD}\]

<table>
<thead>
<tr>
<th>Leucapheresis n.</th>
<th>1.75 (0.86)</th>
<th>2.2 (0.94)</th>
<th>1.70 (0.46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU-GM* x10⁶/ kg (SD)</td>
<td>64 (49)</td>
<td>69 (64)</td>
<td>103 (98)</td>
</tr>
<tr>
<td>BFU-E*</td>
<td>1577±3311</td>
<td>6045±1138</td>
<td>19.080±2968</td>
</tr>
<tr>
<td>CFU-Mk*</td>
<td>693±118</td>
<td>347±64</td>
<td>524±1190</td>
</tr>
<tr>
<td>CFU-Mac*</td>
<td>84±22</td>
<td>89±24</td>
<td>240±164</td>
</tr>
<tr>
<td>CD34+ LTC-IC*</td>
<td>14±5</td>
<td>21±100</td>
<td>22±7</td>
</tr>
<tr>
<td>CFC/LTC-IC</td>
<td>7±1</td>
<td>5±1</td>
<td>22±2</td>
</tr>
</tbody>
</table>

Out of the VD the CD34+ cell number (higher than in AML and NHL, p<0.05) and apheresis number (in NHL > VD, p<0.01) the other data do not show statistical differences. In 3 AML pts the collection was not permissive for a BMT. The biologic potential of AML PBSC is confirmed by the mean neutropenia duration (13 days, range 10-14) observed in 5 pts submitted to autoBMT (all of them are alive in RC). So we conclude that HDARAC in AML patient is efficacy for PBSC collection with biologic characteristics comparable to that observed in the other clinical situations analysed.
Poster Discussions Transfusion medicine

PO-0908 Cost-effective strategy to reduce alloimmune blood exposure in major orthopaedic elective surgery
Tirindelli MC, D’Addiasso AM, Mannili M, Santoni FS, Borgia C
Blood Bank and Orthopaedic Division - Ospedale S Pietro, Rome, Italy

In order to reduce alloimmune blood requirements, several transfusion strategies have been investigated in patients undergoing major orthopaedic surgery. These include the preoperative administration of Epothilone alpha (EPO), preoperative autologous donation (PAD) and intraoperative blood salvage (IS). As the safety of donor blood supply has progressively improved during the last years, the use of technologic products combined with autologous transfusion should be evaluated in terms of cost and efficacy with respect to alloimmune blood transfusion. In this prospective study we evaluated the cost-efficacy of an autologous blood transfusion program combined or not with the preoperative use of EPO (150 IU/kg s.c. twice weekly for 2 weeks) in patients undergoing orthopaedic surgery for hip arthroplasty. Eighty-two patients with unilateral primary replacement (n=64) or revision (n=16) had their hip arthroplasty and more than 2 PAD entered the study. The cost of procedures and the exposure to alloimmune blood transfusion are reported in table:

<table>
<thead>
<tr>
<th>PAD/HS</th>
<th>n pts</th>
<th>Allogenic transf</th>
<th>$/ pt</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPO</td>
<td>35</td>
<td>5.14%</td>
<td>815</td>
</tr>
<tr>
<td>NO EPO</td>
<td>47</td>
<td>27.59%</td>
<td>865</td>
</tr>
<tr>
<td>p &lt;0.001 ns</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The regimen including EPO significantly reduced the exposure to alloimmune blood transfusions in patients with elevated blood loss during hip surgery. The cost of procedures was similar.

PO-0909 Study of clinical and laboratory factors that affect the post-transfusion platelet increment
Fabbri S*, Saini B, Santori A, De Silvestro G, Randi ML, Girolami A
Dept of Medical and Surgical Science, University of Padua, Italy

Background. Long-term platelet supportive care is complicated by development of refractoriness resulting in poor platelet transfusion increment. The main causes of refractoriness are clinically determined (fever, sepsis, splenomegaly, bleeding, DIC), patient related (alloimmunization, gender, IVIg, Amphotericin B) or blood bank determined (storage duration, platelet activation, collection method). Methods. We have evaluated the post-transfusion platelet increment in 20 patients (F: 13 M: 7) with a hypoplastic thrombocytopenia receiving platelet concentrates (PC) (random PC, ABO and Rh matched). Quality of PC was assessed by pH, LDH release, platelet count, glucolitocin levels (ELISA), CD62 and CD42b expression (immunofluorescence). The investigated parameters were: history and clinical status of the patients, platelet count, bleeding time (Duke), (basal and 24h after PC), anti-HLA I and anti-HPA1-4-5 antibodies (ELISA, GIFTI, USA). Refractoriness was defined by 1h post-transfusion corrected count increment (CCI) = 5 × 10^9/L. Results. Five patients were refractory to platelet transfusion. Age, sex, type of disease, previous transfusions or pregnancies, blood groups, basal platelets, or quality of transfused platelets were not predictive of refractoriness.

| CCI 1 h | 2.4±0.8 (msd) | 18.8±3.8* (p<0.01) |
| Bleeding time 24h | 778±57 (sec) | 335±266* |
| Ab. Anti-HLA I | 4/5 (80%)* | 0/5 |
| Ab. Anti-HPA | 13/20 (65%) | 0/1 (5%) |

Conclusions. a) antibodies against alloantigens HLA class I are a major cause of refractoriness to platelet transfusion; b) bleeding time is related to platelet count increment.

PO-0910 Flow cytometric analysis of platelet activation and platelet-leukocyte cell interaction during apheresis
Gutensohn K, Carrero I, Alish A, Greide K, Brockmann M, Kuehn P
Dept of Transfusion Medicine and Transplantation Immunology, University Hospital Eppendorf, Hamburg, Germany

Objective. During apheresis, platelets are activated. Hereby the quality of the platelet concentrate is impaired. To analyze the extent of changes in platelet antigens and platelet-leukocyte interaction, we examined platelets during apheresis by flow cytometry. Design and Methods. During apheresis (n=11; AMICUS™, Baxter, FRG), blood samples were taken at fixed time intervals (0, 5, 10, 15, 30, and 5 min prior the end). For flow cytometry, samples were fixed and stabilised. Platelet antigens were analysed with monoclonal antibodies CD41a, CD42b, CD62p, CD63, and anti-human fibrinogen. Leucocytes were detected with antibodies CD45, CD14, CD3, and CD19. Results. During apheresis, activation-dependent antigens increased slightly in CD62p from 2.00% (±0.94) to 9.97% (±3.97; p<0.05), in CD63 from 1.72% (±0.75) to 7.93% (± 2.68; p<0.05), and in anti-human fibrinogen from 1.99% (±0.10) to 5.2±0.61 (±p<0.05). Changes in CD41a were not significant. An increase in platelet-leucocyte binding was detected in CD14+ cells (p<0.05) and in CD45+granulocytes (p<0.05). The binding of platelets to CD3+ and CD19+lymphocytes was not significant. Discussion. Our results demonstrate that platelets are only activated to a small degree. Parallelwise, only a low degree in platelet-leucocyte binding occurs during apheresis. Conclusions. We conclude that only minor changes in antigen patterns occur during the extracorporeal circulation. Flow cytometry provides a useful method for biocompatibility testing.

PO-0911 Properties of platelet concentrates stored in new polyolefin bags and two media
Galician Transfusion Centre, Santiago de Compostela, Spain

We have evaluated in vitro the effects of using plasma (group A) or additive solution (AS) PAS-2 (group B) in storing platelets, using a new oxygen-permeable platelet bag (L PL-2410), on the metabolism of platelet concentrates (PCs) from pools of five buffy coats. Methods. Forty-five, A and B pools of PCs were studied. The samples were taken before and after 5 and 7 days of storage. Results. The medium of platelet unit in PCs was 3.4±1.0 (r: 2.8 ± 4.3). When we compared groups A and B, we found a lower level of glucose p<0.01 in group B, but it is sufficient to support the platelet metabolism on days 5 and 7 of storage. All PCs showed pH values above 6.8 on day 7. The platelet activation was similar in both groups. Conclusions. The use of new oxygen permeable polyolefin bags and AS, PAS-2 allows us to obtain pools of PCs with suitable metabolic parameters during storage and to have greater availability of plasma for fractionation.

PO-0912 Parvovirus B19 - transfusional aspects
Gaweda J*, Klos M*, Sulek K*, Halota W*
*Central Clinical Hospital Military School of Medicine, Warsaw, Poland; *Medical University, Bydgoszcz, Poland

Objective. The aim of the study was to determine the prevalence of parvovirus B19 infection in young blood donors, in healthy population and in patients with underlying haematological disorders and to evaluate the risk of Parvovirus infection through blood transfusion. Design and Methods. The study groups consisted of 1963 blood donors at 19-23 years of age, 162 patients with haematological disorders, 48 multiply transfused patients and 472 healthy individuals. Anti-B19 IgG and IgM were detected in serum with Parvovirus B19 IgG & IgM ELISA (IBL), and B19 antigen was detectable in 634 (32.3%), and antibodies IgM were detected in 8 (0.4%) in blood donors. In the general population anti-B19 IgG were detected below 40% in people under 30 years of age and over 45% in adults above 40 years of age. Anti-B19 IgM were detected only in one adult from the group over 31 years of age. Among 162 patients with haematological disorders anti-B19 IgG were present in 67 (41.3%) and IgM in 1 (0.6%). No statistically significant differences (p=0.67) have been found in prevalence of anti-B19 IgG between the general population and patients with haematological disorders. This patient showed evidence of acute B19 infection in this patient B19 antigen was also detected. No other case of B19 antigens was detected. Anti-B19 IgG before transfusions were detected in 6 patients in titre 84.8±4.41 IU/mL, and 1 month after transfusion therapy antibodies were detected also in 6 individuals in titre 64.9±3.33 IU/mL. No patients had antibodies IgM. Conclusions. 1) the rate of B19 infection increases through life; 2) the prevalence of anti-B19 IgG in patients with haematological disorders, treated with transfusions, does not differed from prevalence in the general population.

PO-0913 Granulocyte transfusions as a new prophylactic treatment modality against infections in neutropenic patients
Illerhaus G, Dwenger A, Wirth K, Lange W
Department of Internal Medicine I, Haematology/Oncology, Albert-Ludwig-University Freiburg Medical Centre, Freiburg, Germany

Morbidity and a low rate of mortality during neutropenia following bone marrow or peripheral blood-stem-cell transplantation result when progressive
infections occur despite the administration of modern antibiotics. In the 1970’s several studies showed that the effectiveness of additional granulocyte transfusions to treat these complications is mainly dependent on a sufficient number i.e. >2x10^11 of transfused cells. A phase II study was initiated at our institution to evaluate the effectiveness of rhG-CSF mobilised granulocyte transfusions as prophylaxis against infections. Inclusion criteria for the recipient were malignancy, disease associated or chemotherapy-induced prolonged neutropenia and availability of a related ABO and Rh compatible volunteer donor. In case of signs of infections in all patients antibiotics were used according to standard institutional guidelines for neutropenic patients. Exclusion criteria were possible future allogeneic transplantation, severe other diseases and prior organ transplantation. Patients lacking a compatible donor served as a control group. The donor was required to be negative for HIV, HBV, HCV, CMV and HIV. Granulocytes were harvested 12 hours after stimulation of the donor with rhG-CSF (5 μg/kg BW) using a cell separator, irradiated with 20 Gy and, after a negative cross-match and premedication with clemastin and dexamethasone, immediately transfused within approximately 5 minutes. Until today 16 patients received a median of 3 (range 1-4) prophylactic transfusions with a median of 3.2x10^11 WBC (range 0.74x10^11-8.51x10^11) containing 1.81x10^10 neutrophils (median, range 0.22-6.59x10^10). After granulocyte transfusion WBC rose from a baseline of 200 μL (median, range 100-600/μL) to a maximum of 600/μL (median, range 200-4200/μL) 4 hours after transfusion. After 12 and 24 h median WBC of 150x10^6/μL (range 200-3100/μL) and 300/μL (range 100-1300/μL) were observed. Simultaneously, increases in platelet counts were also noted. The only observed side effect was 1/16 WHO grade I erythema. Two patients did not require any antibiotic therapy. Compared to the control group platelet as well as red cell support was reduced. Detailed analyses with regard to platelet counts, days with WBC <1000/μL, temperature >38°C, days with CRP elevation, cytokine support and antibiotic use as well as blood counts of the donors will be presented.

### PO-0914 Use of a modified chromometric method for the assay of a new recombiant factor VIII in severe haemophilia patients.

D’Oiron R,1 Parquet A,2 Bordet J,3 Caron C,3 Rothchild C,3 Négrier C,3 Quilès E,4 Provost JC 5
Centres de traitement des hémophiles de 1Paris Bicêtre, 2Lille ETS2, 3Lyon E Hôpitaux, 4Lille C, Hurlet, 5Paris Necker, France; 6Wyeth-Léderle
Puteaux, France

Introduction/Objective. The reference method recognised by the European Pharmacopoeia is the chromogenic method. However, the assay of factor VIII (FVIII) is routinely carried out by the one step chromometric method, simpler and less expensive. The introduction of recombinant FVIII concentrates for treatment of haemophilia A patients has demonstrated differences between the results obtained with the 2 methods, those obtained with the chromometric method can be 20 to 50% lower than those obtained with the chromographic method. The high concentration (>50 μg/mL) and phospholipid (PL) composition of commercial reagents could be one cause of these differences. The aim of this work is to propose a modified chromometric method that can be used routinely, whose results correlate well with the reference chromographic method.

Methods. During two clinical studies carried out with a modified 2nd generation recombinant FVIII/vIII SQ, plasma samples were taken from severe haemophilia A patients treated with this FVIII. A local FVIII SQ assay by the chromometric method as well as a central determination by the chromogenic method were scheduled. For the local assay, centers 1, 4 and 5 used the commercial kit CK-Prest 2 (Stago) in which cephalin was more diluted: 1/150 (centre 5) or 1/20 (centres 1, 4) in 0.1 M NaOH, while kaolin was added at the recom-}
from apheresis platelets before irradiation and from apheresis platelets after [L 2500 cGy irradiation. In these blood samples with or without incubation of ADP, platelet surface expression of CD41, CD61, CD62P were analysed by flow cytometry (FACS Calibur, BDIS). Results: Expression of CD41, CD61, CD62P fluorescence was not changed significantly after plateletapheresis and after irradiation of platelets, but CD62P positive platelet percentage was significantly increased after plateletapheresis from 12.3±% to 19.4±% and after irradiation of pheresis platelets from 19.3±% to 25.3±%. Platelet activation with ADP was not changed after plateletapheresis but platelets with ADP, CD62 fluorescence and CD62P positive platelet percentage were significantly increased from 409±% to 419±% and from 75±% to 80±% respectively. Interpreation: These data indicate that apheresis procedure and irradiation of platelets cause platelet activation and also irradiation of platelets causes the increasing platelet response to ADP.

PO-0918 Algorithm for estimation of salvaged blood
Inghilleri G, Mercier fails F
G. Pini Orthopedic Institute, Milan, Italy

Intraoperative blood salvage is a safe and effective technique but it becomes cost-effective only when more than 1-1.5 units of RBCs are salvaged, the yield depending on the type of surgery and on a number of other important variables. To offer alternative solutions for all patients (pts) the benefit of salvaging their own blood but maintaining a favourable cost-benefit ratio the stand-by procedure is utilised. It consists in mounting the collection set in all the procedures where transfusion is expected and proceed to the washing cycle only after enough blood has been collected. However, the estimation of the volume of RBCs that can be actually recovered may be fallacious when it simply relies on the volume collected into the reservoir: to estimate the volume of RBCs that can be rendered available for transfusion we defined an algorithm that takes into account the total volume collected into the reservoir [Vol in reserv] the volume of anticoagulant solution [anticoag] the volume of solutions used to irrigate the surgical field [irrig sol] and the expected hemolysis, expressed as a ratio, occurring throughout the process [haemol ratio]; according to the following formula:

expected volume of salvaged RBCs = [(Blood in reserv - anticoag - irrig sol) x Freq Hot] / (1 - hemol ratio)

We prospectively compared the estimated volume of salvaged RBCs (calculated with the algorithm assuming a hemolysis ratio of 0.3) with the volume of RBCs actually salvaged in 99 pts undergoing different orthopedic surgical procedures. An optimal correlation between the estimated and the actually collected salvaged RBCs was obtained (r = 0.957). Out of 27 cases where the expected RBCs yield was >180 mL only in 3 cases (11%) we actually obtained less than 180 mL, while out of 72 cases where the estimated yield was <180 mL of RBCs only in 8 cases (11%) the actual yield was higher (Pearson’s chi square = 54.3; p=0.000). Conclusions: The algorithm seems to represent a simple and precise method to estimate the volume of RBCs that can be saved and can be used during a standby procedure to base the decision to process the collected blood.

PO-0919 Neutrophil-specific antigen and gene frequencies in Tunisian blood donors
Abid S, Naïs B, Sali A, Chehata N, Kibech R, Rekaya Z, Boukou K
National Blood Centre, Tunis, Tunisia; *Military Hospital, Tunis, Tunisia

The biallelic NA antigen system is of special interest, since the NA antigens frequencies are targets of Neutrophil antibodies, causing alloimmune neonatal neutropenia, blood transfusion-reactions, and chronic autoimmune reactions neutropenia of infancy. The purpose of this work is to study, the NA antigen and gene frequencies in Tunisian blood donors. Neutrophils isolated from the peripheral blood of 50 unrelated blood donors, at Blood National Centre, were phenotyped for NA1 and NA2 using granulocyte immunofluorescence test. In the phenotyping study conducted, the NA1 and NA2 antigen frequencies observed, were 0.560 and 0.920, respectively and the NA1 and NA2 gene frequencies calculated, were 0.346 and 0.717 respectively. Our findings attest the Caucasian and African origin of Tunisian population. So to validate these results, we concluded that further investigations are necessary. This information may be helpful in the future for NA antigen and disease association studies.

PO-0920 Molecular characterisation of the D - - phenotype in a family
Pereira I,* Abalo M,° Ribeiro ML,° Abade A,* Tamagnini G,*
*Unidade Haematologia Molecular, Centro Hospitalar de Coimbra, Coimbra, Portugal; °Departamento de Antropologia da Universidade de Coimbra, Coimbra, Portugal; *Scholarship from Fundação Para a Ciência e Tecnologia - Programa “Praxis XXI”

The Rhesus (Rh) blood group locus is composed of two homologous genes - D and CeCe. The D gene encodes the polypeptide D and the CeCe gene encodes the polypeptides E/e by a full length transcription of the gene, and the polypeptides C/c using an alternative splicing of a primary transcript. Rh positive individuals have both D and CeCe genes. Serological Rh negative individuals are homozygous for a deletion of the D gene (dd). Occasionally, they can have incomplete or non functional D genes. The D – individuals do not express the C/c and E/e antigens, however they have a large amount of D antigen than that expressed by common D positive individuals. This results from deletions, gene conversion, in the CeCe gene. In some cases alterations have not been found. Design and Methods. Genomic DNA was isolated from white blood cells by standard methods and studied by Polymerase Chain Reaction (PCR), Single-Strand Conformation Polymorphisms (SSCP), Restriction Fragment Length Polymorphisms (RFLP) and sequencing techniques. Results and Discussion. These 3 individuals have a normal D gene and a normal promotor region, exon 1, intron 1, and exon 10 of the CeCe gene. In the internal region (exon 2 to exon 9) the CeCe gene sequences were not found, they have been replaced by the equivalent region of the D gene. It is not possible to know if the exon 2 belongs to the CeCe gene or to the D gene, because this rearranged gene derived from a C allele.

PO-0921 Autologous fibrin glue in major bleeding surgery
Mercierails F, Inghilleri G
Gaetano Pini Orthopaedic Institute, Milan, Italy

Transfusion requirement (TR) in surgical patients (Pts) is conditioned by perioperative RBC loss and the volume of blood that the Pt can tolerate to lose before reaching a minimum acceptable Hct. Thus the adoption of strategies to limit the surgical induced RBC loss is of critical relevance in reducing TR. Recently the use of fibrin glue has been proposed and reported effective in different settings, particularly in minor surgery. The aim of this work was to evaluate the efficacy of fibrin glue obtained from cryoprecipitate (cryo) of autologous plasma in major elective surgery. Study design. Eighteen pts undergoing primary hip replacement were randomised 1: 1 to receive a) autologous fibrin glue in addition to standard methods to reduce red blood loss; or b) only standard methods. Autologous cryo was obtained in 30’-45’ through an automatic dedicated device (Thromogenesis-Dideco) allowing the production of 7-10 mL of cryo from 250 mL of plasma collected by apheresis or separated from autologous donated units. Cryo was topically applied through a delivery kit allowing the mixing of cryo with a solution of 100 U/mL of human thrombin (Ortho Diagnostics) in a 1:1 ratio. Perioperative RBC loss was calculated as the reduction of the circulating RBC mass from presurgery to the 3rd postoperative day plus the volume of RBCs transfused during this period. Results. Age, body mass, baseline and preoperative Hct were comparable in the 2 groups. The mean RBC loss in fibrin glue Pts was 640±121 mL compared with 904±152 mL of RBCs in the control group (p=0.000) with a mean saving of 264 mL of RBCs. Conclusions. Fibrin glue may represent an effective further strategy to be used in association with the current blood conservation techniques in surgical procedures associated with high blood loss.

PO-0922 Short term low dose perisurgical recombinant human erythropoiesis in trauma patients
Mercierails F, Inghilleri G, Biffi E
G. Pini Orthopedic Institute, Milan, Italy

Trauma is a major health care problem with significant impact on blood bank resources; moreover the only technique applicable in patients (pts) to reduce allogeneic blood utilisation is, in selected cases, perioperative salvage. However when surgery is delayed 3-4 days after the injury the stimulation of erythropoiesis induced by rHuEPO could be used to expand the circulating RBC mass and to accelerate the correction of anaemia postoperatively. Study design: 6 trauma Pts (22±8 years) were selected to participate into the study because of low Hct (<7±%) and an expected perioperative blood loss >1 liter. Pts received a daily recombinant erythropoietin dose of 100 IU/kg of hHuEPO (Eprex, Janssen-Cilag) from day 3 or 4 preoperatively to the 3rd postoperative day, plus an intravenous (iv) bolus of 200 IU/kg of rHuEPO on the first day of treatment (mean total dose: 833±136 IU/kg of hHuEPO). An iv support of iron sucrose was adminis-tered (mean total dose 850 mg, subdivided in daily doses. Results. A significant increase in reticulocyte count was observed after 3 days of treat-
ment (from 2.5±1.1% pretreatment to 7.8±3.3% presurgery) with a peak value occurring between day +1 and day +3 (8.5±2.2%). A consistent increase of preoperative Hct values was achieved in all the Pts (mean ±5.5±2%, range: 4% to 6.2%), accompanied by an 11% increase of the circulating RBC mass (mean 230±8 mL of RBCS). During surgery in 4 out of the 6 patients perioperative blood salvage was performed. Five out of the 6 pts completely avoided allo-blood transfusion. One pt. received 3 units of donor blood. Conclusions. Although preliminary, these results suggest that a short persistent H deity treatment, together with IV iron, is effective in stimulating erythropoiesis, expanding the circulating RBC mass and reducing the transfusion requirement in trauma pts when surgery is planned to take place 4-5 days after injury.

PO-0923 Role of presurgery haematological patient's evaluation in transfusion practice

Mecuriali F, Inghirilli G, Biffi E, Colotti MT

G. Pini Orthopaedic Institute, Milan, Italy

A properly timed presurgery evaluation of patients (Pts) undergoing major elective surgery by transfusion specialists is essential to define the most appropriate transfusion strategies and to optimise the alternatives to allo-transfusion. In our Institute, we set up a protocol allowing to evaluate, 25-30 days prior to surgery, the Pts' specificity, haematological, and clinical conditions and expected transfusion need (TN) according to the formula: TN = perioperative RBC loss - tolerated RBC loss. As the tolerated RBC loss depends on the body mass and the haematoctrit (Hct), all the Pts expected to require transfusion support who have a low baseline Hct are evaluated to define it causes and, when indicated, to correct it. A special care is devoted to detect iron deficiency (ID) conditions. In 1997, out of 1421 pts, 25% were candidates for major elective surgery, 1020 (666 females and 354 males) were referred for pre-surgical evaluation. A total of 52 Pts (49 females, 3 males) had low baseline Hct (36±2.7%) attributable to ID and could be treated with intravenous (IV) administration of iron sucrose (average dosage: 883±302 mg/pt) before preoperative autologous donation (PAD). The treatment allowed the production of 157±87 mL of new RBCS in a mean of 40 days. In 172 Pts ID was not associated with anaemia and 95% of the runs) has been tested on seven independent dilution series and 4 replicates of each dilution have been tested on separate days, giving a total number of at least 24 test results for each dilution. The positive cut-off point has been established by probit analysis in 20.3 IU/mL. One Pt. received 3 units of donor blood. Conclusions. Although preliminary, these results suggest that a short persistent H deity treatment, together with IV iron, is effective in stimulating erythropoiesis, expanding the circulating RBC mass and reducing the transfusion requirement in trauma pts when surgery is planned to take place 4-5 days after injury.

PO-0924 Validation of a nucleic acid amplification technique (NAT) assay for the detection of hepatitis C virus RNA in plasma pools

Curto S, Gajardo R, Xairó D,* Jorquera JI

Research & Development Area, Institute Grifols, S.A, and *Analytical Department, Biomat, S.A, Barcelona, Spain

According to the European Agency for the Evaluation of Medicinal Products (EMEA, CPM/ BWP 390/97), from July 1999 only plasma pools tested and found non-reactive for hepatitis C virus (HCV) RNA using validated NAT assays are recommended in the manufacture of plasma products. A non-reactive plasma pool is a pool found non-reactive in a run able to detect a HCV RNA control with a content equivalent to 100 International Units (IU) per mL. In Grifols, a Qualitative NAT assay for the routine detection of HCV RNA has been applied to plasma pools for fractionation since 1997. Briefly, it consists of a quenched thioconan extraction and a reverse transcription nested PCR performed by electrophoresis. A study addressing validation issues has been carried out. The characteristics regarded as the most important for NAT assay validation are specificity, sensitivity and robustness. Two aspects are considered for the assessment of specificity: 1) the ability of the assay to unequivocally detect HCV RNA and, 2) as the HCV RNA is a high degree of variability which leads to classification into geno-types, the major variants should be identified. For the evaluation of sensitivity, the positive cut-off point (the minimum number of target sequences per volume sample which can be detected in 95% of the runs) has been calculated. As specificity is dependent on the choice of primers, the sets used have been chosen from the highly conserved 5' non-coding region of the genome. To increase specificity, amplification with nested primers is performed. With these primers, the assay has been able to distinguish between HCV genotypes tested so far, 1a, 1b, 2a, 3a and 4, which are the most prevalent in a suitable level. To determine the positive cut-off point, 2-fold serial dilutions of the Working Reagent for HCV NAT Assays (96/586), sup-

PO-0925 Robustness study of specific virus inactivation steps in the manufacturing process of a high purity factor VIII concentrate, FANHD®

Bionaca H, Ruiz P, Ristol P, Gensana M, Massot M, Duft K,*Jorquera JI

Research & Development Area, Instituto Grifols, S.A, Barcelona, Spain; *MA Bioservices, Stirling, UK

FANHD® is a human high purity FVIII:WF concentrate that combines Solvent Detergent (SD) (6H, 25°C) and Freeze-drying + heat treatment (FD + HT) at 80°C for 172 h, to inactivate potential blood borne viruses. In order to evaluate the consistency and robustness of both inactivation steps, the issue of the influence of in-process variables is addressed. For the SD step a H Paz C virus model, Bovine Viral Diarrhoea virus (BVDV) was spiked into the starting material. A load sample was taken and sequential concentrations of SD were added until 100%, 25%, 50% and 90% of the final industrial concentration was achieved (0.3%). The Residual virus titres were evaluated at different kinetic timepoints (0, 15, 30, 60 min). For the FD + HT step, experiments varying protein, excipients concentration and residual moisture were carried out in order to investigate the effects of those parameters on the inactivation of hepatitis A virus (HAV). Different compositions of the final product were evaluated i.e. low and high concentration of the principal proteins (albumin and factor VIII:WF) and excipients. In addition, different lyophilisation conditions were investigated, to know their effect on HAV killing. The experiment included a fully detailed kinetics inactivation during the 72 hours period. For the SD step, at 90% of SD, BVDV detectable infectivity disappeared before 30 min of treatment. Robustness of this step was demonstrated: even after reducing the SD concentration to 50%, significant amounts of BVDV were inactivated (2.38 log for the first hour). For all the conditions assayed, the FD + HT treatment provided a significant viral reduction factor (RF) ranging from 4.36 log (low protein concentration, low moisture) to ≥ 8.4 log (high protein concentration, low moisture). Compositions with high protein concentration and high and intermediate moisture displayed RF of 5.67 and 4.55 log, respectively. No clear correlation between protein and excipient content, final moisture and viral RF was evidenced. Our studies show that both viral inactivation steps, SD and F + HT, used in the manufacturing process of FANHD® are highly effective inactivating enveloped and non-enveloped viruses.

PO-0926 Stability of hepatitis C virus RNA in plasma samples during storage

Curto S, Gajardo R, Xairo D,* Jorquera JI

Research & Development Area, Institute Grifols, S.A, and *Analytical Department, Biomat, S.A, Barcelona, Spain

In Grifols, a PCR assay for the detection of hepatitis C virus (HCV) RNA has been applied to plasma pools for fractionation since 1997 to increase safety of plasma products. In addition, samples with known HCV RNA titre are used as run controls for routine analysis. There is concern that storage conditions of the plasma samples might influence the stability, and hence, the detectability, of HCV RNA. A study addressing this issue was carried out. Two citrated plasma samples identified as HCV RNA positive were divided into an appropriate number of aliquots and were stored at -70°C and -20°C. Approximately once a month, the titre of one aliquot of each sample at both conditions was simultaneously quantified by the branched DNA (BDNA) signal amplification method (Quantiplex HCV-RNA; Chiron Corporation) and by a PCR amplification method (Amplipcr HCV Monitor; Roche Diagnostics). Our data suggest that the viral RNA titre is stable over a period of at least 3 years.
PO-0927 Blood transfusions and postoperative complications
van de Watering LMG, *Brand A,* Hermans J*
*Sanquin Blood Foundation, Blood Bank Leidenhage; °LUMC (University Medical Centre Leiden), The Netherlands

Methods. Since 1987 we conducted 3 trials in surgical patients to investigate a possible deleterious role of alloimmune leucocytes in red blood cell products without buffy-coat (ST-RBC) and leucoreduced filtered red cells (LD-RBC). The first study (1987-1991) conducted in colorectal cancer patients evaluated a difference in cancer recurrence. The second trial (1992-1994) was designed to detect postoperative infections and alloantibodies in patients who underwent coronary bypass and valve surgery. The last study (1997-1998), a pilot study for a large multicenter trial, was a not-blinded randomised study in 152 patients with aorta aneurysm surgery and large digestive tract surgery. The primary endpoint was mortality and multi organ dysfunction syndrome. We performed a meta-analysis of these three studies on the incidence of postoperative mortality. Results: In the combined studies there was a significant difference in mortality between the transfusion protocols, in favour of the use of leucoreduced products (3.8% versus 7.4%, p<0.004). Mortality in both transfusion arms increased with the number of transfusions (BT). The advantage in survival by using leucoreduced RBC was only seen when >3 transfusions were given.

<table>
<thead>
<tr>
<th>N</th>
<th>LD-RBC</th>
<th>ST-RBC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>340</td>
<td>845</td>
<td>578</td>
<td>1763</td>
</tr>
<tr>
<td>4-10 BT</td>
<td>2.1%</td>
<td>7.1%</td>
<td>4.1%</td>
</tr>
<tr>
<td>&gt;10 BT</td>
<td>18.9%</td>
<td>31.6%</td>
<td>24.2%</td>
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* In both study arms, non-transfused patients (NT) were unbalanced for prognostic factors compared to transfused patients.

Conclusions. We speculate that no difference in mortality between leucoreduced and standard RBC is observed when the number of transfusions for a given operation is small as the effect of allogeneic WBC is high-dose dependent and not occurring if <4 units are transfused.

PO-0928 Successful treatment of progressive infections in neutropenic patients by granulocyte transfusions
Illerhaus G, Dwenger A, With K, Lange W
Department of Internal Medicine I, Haematology/Oncology, Albert-Ludwigs-University Freiburg Medical Centre, Freiburg, Germany

Bacterial and fungal infections are the main cause of morbidity and mortality in neutropenic patients. To resolve infections an adequate number of peripheral circulating granulocytes is required. Clinical studies in the 1970s showed, that granulocyte transfusions (GTX) could transiently increase the number of circulating granulocytes. The success of the treatment is strongly dependent on the number of transfused granulocytes, ideally >2×10^10 cells. The availability of haematopoietic growth factors like rhG-CSF and the observation that they could substantially increase the number of circulating granulocytes and myeloid precursor cells led to their use to recruit cells for granulocyte transfusions. At our institution 23 neutropenic patients, median age 35.5 years (range 12-67) with life-threatening infections or multi organ dysfunction were given prophylactic granulocyte transfusions. The donor was required to be negative for HAV, HBV, HCV, CMV and HIV. Granulocytes were harvested 12 hours after stimulation of the donor with G-CSF (3 μg/kg BW) using a cell separator, irradiated with 20 Gy and, after clemastin and dexamethasone premedication, immediately transfused. The median number of transfused cells was 2.35×10^10 (range 0.3-8.44×10^10) containing a median of 41.7% neutrophils (range 17-93.8%). Five of 9 patients with invasive pulmonary aspergillosis during prolonged neutropenia had favorable responses with pulmonary resolution before endogenous recovery of granulocytes. Three of 6 patients with severe septicemia or pneumonia improved clinically and survived the infection. All patients (8 of 8) who received prophylactic granulocyte transfusions due to high risk situation (severe bacterial or fungal infections during previous neutropenia) showed an uneventful clinical course. Seven of 15 patients with severe infections deteriorated during or after granulocyte transfusion despite maximal antiinfective therapy. Apart from mild bone pain no side effects were observed in the donors. In conclusion we feel that granulocyte transfusions with sufficient numbers of cells are of clinical benefit for neutropenic patients with proven severe infections or high risk situation. We shall present detailed analyses of all 23 patients.

PO-0929 Ultralow platelet counting using flow cytometry and TruCount®
Kraliadzi P, Soghatchian J, Williamson L
National Blood Service-London & South East Zone, UK

Apart from leucocytes, platelets are also known to express and release proinflammatory cytokines. Therefore the evaluation of leucocyte filters should take into account not only the number of residual leucocytes but also the number of residual platelets in filtered products. Most of leucocyte filters for whole blood and red cells are capable of removing platelets below the detection limit of automated cell counter. However, various filters made from different materials may remove variable levels of platelet contents. Objective. To develop a new procedure for the enumeration of ultra low platelets in filtered whole blood, i.e. below 5000/μL. Design and Methods. Using whole blood, platelets were labelled with anti-CD41-1-RPE in a tube containing a known number of lyophilised multi-fluorescent beads (TruCount®, Becton Dickinson, UK) and analysed by a flow cytometer (Coulter Excel). A serial dilution of double filtered whole blood spiked with platelets were used to assess the linearity and the sensitivity of the assay. Each dilution was measured in triplicate. Results. The means of the triplicate runs are shown in the figure (r² = 0.99, p<0.0001) (solid line-observed counts, broken line-ideal counts). The coefficient of variation of each dilution was ≤10%. The observed count started to deviate from the ideal line at approximately 50 platelets/μL. Considerable variations in residual platelets counts were found among different types of filters as well as different filtering conditions. Conclusions. We developed a simple and practical method for the enumeration of ultralow platelet count, i.e. 50/μL in whole blood. The procedure is useful for the evaluation of leucocyte filters, in process control, as well as for an accurate platelet counting in severe thrombocytopenic patients.
**Poster Discussions: Infections**

**PO-0930 Totally implantable catheter system versus central venous catheter placed before induction chemotherapy in patients with acute leukaemia**

Johansson E, Haas R, Bjoekholm M, Engervall P. *Departments of Medicine, Karolinska Hospital and Danderyds Hospital, Stockholm, Sweden*

Introduction. The most common access used in patients with acute leukaemia (AL) is the central venous catheter (CVC). Another access device available is the implantable catheter system (Port-a-cath® (PAC)).

The objectives of this randomised study were to compare function, complication rate, and patient acceptance between the use of PAC or CVC placed before induction therapy in patients with AL. Methods. Forty-three patients (M/F=24/19, median age 65 yr, range 24-85) were randomised to receive either a tunnelled double lumen CVC or a double lumen PAC. Patients were excluded due to protocol violation. In the remaining 40 patients, a CVC (n=21) or a PAC (n=19) was inserted 3 days (median, range 0-24) after date of diagnosis. Results. Two PAC could not be placed due to technical difficulties. Local bleeding after placement of PAC occurred in four patients, necessitating simultaneous venous access. The median catheter stay was 113 days (range 2-634) for the PAC group and 55 days (range 11-223) for the CVC group. Reasons for PAC explantation were infection (n=4) and death (n=13). Reasons for removal of the CVC were infection (n=6), occlusion (n=2), technical problems (n=2) and elective or death (n=11). Patients expressed greater satisfaction with the PAC compared to CVC. Conclusions. There was no apparent difference between the two groups with regard to complication rate. However, a high risk for initial local bleeding in patients, receiving a PAC has to be considered.

**PO-0931 Nutrition and acute leukaemia in adults: relation to remission rate and survival**

Palmblad J, Eriksson K, Cederholm T. Departments of Haematology and Department of Geniatric Medicine, Karolinska Institutet, Huddinge University Hospital, Huddinge, Sweden

Because malnutrition may augment myelotoxicity of antileukaemic drugs we tested whether changes of the nutritional state of 37 adult patients with acute leukaemia during first remission induction chemotherapy were related to remission rates and times. During the induction period, i.e. until they died or achieved complete haematological remission, patients lost in mean 5.1 kg body weight, spent 25-28% of this time with severe neutropenia (i.e. <0.1-10/L) and fever. Weight changes were classified into one of four separate groups, ranging from minimal weight changes to >10 kg loss. Patients in the two middle groups, exhibiting moderate weight loss (i.e. 2-5.9 and 6-9.9 kg; n=22), were neutropenic and febrile for 23% of the induction period; they displayed the highest remission rates, 90 and 92%, respectively. In contrast, patients in the minimal or maximal weight change groups (i.e. a loss of <2 kg; n=8, or >13 kg; n=7), showing shortest or longest duration of neutropenia (26% and 36%, respectively) and fever (25% and 42%), had the lowest remission rates, 50 and 63%, respectively. By logistic regression analyses, change of weight and time with fever (25% and 42%), had the lowest remission rates, 50 and 63%, respectively. By logistic regression analyses, change of weight and time with fever predicted less favorable short-term outcome. Due to profound immunosuppression and neutopenia infections contribute mainly to the morbidity of the procedure. To assess the impact of this complication we analysed data of 73 autologous blood stem cell transplants which were performed at our institution in 1998. Results. Treatment regimen was BEAC or TBI/CY for NHL (n=17), Melphalan or Megace for MM (n=9), and Autograft (n=39) for AL. Infections contribute mainly to the morbidity of the procedure. To assess the impact of this complication we analysed data of 73 autologous blood stem cell transplants which were performed at our institution in 1998.

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**PO-0932 Infectious complications after autologous peripheral blood stem cell transplantation**


High-dose chemotherapy followed by autologous stem cell support is increasingly used. Due to profound immunosuppression and neutopenia infections contribute mainly to the morbidity of the procedure. To assess the impact of this complication we analysed data of 73 autologous blood stem cell transplants which were performed at our institution in 1998. Treatment regimen was BEAC or TBI/CY for NHL (n=17), Melphalan or TBI for multiple myeloma (n=15), Melphalan for multiple myeloma (n=15), Melphalan (n=15), Melphalan (n=15), Melphalan (n=15). Cytokine (Taxol) for ovarian cancer (n=15), CMV or a similar regimen for breast cancer (n=11), ICE for soft tissue carcinoma (n=11), ICE or dose escalated PEI for germ cell cancer (n=6) and for acute leukaemia (n=5). The overall incidence of neutropenic fever after transplantation was 53%. Incidence for patients treated with NHL was 76%, multiple myeloma 47% ovarian cancer 0%, breast cancer 63%, soft tissue sarcoma 100%, germ cell cancer 40% and acute leukaemia 100%. Fever of unknown origin was documented in 89% (n=35) of febrile patients while in 4 patients positive blood cultures could be obtained: in 3 cases with coagulase negative Staphylococci and in one case with Pseudomonas aerugi.*no. Invasive fungal infection or serious viral complication was observed. All patients fully recovered on broad spectrum antibiotics, no patient died due to infection. Incidence and severity of neutropenic fever correlates with intensity of conditioning regimen, duration of neutropenia and severity of mucositis. In conclusion, neutropenic fever is a major complication after autologous blood stem cell transplant. High risk patients should be monitored closely with broad anti-microbial prophylaxis while patients at low risk can be evaluated for transplantation in an outpatient setting.

**PO-0933 TH1/TH2 cytokine profiles - effect of IFN gamma and GM-CSF in acute leukaemia patients with invasive fungal infection**

Poynton CM, Mickleard U, Barnes R, Jackson S. Departments of Haematology and Microbiology, University Hospital, Cardiff, Wales, UK

Eradication of advanced deep seated fungal infection (DFI) in patients with acute leukaemia with current antifungal agents is difficult. With prompt treatment successful eradication is possible, yet why some patients recover (with or without neutrophil recovery) and others progress to DFI is unclear. Prior periods of severe neutropenia of greater than 21 days are strongly predictive for the development of invasive fungal infection, but factors influencing recovery are more subtle and include other immune effectors cells - monocytes and T-helper cells. We have treated 17 patients with DFI (invasive aspergillosis as well as hepatosplenic candidiasis) with Ambisome or flucytosine together with one or both of the prophylactic cytoxins GM-CSF and IFN gamma for periods of up to 14 months (range 1-14). Serial measurements of whole blood cytokine production at a single cell level were performed by multiparameter flow cytometry as previously described for IL-2, IL-4, IL-10, IL-12, IFN gamma and TNF alpha in T-cell subsets (as well as other immune cells including monocytes, NK cells and neutrophils). The ratio of T-helper cells that produce IL-12 to those where IL-4 is detected was the most discriminatory parameter associated with recovery (high IL-12: IL-4) or progression and death (low IL-12: IL-4). Moreover, several features of the underlying immune defect suggested a pre-existing TH2 profile in patients with DFI associated with chronic graft-versus-host disease. Prior fludarabine therapy with a CD4 count below 0.2 x 10⁹/L, coexistent CMV disease and steroid usage. Patients treated with GM-CSF showed a shift upwards in their IL2/IL4 ratio in CD4+ cells. Two patients with invasive pulmonary aspergillosis received flucytosine solution at a dose of 400 mg daily together with GM-CSF three times weekly and showed steady resolution of lung lesions. After stopping GM-CSF (not flucytosine) both patients showed progression of the lesions again and recurrence of fever and a rising CRP which again resolved with restarting GM-CSF. These data suggest not only that GM-CSF and IFN gamma have a role in the treatment of deep seated fungal infection, but also that recovery is associated with a change in cytokine profiles from TH2 to TH1. More data on the potential for long term flucytosine and GM-CSF in the treatment and prophylaxis of DFI is needed.

**PO-0934 Microbiuria is not predictive of infection in patients receiving bone marrow allografts**

Auer A, Durakovic N, Kalenic S, Bogdanic V, Labar B. University Hospital Center Rebro, Zagreb, Croatia

Asymptomatic bacteriuria (isolation of pathogenic bacteria from the urine in the absence of symptoms and signs of disease) is in most cases not predictive of disease and treatment is not indicated. The value of treatment of bacteriuria in immunocompromised and neutropenic patients is unknown. We performed a retrospective study on asymptomatic bacteriuria and candiduria in neutropenic patients receiving allografts during a ten-year period when regular weekly bacteriologic and mycologic surveillance cultures were performed. We identified 114 cases of asymptomatic microbiuria. Most were left untreated at the discretion of the attending physician. The cases were divided in 3 groups. The 1st consisted of 36 cases of significant number of pathogenic bacteria isolated from the urine. The 2nd consisted of 29 cases of candiduria and the 3rd 58 cases with insignificant bacteriuria (between 10¹⁰ and 10¹⁰⁹/mL) or bacteriuria with probably apathogenic bacteria (coagulase negative Staphylococci or diphtheroids). There was no difference in the number of febrile days or outcome between those treated and untreated in any of the regimens. Isolation of a particular bacteria or Candida from urine was not predictive for systemic infection with that strain. The routine treatment of asymptomatic microbiuria in allo-transplanted pts. is not warranted.
PO-0935 Prior fungal infection is not a contraindication to bone marrow transplantation (BMT)
Avivi I,* Fineman R,* Oren I,* Rowe JM,* Dann EJ*
*Dept of Haematology, *Dept of Infectious Diseases, Rambam Medical Center, Haifa, Israel

Objective. To assess the feasibility of BMT in patients with haematological malignancies, who had prior systemic documented fungal infection, treated with amphotericin B, liposomal amphotericin, itraconazole or surgical resection. Design and Methods. Seven patients ages 23-47 with haematological malignancies (5 AML, 1 MM and 1 T-ALL), during the period 1.1.97-1.1.99 underwent BMT (3 allo; 4 auto) following treatment for an invasive fungal infection. Sites of involvement included lung (5), sinuses (1), liver and spleen (1). The fungal pathogens isolated were: aspergillus (5), fusarium (1) and candida (1) respectively. All patients with lung aspergillosis except one were treated with amphotericin B 1 mg/kg, for 2 weeks, or until marked clinical and radiological improvement. Amphotericin B was then changed to itraconazole 400 mg/d until BMT, except during recurrent neutropenia in which full dose amphotericin B was given. Two patients underwent surgical resection of aspergillus fungal balls. Results. Five patients - all with undetectable fungal disease prior to BMT survived the transplant uneventfully. All these patients were also in continued haematological remission. Two patients developed fungal infection during the peri-transplant period and died due to reactivation of fusarium sinustis and pulmonary aspergillosis. These patients were transplanted in relapse of their primary haematological disease. It is worth noting that these two patients had a particularly prolonged period of peri-transplant absolute neutropenia (60 and 38 days respectively). Conclusions. Prior fungal infection is not a contraindication to BMT in patients with haematological malignancies, provided the disease is undetectable at initiation of transplantation. Two patients died, both with detectable residual fungal disease at onset of transplantation, and associated with protracted neutropenia. Aggressive therapy of prior fungal infection followed by full dose antifungal treatment during transplantation may allow the performance of BMT without reactivation of the fungal disease.

PO-0936 Prophylaxis of pulmonary mycosis with intravenous amphotericin B (AMB) in patients with intensive chemotherapy
Boehme A, Hoelzer D
Med. Clin III, J.W. Goethe-University, Frankfurt, Germany

Systemic fungal infections are a main cause of morbidity and mortality in patients with haematologic malignancies receiving intensive chemotherapya. Due to an increased incidence of aspergillus/fusarium infections we started a study to investigate the efficacy and tolerability of an intensified antifungal prophylaxis with intravenous AMB in patients with expected severe neutropenia of ≥8 × 10^9/dL for ≥2 weeks. For primary prophylaxis (no previous systemic mycosis) the patients received AMB 0.5 mg/kg three times a week (low dose). For secondary prophylaxis (previous systemic mycosis) the AMB dosage was adjusted to the residual size of pulmonary infiltration (< or >35% of initial manifestation). Until today, 16 patients (AML 12, ALL 1, CML-BC 1, MDS RAEB-T1, NHL 1) with 17 neutropenic episodes received a primary AMB prophylaxis. Seven available patients (median neutropenia <100/µL: 9.5 days) finished the neutropenic period without any signs of pneumonia or other manifestations of fungal infections. Two patients were withdrawn due to side effects of AMB (severe allergic exanthema, hypotension). For secondary prophylaxis 9/10 patients (all AML) with 15 neutropenic episodes (median duration of neutropenia <100/µL: 8 days) are evaluable. In 2 episodes with >50% and 7 with <50% of the initial pulmonary infiltration a further regression or stability of mycosis was seen during the following neutropenic period with an AMB dosage of 1 mg/kg, daily or thrice a week. One patient with >50% and 3 with <50% of initial mycotic manifestation who were only treated with AMB 0.5 mg/kg thrice a week for different reasons, had a progression of aspergillosis. One patient with a previous resection of aspergillus node and 1 patient with complete regression of pulmonary infiltration was a patient with prophylaxis with low dose AMB. Due to careful preventive measurements a break-off of the secondary AMB prophylaxis was not necessary. In summary, considering the limited results, intravenous low dose AMB seems to be a promising manner for primary antifungal prophylaxis. In patients with previous and residual pulmonary mycosis AMB 1 mg/kg daily (at least thrice a week in only small manifestations) may enable further chemotherapy cycles without progression of mycoses.

PO-0937 Meropenem (M) versus piperacillin-tazobactam/amikacin (PTA) for febrile neutropenia: Influence of response definitions on interpretation of results
Hospital dos Capuchos, Lisboa, Portugal

Objective. a) to compare in a prospective study the efficacy and tolerance of M and PTA as empirical therapy for febrile neutropenia in cancer pts; b) to analyse the influence of different definitions of response used in the literature on the interpretation of data. Methods. Between 10/97 and 5/98, 60 consecutive episodes of fever with neutrophils <1000/µL were randomised to M lg q8h or PT 4g q6h plus A 20 mg/kg/d. Response was assessed at 72 h and at the end of treatment, and the EORTC criteria for response were used. Results. Fifty-eight episodes in 52 pts were evaluable (28 in the M arm, 30 in the PTA arm) which were comparable for underlying (89/83% acute leukaemia [AL]), median neutrophil count at entry (both 100 µL) and median total duration of neutrophil (both 23 days). Barteremia occurred in 32/37% of the episodes (9 organisms were resistant to M and 3 to PT, mainly coagulase-negative staphylococci). Tolerance was good in the 2 arms (no discontinuation due to adverse effects), skin reactions tending to be more frequent in the PTA arm and auditory toxicity only occurring in this arm. Rates of success, defined as resolution without any modification, were equivalent (18% for M and 30% for PTA, n.s.). Amphotericin B was given in 68 and 53% of episodes, and vancomycin in 46 and 37%. Success rate was significantly higher for pts not undergoing AMB-type chemotherapy (42 vs 9.5% in the M arm, 67 vs 14% in the PTA arm both p<0.05). However, survival-based definitions of response (Pizzo criteria) would lead of success of 93 and 97% (success defined as no death from the primary infection) or 79 and 90% (success defined as no infectious death) (n.s.). Applying the Immunocompromised Host Society (ICHS) Consensus Panel definition of an initial response but regimen modified (in our trial, success at 72h but subsequent need for modification), response would be 28 and 57% (p<0.05). Conclusions. The wide variation in outcomes with different response criteria underscores the need for uniform definitions if comparisons between trials are to be meaningful. In pts with severe and prolonged neutropenia (such as the AL pts in this study) in whom virtually all episodes require late modifications due to subsequent infections, the ICHS guidelines should be adopted.

PO-0938 Fungal surveillance of an open haematology ward
Soultar R,* Richardson M,* Shankland G,* Rennie S,* M arshall I,* Morgan M,* Murphy J,* Watson W
Departments of Haematology, and Microbiology, Monklands Hospital, Airdrie, Scotland and Department of Mycology*, Western Infirmary, Glasgow, Scotland

Objective. Fungal infections are a serious problem in neutropenic patients, carrying a high mortality. Treatment is costly, associated with side-effects and not guaranteed to have successful outcome. We assessed rates of fungal infection in a population of neutropenic patients and focused on the hospital admissions. Methods. Between 5/97 and 4/98, 105 consecutive neutropenic admissions to the open haematology ward with a neutrophil count <100/µL were screened for the presence of any fungal pathogen. Fungal invasion was defined as isolation of fungi from any specimen. Infections were classified as nosocomial or community-acquired. Results. A total of 555 swabs were taken from 69 patients; 20% of the swabs had a positive culture. 9% were positive for nonalbicans species of Candida, 7% for C. albicans and 4% for Aspergillus fumigatus. Candida species were the most frequent oral and perineal pathogens, while Aspergillus fumigatus and non-albicans species of Candida were the commonest nasal pathogens. Thirty-three (48%) of patients were colonised on admission. Of the 15 patients colonised with Aspergillus fumigatus (33%) were colonised on admission. Conclusions. 1) Fungal carriage is common in haematological patients; 2) previous outbreaks of Aspergillus have focused on the hospital as an infection source. This study indicates that patients are often admitted pre-colonised with Aspergillus and other fungal pathogens. Greater antifungal prophylaxis between hospital admissions may therefore be indicated; 3) determination of fungal carriage may identify at risk patients for whom early antifungal therapy would be beneficial.
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Infections

PO-0939 A randomised prospective multicenter trial of cefpirome
versus piperacillin-tazobactam in febrile neutropenia
Bauduer F, Cousin T, Boulat O, Rigal-Huguet F, Molina L, Fegueux N,
Jourdan E, Reiffers J
for the BGMT collaborative group, CHU Bordeaux, Pessac, France

From 06/96 to 03/97, 208 febrile neutropenic episodes (FNE) ≥ 38.5°C
occurring in patients more than 18 years with ANC ≤ 0.5 giga/L or anticipated to fall below this level within 48 h were eligible for this study. Exclusion criteria included known allergy or previous documented resistance to
protocol antibiotics, septic shock, hepatic or renal failure, HIV seropositivity or pregnancy. There were 131 men and 77 women aged between 17
and 83 years (median: 49) originating from 7 different haematological
centers. Underlying diseases were: acute myeloid (n=106) or lymphoid
(n=25) leukaemia, Hodgkin (n=1) or non-Hodgkin’s (n=32) lymphoma,
multiple myeloma (n=16), solid tumour (n=8), myeloproliferative disorder
(n=9), chronic lymphoid leukaemia (n=5), aplastic anaemia (n=3),
myelodysplasia (n=3). Neutropenia occurred after chemotherapy (160
FNE) or allogeneic (10 FNE) or autologous (38 FNE) stem cell transplantations. A central venous line was used in almost all cases. The patients
were randomly assigned to receive as first line therapy, either cefpirome (C)
2 g 3 2/day (105 cases) or piperacillin-tazobactam (PT) 4 g 3 3/day (103
cases) which were used alone (C: 15/PT: 15) or in combination with an
aminoglycoside (165 cases, C: 82/PT: 83) or a quinolone (C: 2/PT: 2)
according to each center’s policy. Granulopoietic growth factors were used
in 89 cases. Distribution of age, neutropenia duration (median: 17 days),
underlying disease, oral antimicrobial prophylaxis and protocol therapy
duration (median: 11 days) was comparable in both arms. A microbiologically documented infection (MDI) was evidenced in 57 cases (27%). Bacteria were isolated from blood cultures in 48 cases including: coagulase
positive (3) or negative (12) staphylococci, streptococci of various species
(14), E. coli (7), P. aeruginosa (5) and other gram-negative bacilli (7). The
in vitro susceptibility rate was 53% for C and 59% for PT. Two days after
antibiotics initiation, clinical (fever disappearance) and microbiological
(culture becames negative) success rates (SR) were 62% for C versus 61%
for PT and 50% versus 55% respectively in case of MDI (p = 0.89). Two
deaths and 77 failures were registered. At the end of the protocol, SR (no
antibiotic change/absence of superinfection) was 59% with C versus 50%
with PT (p 0.27) and 53% versus 40% respectively in the 151 cases with
neutropenia ≥ 10 days (p 0.17). The overall occurrence of side effects was
similar in both arms (the most frequent being cutaneous rash: 10 cases).
In conclusions, there was no statistically significant difference between C
and PT concerning the management of FNE.
PO-0940 Penicillin or vancomycin plus ceftazidime in febrile
neutropenia after stem cell transplantation

PO-0941 Severe toxicity of amphotericin B colloidal dispersion
(Amphocil®) in patients with haematological malignancies
Timmers GJ, Simoons-Smit AM, Touw DJ, Van Loenen AC, Huijgens PC
University Hospital Vrije Universiteit, Amsterdam, The Netherlands

The prevention of fungal infections, especially those caused by Aspergillus
spp. presents an ongoing challenge in the treatment of haematological malignancies. Objective and Design. An open label, randomised clinical trial was
designed to compare amphotericin B colloidal dispersion (ABCD, Amphocil®)
2 mg/kg/day intravenously, with fluconazole (F) 200 mg/day orally, for the
prevention of fungal disease in neutropenic patients. In case of febrile neutropenia, not responding to antibacterial treatment within 96 hours, patients
in both treatment groups were to receive a therapeutic dose of ABCD, 4
mg/kg/day. However, the study had to be stopped in an early phase, due
to severe side-effects of ABCD. Patients. Twenty-four patients were included, with the diagnoses of multiple myeloma (n=8), aplastic anaemia (n=l),
HD (n=1), NHL (n=5) CML (n=1), ALL (n=2), and AML (n=6). There were 10
females and 14 males, with a median age of 51.5 years (32-65), receiving
cytotoxic chemotherapy (n=7), syngenic (n=1), allogeneic- (n=1) or autologous (n=15) stem cell transplantation. Results. Prophylactic ABCD 2
mg/kg, was randomly assigned to 12 patients and administered for a median of 16 days (2-27), mean dose: 155 mg/day (100-300), median total
dose: 2.31 g (0.15-8.1). Therapeutic ABCD, 4 mg/kg, was initiated in 4
patients, all receiving F, for a median of 2.5 days (1-3), mean dose: 272
mg (220-400). Infusion related chills were observed in 15/16 patients
(94%). This was accompanied by a temperature rise of ≥ 2°C in 4/16
patients and of ≥ 1°C but <2°C in 10/16 patients. The mean temperature
during the first 3 days of prophylaxis was 37.49°C in ABCD patients, versus
36.53°C in patients receiving F (p=0.01). Patients received for premedication: hydrocortisone (9/16), morphine (9/16) andlor antihistamines
(12/16). Despite premedication chills persisted in 6/16 patients. Other
ABCD related adverse events were tachycardia (7/16), dyspnoe (2/16),
nausea with vomiting (6/16), headache (3/16) and hypotension (4/16).
ABCD was discontinued in 9/16 patients (56%) due to side effects. Conclusions. ABCD is not suitable for antifungal prophylaxis in neutropenic
patients due to severe side effects, which are mainly infusion related.

PO-0942 Central venous catheter infections in patients with acute
leukaemia

Kargar Samani K,* Vandercam B,° Straetmans N,# Michaux L,# Ferrant A#
*Division of Haematology, Cliniques La Dorcas, Tournai; °Division of
Internal Medicine and #Haematology, Saint Luc Hospital, Brussels, Belgium

The incidence of gram-positive bacteriaemia has increased in neutropenic
patients. Empiric antibiotherapy regimens in febrile neutropenic patients
often include a third generation cephalosporin, but the response of grampositive cocci to therapy is not optimal. We conducted a retrospective
study in febrile neutropenic patients after stem cell transplantation to evaluate the safety and efficacy of ceftazidime plus penicillin (C+P) combination and compared it with ceftazidime plus vancomycin (C+V) combination.
The study includes 64 patients admitted to the Aseptic Unit of Haematology at St. Luc hospital. Thirty-six patients were treated with C+V and 28
patients with C+P. Success of initial antibiotic therapy was observed in 78%
of the patients in the C+V group and in 57% of the C+P group (p = 0.5).
All patients were alive at day 3. Infection was the cause of death of 1
patient in each group.
Clinical characteristics and outcome of the patients.

N° of patients
PBSC transplantation
BMT
Duration of neutropenia <500/days (Range)
Duration of C days (Range)
Duration febrile episode
Succes of initial antibiotherapy
Alive at day 3
Death due to infection

The combination of C+P is as safe as the combination of C+V in febrile neutropenic patients after stem cell transplantation. The morbidity and mortality were identical in both groups and cost effectiveness was in favor of
the C+P group.

C+V

C+P

P

36
18 (50%)
18 (50%)
11 (5-32)
11 (4-28)
4.5 (2-23)
28 (78%)
36 (100%)
1

28
18 (64%)
10 (36%)
8 (3-27)
12 (2-29)
5.5 (1-15)
16 (57%)
28 (100%)
1

0.7
0.7
0.5
0.4
0.1
0.5
1
1

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Karthaus M, Döllmann T, Klimasch T, Elser C, Heil G, Ganser A
Hannover Medical School, Dept. of Haematology and Oncology, Hannover, Germany

Objective. Over the last decades, central venous catheter (CVC) have been
used with increasing frequency in leukaemia pts. CVC-infections are an
important complication and may be the cause of fever during neutropenia
(<500/µL). However, data regarding the incidence of CVC-infections are
rare in acute leukaemia pts. Methods. Untunneled CVC were analysed in
58 pts with acute leukaemia (22 M/36 F) within 119 consecutive
chemotherapy cycles from 4/96 to 1/98. CVC-related infection was proven,
if the same organism from peripheral blood and CVC-tip was isolated. CVCinfection was suspicious or possible when exit-site inflammation and positive blood culture or micro-organisms typical for CVCL infection were
observed. Results. Mean duration of neutropenia/chemotherapy cycle was
16.7 days (SD 7.7). 178 CVC with a total of 2576 CVC days (mean 14.5d,
SD 7.2d) were observed in 119 cycles. Fever occurred in 87 cycles (73%).
Bloodstream infection was proven in 31 out these 87 eps (26.1%) with 40
isolates (8 gram neg, 31 gram pos, 1 Candida ssp.). Microbial colonisation of the CVC-tip was observed in 24 CVC-lines with 28 isolates (27
gram-pos, 1 gram-neg.). CVC related infections were observed in 5 eps
only, all with coag.-pos. Staphylococci. CVC-related infection was assumed
in another 6 eps (local inflammation and gram-pos. blood culture). Six further eps had typical isolates (4 coag-pos staphylococci, 1 Candida ssp)
from the blood and were considered possible CVC related infections. In
none of the remaining afebrile 32 cycles a CVC- infection was proven or
suspected. Conclusions. The overall incidence of CVC-infections in acute
leukaemia pts was low 6.5/1000 CVC-days (1.9 proven/2.3 suspected/2.3possible/1000 CVC-days). Although infectious CVC complications
were assumed in 15 febrile episodes (17.2%), fever was proven to originate from CVC in leukaemia pts in 5.7% only.


Objective. Systemic fungal infections (SFI) are a frequent cause of morbidity and mortality for pts with acute leukemia and febrile neutropenia (FN). Since mortality of proven SFI is up to 50%, an effective prophylaxis is necessary. Conventional dosages of AMB (0.75 mg/kg/d) are standard treatment for proven SFI, but limited by side effects. Design and Methods. In a prospective trial, the efficacy and safety of intensive i.v. AMB (1 mg/kg every second day) on the risk of development of SFI was tested in 69 patients with acute leukemia. Patients with diagnosis of sarcoma were identified and grouped by presence or absence of neutropenia. Prophylaxis was applied for a mean of 17.1 d (SD 1.1) of AMB treatment in the control group (n=50), mean total dosage of AMB in the AMB group was 695 mg/cycle (n=49), compared with a mean of 647 mg/cycle in the control group (n=49), and a mean of 1071 mg/cycle in pts receiving AMB (n=29). Infusion related toxicity of AMB was low, documented in 13 cycles (29%) with prophylaxis compared with 16 out of 29 treatment cycles (52.5%) with AMB in the control group. Neutrophilia of AMB was maximal with a maximum of WHO II reported in 1 out of 4 treatment cycles only. Conclusions. Intensive i.v. AMB is effective regarding the reduction of fungal microabscesses in liver or spleen as well as pulmonary infiltrates and FN. Intensive i.v. AMB was safe with moderate and tolerable side effects.

### Table 1: Median charges (US$) and LOS (days)

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<th>Year</th>
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<tr>
<td>1992</td>
<td>8225</td>
<td>4.00</td>
</tr>
<tr>
<td>1993</td>
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<td>4.00</td>
</tr>
<tr>
<td>1994</td>
<td>17264</td>
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### Table 2: Sarcoma admission with neutropenia

<table>
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<th>Year</th>
<th>Attrib.</th>
<th>Febrile</th>
<th>Attrib.</th>
<th>Febrile</th>
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<tr>
<td></td>
<td></td>
<td>(53.4%)</td>
<td>(46.6%)</td>
<td>(53.4%)</td>
<td>(46.0%)</td>
<td>(53.5%)</td>
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<tr>
<td>1993</td>
<td>Median</td>
<td>8225*</td>
<td>14710*</td>
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<tr>
<td></td>
<td>charges</td>
<td>(53.4%)</td>
<td>(46.6%)</td>
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<td>4.00*</td>
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</table>

### Table 3: Patients with CNS bacteremia

- No adult patient with a haematological malignancy and profound neutropenia (number of episodes with >38.5°C 88% vs 50%; p<0.05), 2) had higher body temperature (number of episodes with >38.5°C 88% vs 50%; p<0.05), 4) showed a trend to have more frequent relapses of CNS bacteremia (number of episodes with new CNS, 16 vs 10 p=0.05). There were no differences between the two groups with regard to underlying diagnosis, age or proportion of patients with central venous lines. Conclusions. The results support the concept that there are clinical differences in patients with CNS bacteremia based on the number of positive blood culture bottles. Growth in two or more bottles was associated with longer usage of the central venous line, relapse of CNS bacteremia, higher body temperature and less granulocytopenia. The findings favour the notion that growth of CNS in only one bottle/pair of bottles may be considered as a contamination also in this patient population.

### Table 4: Prophylaxis against Pneumocystis carinii in patients with haematological malignancies

<table>
<thead>
<tr>
<th>Year</th>
<th>Prophylaxis</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
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<td>100</td>
</tr>
<tr>
<td>1993</td>
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<tr>
<td>1994</td>
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### Table 5: Prophylaxis against Neutropenia and Febrile Neutropenia

<table>
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<tr>
<td>1994</td>
<td>150</td>
<td>150</td>
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### Table 6: Pulmonary mucormycosis

- In a prospective trial, the efficacy and safety of intensive i.v. AMB (1 mg/kg every second day) was tested in 69 patients with acute leukemia. Patients with diagnosis of sarcoma were identified and grouped by presence or absence of neutropenia. Prophylaxis was applied for a mean of 17.1 d (SD 1.1) of AMB treatment in the control group (n=50), mean total dosage of AMB in the AMB group was 695 mg/cycle (n=49), compared with a mean of 647 mg/cycle in the control group (n=49), and a mean of 1071 mg/cycle in pts receiving AMB (n=29). Infusion related toxicity of AMB was low, documented in 13 cycles (29%) with prophylaxis compared with 16 out of 29 treatment cycles (52.5%) with AMB in the control group. Neutrophilia of AMB was maximal with a maximum of WHO II reported in 1 out of 4 treatment cycles only. Conclusions. Intensive i.v. AMB is effective regarding the reduction of fungal microabscesses in liver or spleen as well as pulmonary infiltrates and FN. Intensive i.v. AMB was safe with moderate and tolerable side effects.
Infections

Caninii infection for 3 months post-transplant. Infectious complications from day 30, at which time all patients had a total granulocyte count exceeding 0.5 x 10^9/L. Six patients were included in group A (n=3), septicaemia (n=1) and pneumonia (n=3). One hundred days to 1 year infectious episodes were recorded in 13 patients. Early infections (30-100 days post-ASCT) occurred in 7 patients who developed herpes zoster (HZ, n=2), varicella zoster (VZ, n=1) and pneumococcal (n=3). One hundred days to 1 year all patients died of post-ASCT2 patients were diagnosed with generalised varicella zoster (GVZ), 3 H Z, 2 pneumococci. 5 pneumonia and 3 other bacterial infections. More than 1 year after ASCT, 1 patient developed GVZ 2, sepsaisma, pneumonia and 3 other bacterial infections. Six of these patients had Streptococcus pneumoniae bacteremia, one of whom on two occasions. Two patients had signs of CMV reactivation. Three patients died in remission. 1 of pneumonia and sepsaemia (700 days post-ASCT), 1 of progressive lung fibrosis (330 days post-ASCT) and 1 of encephalitis (660 days post-ASCT). No association between T and B cell restitution and infectious complications post-ASCT was recorded (data not shown). Treatment of patients with MM undergoing tandem ASCT should receive long-term anti-viral prophylaxis and in the individual patient anti-bacterial prophylaxis, gammaglobulin administration and pneumococcal vaccination should be considered.

PO-0948 Fiberoptic local endobronchial instillation of antymycotic agents as an additive treatment of invasive aspergillosis and aspergillosis of the lung

Pöllnisch W,* Winkler J,* Vogtmann M,* Nenoff P,* Becker C,* Woff D,* Lange T,* Friedrich Th,* Borte G,* Klöppel R,* Schwenke H,* Nieder- wieser A* and Schwaiger T* (1)*Dept of Internal Medicine, *Dept of Dermatology, *Inst of Pathology, *Dept of Diagnostic Radiology, Univ. of Leipzig, Germany

Invasive bronchopulmonary infection with Aspergillus species is a major and increasing complication in immunocompromised patients. Over the last 30 years amphotericin B has remained the drug of choice for invasive aspergillosis despite its toxicity and low response rates of 20-55%. Resistance of the fungi to an inadequate concentration of the drug at the infection site might be responsible for the high mortality rate. We report our experiences with local fiberoptic instillation in five patients (2AML, 1CML, 1NBL, 1ALL). Two patients suffered from aspergillosis and three from dissease Aspergillus pneumonia. Response to systemic antymycotic treatment was insufficient. Therefore, amphotericin B (5-40 mg) or miconazol (25-200 mg) was applied between 3 and 9 times into the involving segmental bronchi via fiberopticoscopy in wedge position, in addition to systemic therapy. Side effects of instillation were transient cough and mild bronchospasm. In one case with aspergillosis a self-limiting endobronchial bleeding after the second instillation occurred. The follow up showed improvement of the clinical performance and accelerated regression of pulmonary infiltrates without lethality and nearly complete resolution in three cases. Our data provide evidence of the bronchoscopic instillation of antymycotic agents were used in addition for the treatment of invasive Aspergillus infections of the lung.

PO-0949 Antifungal prophylaxis in lymphoma patients (pts) submitted to autologous haematopoietic stem cell transplantation

Silva M.RG,* Leal-da-Cocta E, Passos-Coelho JL, Miranda N, Parreira A (1)*Servico de Hematologia, Instituto Portugues de Oncologia, Lisbona, Portugal

Between September 1994 and October 1997 we evaluated the impact of itraconozole prophylaxis on the incidence of fungal infections and the use of Amphotericin B in 48 pts (30 men and 18 women, median age 31, range 13-61) with Hodgkin’s disease (25) or non-Hodgkin’s lymphoma (23) who underwent high-dose chemotherapy with BEAM supported with bone marrow (17), peripheral blood progenitor cells (25) or both (6). Twenty five pts were randomly assigned to receive itraconozole capsules (200 mg bd) starting on day-5 (group A) and 23 to no antifungal prophylaxis (group B). In group A, 13 pts were switched from itraconozole to iv fluconazole due to inadequate concentration of the drug at the infection site. All patients had a total granulocyte count exceeding 10000/µL was 14 (8-119) and 15 (8-32), to ANC ≥ 500/µL was 14 (10-118) and 16 (9-35), and with ANC <500 was 11 (8-117) and 14 (6-32), respectively. Seven patients in group A (26%) and 12 in group B (52%) received Amphotericin B (p<0.05), a median of 10 days after transplantation in both groups. Although documented systemic fungal infections after transplantation were not observed, fungi (Can-zid spp and one case of Aspergillus) were isolated from 17 patients, 14 of whom were not taking antifungal prophylaxis. The incidence of fungal isolation was 12% in group A and 61% in group B (p<0.001). One infectious death occurred in group A 7 days after transplantation. The patient died with severe pneumonia with Candida albicans in bronchial secretions while on Amphotericin B. Although we could not demonstrate a difference in the frequency or timing of prescription of Amphotericin B, we conclude that the prophylactic use of azoles reduced the number of fungal isolates in this group of lymphoma pts treated with BEAM.

PO-0950 Clinical follow-up of pseudotumour cerebri induced by ATRA in acute promyeloicytic leukaemia adult patients

Rousselier Ph, Jablon L, Takis AL, Miéla M, Dombre H, Degos L,*Hôpital Saint-Louis, Paris, France

Background. Pseudotumour cerebri (PTC) is a well-known complication of all-trans retinoic acid (ATRA) treatment in paediatric patients and very few cases of PTC have been reported in adults under ATRA to date. PTC is characterised by a clinical syndrome of intracranial hypertension with a normal cerebral CT scan. Usually regression of all the manifestations is noted within 3 weeks. We report 2 cases of PTC in APL adult patients with ophthalmological disturbances for more than 6 months. Case #1. A 19 years old male with APL received a first induction with a combination of ATRA and chemotherapy. She presented at day 12 a severe headache, vomiting and diplopia related to VI cranial nerve palsy. Fundus ocular examination showed that both optic discs were blurred and elevated with tortuous vessels. The CT scan and MRI cerebral imaging were normal, with normally sized and shaped cerebral ventricles. ATRA therapy was reduced at day 14 and stopped at day 18 concomitantly with methylprednisolone therapy. Clinical manifestations resolved after 10 days. However, the Goldman visual field demonstrated a persistent enlargement of the blind spot with generalised isopters constriction during 6 months. Case #2. A 55 year old male with APL was initially treated with ATRA (45 mg/m²). He started to develop PTC at day 13. Fundus ocular examination showed an asymmetric papillaeama associated with bilateral retinal haemorrhages. ATRA therapy was pursued and stopped at day 17 because of the occurrence of an ATRA syndrome. 6 months later the fundus was not restored and the Goldman visual field was normalised at month 9. During the ATRA maintenance treatment, the patient regularly complained of headache. Discussion. Contrary to previous case reports, our patients had a prolonged evolution before complete recovery. Confronted to PTC, corticosteroid therapy is recommended to rapidly control the papillaeama concomitantly with the interruption of ATRA therapy and therapeutic lumbar punctures. In one report, evacuation of the CSF permitted to maintain the ATRA therapy schedule. Ophthalmologically neglected patients are those apt to develop chronic atrophic papillaeama and visual loss. In this situation, optic nerve sheath decompression can be performed. The patient developed ATRA syndrome after the occurrence of PTC but no relationship between these two manifestations has been demonstrated to date. Conclusions. A prolonged ophthalmological follow up is recommended after PTC associated with ATRA treatment.

PO-0951 The role of a haematological emergency unit (HEU) in the management of patients with acute leukaemia


At the Department of Cellular Biotechnologies and Haematology in Rome a haematological emergency unit (HEU) is active and operative 24 hour-a-day for emergencies occurring in patients with haematological diseases. Between March 31, 1996 and August 31, 1998 we observed 850 patients in this HEU with acute leukaemia in various phases of their disease: 464 had AML and 386 had ALL. No admission to the ward was needed in 501 (59%) cases which were discharged within 12 hours: in particular, 208 had febrile episodes (113 AML, 95 ALL), 45 had haemorrhagic complications (31 AML, 14 ALL) and 248 had other medical problems. Thirty four and forty-nine (41%) patients needed admission to the HEU ward: in detail, acute cytopenia or severe sepsis and severe infection: 20 had haemorrhagic episodes (11 AML, 9 ALL), 159 had other medical problems (85 AML, 74 ALL): 80 patients were seen at the onset of the disease (52 AML, 28 ALL). Of the admitted patients, 336/349 (96%) were hospitalised in the HEU ward, 9 in one of the regular haematological wards of the same institute and 4 were referred to other hospitals. Of the 336 HEU hospitalised patients, 191 were discharged after a median time of hospit
PO-0952 GM-CSF may have a role in the management of invasive aspergillosis (IA) in the neutropenic patient

Prentice GH,* Moorej *, Potter M,* Herbert L,* Kibbler C,* Scarfe H,* Poynton CH†
*Departments of Haematology and *Microbiology, Royal Free and University College Medical School, RF Campus, London; °Department of Haematology, Christie Hospital, Manchester; †Department of Haematology, University Hospital of Wales, UK

Fluconazole prophylaxis has substantially prevented infection due to Candida albicans. Invasive (90% pulmonary) aspergillosis (IA) is now the most common invasive fungal infection in the neutropenic patient. The main risk factors are the use of corticosteroids and extended periods of neutropenia. Prophylaxis by HEPA filtration and a broad spectrum triazole (e.g. itraconazole) will reduce the incidence. The treatment of established IA is complex with no single effective drug (e.g. amphotericin B: galactomannan/CT scanning); and improvements due to the liposomal entrapment of the otherwise toxic standard drug Amphotericin B, sometimes combined with surgery. Mortality has improved from about 50% to around 10% with these measures. From April, 1994 to February, 1998 we treated 31 episodes of IA in 30 patients with haematological malignancies (63% beyond CR, 10 post-BMT). Diagnosis was proven based on radiology (CT scan), histology or microbiology. Patients received Ambisome, or conventional Amphotericin B combined with GM-CSF 300mcg/kg/day IV. The period of neutropenia was from D-30 (median 16) days and treatment was given for 5-113 (median 22) days. 62% were improved at 14 days. Cure has been seen in 48% of episodes, 42% for proven/probable, 58% for possible. The 100 day survival was 55% and only 8 patients proceeded to surgery. This result is comparable to the RHIF previous outcome with surgery as part of the treatment (survival) 76% for chemo, 35% post-BMT patients at 60 days). This apparent benefit forms the basis for the current MRC randomised trial of GM-CSF.

PO-0953 Catheter related complications in pediatric haematology/oncology patients

Ertem M, Aysev D, Tayylidiz N, Gozdasoglu S, Yavuz G, Unal E, Cin S
Ankara University, School of Medicine, Department of Pediatrics, Division of Haematology, and Oncology, Ankara, Turkey

The use of right atrial catheters (RACs) for long term venous access has gained wide acceptance in the management of children with malignant diseases. Despite the obvious benefits, there are disadvantages to indwelling catheters, including obstruction, dislodgement, and catheter-related infection. The complications of RACs in pediatric haematology/oncology patients are unknown for centres in developing countries. Due to the differences in the health care practices between developed and still developing countries, the rates and patterns of complications of RACs may differ significantly. Therefore, the purpose of this study was to examine the frequency of complications of external RACs in pediatric haematology/oncology patients at a major center in Turkey and to compare our results with those reported from developed countries. The records of all the children who required intensive chemotherapy and RACs between May 1994 and May 1998 were reviewed to obtain data on primary diagnosis, duration of catheter, mechanical complications, and the frequency of catheter infections. A total of 90 RACs were placed in 61 children with a total experience of 13,536 catheter days. Seventy-four of total 90 catheters (82.2%) were required in a total of 46 patients (75.4%) with leukemia and lymphoma. The rate of catheter-related sepsis was 4.9 episodes per 1,000 catheter days or 9 episodes per 10,000 catheter days. The most common complications were obstruction, dislodgement, and catheter-related infection. The complications of RACs in a developing country, such as ours necessitates an appraisal of the benefits and risks for each patient and improvement of catheter care procedures.

PO-0954 Fungal colonisation and infection in children with leukaemia/lymphoma during induction therapy

Ankara University School of Medicine, Departments of Pediatrics and Microbiology, Ankara, Turkey

Fungal infections are increasingly recognised as major causes of morbidity and mortality in patients treated for haematological malignancy. In absence of sensitive and specific markers of invasive fungal infection, treatment of neutropenic pyrexia of unknown origin based on apropto-based antimicrobial approach resulting in the empirical use of anti-fungal therapy if no resolution of fever is demonstrated. The main-stay of anti-fungal therapy is conventional amphotericin B (Con AmB) whose main limitation is toxicity. Abelcet (ABLC), a lipid formulation of amphotericin B, has been shown to be effective in patients failing to respond to Amphotericin B with reduced toxicity: the standard dose of ABLC is 5 mg/kg/day. This prospective study investigated the efficacy and toxicity of the use of low dose ABLC (LD ABLC, 2 mg/kg/day) in patients experiencing toxicity (pre-existing renal disease, serum creatinine >170 µmol/L, whilst receiving ConAmB or development of ConAmB related toxicity) or failing to respond to ConAmB. Patients considered at high risk of invasive fungal infection or with documented fungal infection were excluded from this study. Patients received 100 mg/day, 100 mg/200 mg alternate days or 200 mg/day depending on body weight and response to ABLC was assessed. To date, 15 patients have been treated (allo-BMT n=4, auto-BMT n=6, chemotherapy n=5) with a median age of 39 yrs (range 18-66). The median time to neutrophil recovery to >0.5 x10^9/L was 12 days in total (range 8-45). Thirteen patients received oral fluconazole and/or oral itraconazole with 7 patients receiving ConAmB prior to LD ABLC. Patients received a mean dose of 2.32 mg/kg/day ±0.13 (±SEM) for a median of 3 days (range 1-12). 10 of 15 patients responded to LD ABLC (resolution of fever for >3 days) with a median time to response of 4 days (range 3-5). No patient died whilst on study. All patients received additional potentially nephrotoxic drugs with a median of 3 (range 1-5) drugs administered per patient. Our findings suggest that low dose ABLC is effective in patients failing to respond to Amphotericin B with reduced toxicity.
toms of anaphylaxis. This study demonstrates that LD ABLC is a safe and generally effective alternative to full dose ABLC in febrile neutropenic patients at low risk of fungal infections in whom blood cultures have resulted in toxicity or has been ineffective. The regimen clearly offers reduced drug costs and warrants more intensive study in this population.

PO-0956 Leukapheresis in small children with initial hyperleukocytosis in acute leukaemia: is it still a therapeutic option?

Weigel S, Reddemann H, Bernig T, Mukodzi S
Department of Pediatric Haematology and Oncology of the Ernst-Moritz Arndt University Greifswald, Germany

Objective. Cytotherapy for hyperleukocytosis before the initiation of primary therapy may reduce morbidity and mortality from blast cell lysis in children with acute lymphoblastic leukaemia (ALL) and from leukostasis in children with acute nonlymphoblastic leukaemia (ANLL). Design and Methods. We performed six leukapheresis procedures in three children with newly diagnosed acute leukaemia (2 ALL, 1 ANLL), presenting with initial hyperleukocytosis. The median age was 3.3 years (range: 24-53 months) with low body weight (median 16.9 kg, range: 13.1-19.9 kg). The procedures were carried out on two consecutive days. We used the CS 3000plus (BAXTER) machine, with a collection volume of 55 mL. The venous access were the peripheral veins. For a better compliance, the little children received a continuous Midazolam-infusion before and during apheresis. The blood and albumine substitution was done through a bypass system using a peripherally inserted central line. The cumulative volume that was infused through the bypass system was 5 L (range: 4.5-5.7 L) in the two patients who received apheresis before being 139 (128-220) min. The median decrease in leucocyte count was 62%, reflecting the effectiveness of the cytoreductive procedure. Start of the leukapheresis we witnessed a moderate increase of the leucemic cells in the peripheral blood. The procedures were well tolerated by all the children. Conclusions. We have proved that it is both safe and effective to perform initial leukapheresis in hyperleukocytosis in small children. The reduction of the initial tumour load is effective and there are no life-threatening complications or tumour lysis symptoms.

PO-0957 Amphotericin B lipid complex at 3 mg/kg/day for treatment of invasive fungal infections in adults with haematological malignancies

Martino R, Subirà M, Sureda A, Brunet S, Sierra J
Division of Clinical Haematology, Hosp. de la Santa Creu i Sant Pau, Barcelona, Spain

Amphotericin B lipid complex (ABLC) has been shown to be better tolerated than conventional amphotericin B (c-AMB) in the treatment of invasive fungal infections. The standard dose studied to date has been 5 mg/kg/day, with few data on the efficacy of lower doses. We treated nine consecutive adults with a haematological malignancy and an invasive fungal infection with ABLC at 3 mg/kg/day. The median age was 46 yr. (29-62) and sex 5 F/4 M. Underlying malignancies were AML (n=5), ALL (n=1), CLL (n=1) and CML (n=2). Five patients had recently received intensive chemotherapy and four an allogeneic stem cell transplant. Seven patients suffered an invasive aspergillosis, pulmonary in five and primary cutaneous in two. Two patients suffered an invasive candidiasis, chronic systemic in one and acute disseminated candidiasis in another. All 9 patients had previously received another systemic antifungal prior to switching to ABLC: 8 c-AMB and 4 fluconazole, and 4 were on immunosuppressive therapy at start of ABLC. Eight patients responded (5 complete and 3 partial responses), and one died from invasive aspergillosis. Treatment was well tolerated, with only 4 infections followed by infusion-related adverse events, and in six patients who had an elevated serum creatinine before therapy (median 142, range 138-169 (normal <94 µmol/L)), the level decreased by the end of therapy (median 113, range 73-114). These data suggest that lower doses of ABLC may be equally effective but less toxic and cost than higher doses, and comparative dose-finding studies are justified.

PO-0958 Medical treatment for invasive aspergillosis avoids a recurrent infection in PBSCT Transplantation

Hospital “La Paz”, Madrid, Spain

Objective. The existence of a previous invasive aspergillosis (IA) in haematological patients undergoing bone marrow (BM) transplantation requires prophylactic measures to avoid the reactivation of infection during the procedure. Most authors propose surgical resection of residual lesions, if possible, and the prophylactic administration of amphotericin B. Such clear recommendations are not easy to follow in patients in which transplant is the only treatment for fungal infection. All cases received treatment until clinical and radiological resolution. Conditioning treatment for transplantation was BUCY (6 ILA and 5 LALN), BEAC (1 Hodgkin’s disease); BU-MF (1 MM) and busulphan 16 mg/kg (1 CML). Patients began their transplantation procedure with prophylaxis for fungal infections with low dose of liposomal or lipidic amphotericin, and in some cases itraconazole was added. One transplant was done without prophylaxis. No relapse of IA was demonstrated in these patients during the transplantations and peritransplantation period (before day +100). This result is better than expected in BM transplants, probably based on two reasons: i) the risk of invasive aspergillosis is directly related to the duration of neutropenia, and PBSCT neutropenia period is shorter than in BM transplants; ii) an effective medical treatment of primary IA and secondary prophylaxis can prevent IA from recurring. Conclusions. Effective medical treatment of primary IA and secondary prophylaxis can prevent from IA recurring in patients who underwent autologous H-BSC transplantation. In our experience surgical resection is not necessary to avoid IA from recurring in these patients.

PO-0959 Chronic coinfection with HBV, HCV, HDV and HGV in children with malignancy

Research Institute of Pediatric Haematology, Sanatorium “Ruskeoe Pole”, Moscow, Russia

We observed 49 children in age from 4 to 17 years with chronic coinfection (HBV+HCV; 36 patients; HBV+HCV+HDV; 10; HBV+HCV+HGV; 1; HBV+HCV+HDV+HGV; 1; HBV+HDV; 1 patient). The patients had malignancy in the remission from 1 to 15 years: ALL 9, AML 11, NHL 3, nephroblastoma 4, HD 1. Twelve children received supportive chemotherapy by two medicines (6 MP, Mdb). For the identification of viruses in serum we used ELISA and PCR. 37 patients (75.5 %) had the replication of all viruses of hepatitis at the same time, the replication only of HBV was marked in the 3 patients (6.1%), HCV in 8 patients (16.4%). The coinfection was combined with 1b genotype of HCV. Three patients had coinfection with the precursor mutant of HBV, 3 patients with prep-5 mutant of HBV. In the serum biochemical assays increased levels of aminotransferase (ALT AST) were changed from 1.5- to 8.7- in 45 patients, and only 4 patients had normal levels of the ALT AST. Most high level of ALT AST was marked in the patients receiving chemotherapy. Increased level of fibrin from 1.3- to 3.3- is fixed in the 3 cases. Twenty-four patients received a interferon-alpha (5 ME/M² TIW for 12 months), from them 10 children were on the supporting chemotherapy, the inhibition of replication of one of the viruses was observed in 10 patients (HCV- in 9 cases; HGV- in 3 cases; HDV- in 2 cases), from them 4 patients received supporting chemotherapy. The elimination of HBV was marked and thus, the partial effect was observed in 41.7 % of cases. The normalisation of serum aminotransferases was marked in 4 patients (16.7%), not receiving chemotherapy. The elimination of all viruses was not marked.

PO-0960 The comparison of ceftazidime-amilcarecin and cefepime-amikacin treatments in patients with febrile neutropenia

Tasova Y, Sahin B, Paydas S, Inal S, Yaman A
Cukurova University Faculty of Medicine, Departments of Medical Oncology and Infectious Diseases, Balcal, Adana, Turkey

Ferbile neutropenia (FN) is a common and life-threatening complication of chemotherapy for haematologic neoplasia and a real problem to be managed empirically. In this Single Institute Haematological Centre, the efficacyp of ceftazidime (CEF) + Amikacin(AM) and Ceftazidine (CEF) + AM was compared in 72 episodes of FN in 62 patients with haematological neoplasia (HA) during the period from March 1999 to March 1999. CEF-AM and CEF+AZAM were begun in 39 and 33 episodes, respectively. Mean ages and female/male ratios were different in both CEF group and CEF+AZAM groups. CEF+AZAM were used in greater 35.2±1.6 patients with severe neutropenia (count <1000/mcL). The result was as follow: CEF+AZAM was superior to CEF-AM in terms of efficacy and safety. CEF+AZAM group had a shorter duration of fever (15±2 days), and a shorter duration of neutropenia period (98±16 days), compared to CEF-AM group (5±1 days).
The functional activity of leucocytes was studied in spontaneous nitroblue tetrazolium reduction test (NBT) which was performed cytochemically. Results. In all the period of observation 17 episodes of positive PCR with CMV DNA were demonstrated (7 in AUBMT pts, 8 in ABMT pts and 2 in pts underwent antileukaemic chemotherapy) in control group DNA of CMV was determined in not a single case. The level of CD determinants and functional activity of leucocytes were determined separately in the cases positive for DNA CMV in PCR (CMV+ group) and negative in this test (CMV- group) in comparison with control. The level of investigated CD markers in CMV+ and CMV- group was similar. The possible influence of CMV infection on the level of these parameters. On the other hand the difference was demonstrated in functional activity of leucocytes. In NBT in CMV+ group it was 34.5±3.4% - significantly higher (p<0.01) and in CMV- group - 8.8±1.3%, significantly (p<0.01) lower than in controls (14.3±2.4%). We suppose that this activity is the result of CMV metabolism in infected leucocytes. Conclusions. Spontaneous NBT is a sensitive parameter of CMV metabolism in leucocytes and it may have certain prognostic and diagnostic importance.

PO-0961 Incidence of Blastocystis hominis in patients with haematological neoplasias

Tasova Y, Koltas S, Sahin B, Inal S, Midikli D, Ozcak K, Paydas S
Cukurovo University Faculty of Medicine, Departments of Medical Oncology and Infectious Diseases, Balcalı, Adana, Turkey

Although Blastocystis hominis (Bh) has been found in stools of asympto- matic healthy subjects, the pathogenic role of this intestinal parasite in symptomatic patients with haematological neoplasia has not been clearly defined. It was reported that it could cause severe and prolonged diarrhoea, abdominal pain and distension in patients with renal transplants and AIDS. In this study the incidence of Bh in patients with haematological neoplasia was investigated in stool samples of 209 patients with various intesti- nal symptoms during the period from June 1997 to November 1998. Fresh stool samples were investigated with Native Lugol, Concentration, Trichome Staining and Modified Ziehl-Nelson methods by a parasitolog. Although various intestinal parasites were found in 52 (24.9 %) patients, Bh was found out in 23 (11 %) patients. The parasite distribution composed of 23 (44 %) Bh, 17 (32.7 %) Entamoeba histolytica, 6 (11.5 %) Entamoeba coli, 3 (5.8 %) Blastocystis hominis, 1 (1.9 %) Ehri- chomonas hominis, 1 (1.9 %) Chilax mesnilii. Of the patients with Bh + Stool, 16 had diarrhoea more than 5 times in a day, abdominal pain, distension, and vomiting symptoms and 5 had diarrhoea with less than 5 bowl move- ments a day and other intestinal symptoms. Only 2 patients had no diar- rhea but abdominal pain. All patients received Metronidazole 500-750 mg PO IV every 8 hours. At the end of treatment, all but 2 patients had Bh(-) stool without any symptoms. As a result, patients presenting with diarrhoea and abdominal pain should have stool examination since Bh could be the agent responsible for the symptoms.

PO-0962 The role of CD determinants and function of leucocytes in cytomegalovirus infection in oncohaematological patients

Tchekolekovitch V, Gonchar V, Volkova S, Abduolkadirov K
Russian Institute of Haematology, St-Petersburg, Russia

Objective. To evaluate the expression of CD3, CD4, CD8, CD28 determi- nants and the functional activity of leucocytes in immunocompromised onco- haematological patients (pts) with and without cytomegalovirus (CMV) infec- tion. Design and Methods. Forty patients with different forms of leukaemia underwent autologous (AUBMT) (n=17), allogeneic (ABMT) (n=11), bone marrow transplantation (BMT) or intensive antileukaemic chemotherapy (n=12) and a group of healthy blood donors (n=50) (controls) were studied. The material (venous blood) was collected each 2 weeks during 4-6 months in pre- and post-transplantation period in BMT patients, during 1.0-1.5 months in the cases of antileukaemic chemotherapy and once in controls. The patients and healthy donors were screened for the presence of CMV DNA in the buffy coat and urine by polymerase chain reaction (PCR). For identification of CD determinants standard immunofluorescence (IF) assay with monoclonal antibodies (CD3, 4, 8, 22 and 38) was used.

The functional activity of leucocytes was studied in spontaneous nitroblue tetrazolium reduction test (NBT) which was performed cytochemically. Results. In all the period of observation 17 episodes of positive PCR with CMV DNA were demonstrated (7 in AUBMT pts, 8 in ABMT pts and 2 in pts underwent antileukaemic chemotherapy) in control group DNA of CMV was determined in not a single case. The level of CD determinants and functional activity of leucocytes was determined separately in the cases positive for DNA CMV in PCR (CMV+ group) and negative in this test (CMV- group) in comparison with control. The level of investigated CD markers in CMV+ and CMV- group was similar. The possible influence of CMV infection on the level of these parameters. The other hand the difference was demonstrated in functional activity of leucocytes. In NBT in CMV+ group it was 34.5±3.4% - significantly higher (p<0.01) and in CMV- group - 8.8±1.3%, significantly (p<0.01) lower than in controls (14.3±2.4%). We suppose that this activity is the result of CMV metabolism in infected leucocytes. Conclusions. Spontaneous NBT is a sensitive parameter of CMV metabolism in leucocytes and it may have certain prognostic and diagnostic importance.
Poster Discussions  
Multidrug resistance and chemosensitivity

**PO-0964** The effect of P-gp and Ki-67 to induction chemotherapy in acute leukemias

Timuragoulu A, Yanarici M, Savas B, Karadogan I, Undur L
Akdeniz University Medical School, Antalya, Turkey

Objective. P-glycoprotein (P-gp) is one of the multidrug resistance protein which reduces accumulation of different drugs such as anthracyclines, epipodophyllotoxins, vinca alkaloids and some of the alkylating agents. Increased expression of P-gp not only first in relapsed or seconder leukemias but also in de novo acute leukemia and correlates with a poor response to therapy. Ki-67 is a monoclonal antibody, which recognises a protein present in the nucleus of proliferating cells especially in the GI, G2, S, and M phases and correlates with proliferation rate of tumour cells. Although high Ki-67 expression has been known to be a poor prognostic marker especially in solid tumors, it was suggested that to be in proliferation phase, the blast cells would be sensitive to cell-cycle-specific drugs leading remission. Based on this suggestion MLM studies were performed by using rhGM-CSF. The aim of our study is to determine the expression of p-gp and ki-67 in acute leukemia blasts and to evaluate their effect on remission induction therapy. Design and Methods. Twenty-two patients with acute leukemia (18 acute myeloblastic leukemia (AML), 4 acute lymphoblastic leukemia (ALL), median age 38 years (15-72)) were included in the study. P-gp and Ki-67 monoclonal antibodies were used in immunohistochemical staining (APAAP).AML patients were treated with 3 + 7 protocol, and BMI-84 were used to treat ALL patients. Remission was induced by peripheral blood stem cells transplantation (PBSC) in some of patients. Results. Median P-gp and Ki-67 positive cell percentage were 2 (0-80) and 40 (1-70) in patients who get remission (R+) respectively. The median of P-gp and Ki-67 positive cell percentage in patients who did not get remission (R-) was 50 (10-90) and 4 (1-50) respectively. The difference in p-gp between R+ and R- patients was significant (p=0.02) but no difference was found in Ki-67 (p=0.33). Conclusions. Our findings support the negative effect of P-gp expression on remission induction therapy in acute leukemias but to be in proliferation phase, did not have a significant effect on remission induction therapy in our study.

**PO-0965** Implication of fas/ APO-1 and NF-kB in resistance to drug induced apoptosis in leukemia cells expressing the mdr phenotype

ToIomno M, Grimaudo S, Meli M, Dusonchet L, Perricone R, Cajozzo A
Centro Interdipartimentale di Ricerca in Oncologia Clinica, and Istituto di Farmacologia, Università di Palermo, Italy

In this work we observed that HL60R, a multidrug resistant (MDR) variant of HL60 cells, selected by exposure to increasing concentrations of daunorubicin (DNR), was resistant to apoptosis induced by anticancer drugs by a MDR-related mechanism. The MDR reversing agent verapamil was able to reverse partially the resistance to DNR, leading to a complete block in G2-M, but it was unable to restore the sensitivity to drug induced apoptosis. This G2-M block was reversible after resuspension of cells in drug-free medium. Furthermore, DNR induced the cytosol release of cytochrome c, the activation of caspase 3, and apoptosis in HL60 cells but not in HL60R, indicating a block in the earlier phases of apoptotic pathway in resistant cells. While the expression of p53 and BCL-2 was the same in both cell lines, HL60R cells, in contrast to HL60, did not express Fas/APO-1 (CD95) and were resistant to the anti-CD95 agonistic MoAb CH11. To investigate the possible implication of the Fas/Apo-1 system in drug-induced apoptosis, HL60 cells were treated with DNR in combination with two anti CD95 blocking MoAbs, (D2X or Z4), two anti Fas/Apo-1 blocking MoAbs (OK-1 or NOX-2), and three caspase inhibitors (ZVAD-CMK, DEVD-CHO, ZVAD-FMK). Only the pan-caspase inhibitor ZVAD-FMK partially inhibited DNR induced apoptosis. However, we observed that resistance to apoptosis could be inhibited when HL60R cells were treated with the NF-kB inhibitor pyrrodimidindohydrocarbamide (PDTC). Interestingly, PDTC conferred a slight but significant protection toward drug induced apoptosis in sensitive HL60 cells. These data indicate that the Fas/Apo-1 system does not play an important role in drug induced apoptosis and suggest an implication of NF-kB in resistance to apoptosis in cells expressing the multidrug resistant phenotype. This work was supported by AIRC, Italy.

**PO-0966** Evaluation of mrp1/p-190 and mdr1/p-170 activities comparing three functional tests in human tumour cell lines

Dogdan AL, Legrand O, Faussat AM, Perrot JY, Marie JP
EA 3529, Université Paris 6, Formation de Recherche Claude Bernard and Service d’Hématologie, Hôpital Hôtel-Dieu AP-HP, Paris, France

Objective. The fluorescent dyes Rhodamine 123 (R123), calcine axetoxyethyl ester (calcine-AM) and carboxyfluorescein diacetate (CFDA) can be used to assess MRPl/P-190 and/or MDR1/P-170 functionality. This study aimed to determine the best functional test for MRPl/P-190 measurement and discriminate between MRPl/P-190 and MDR1/P-170 functions in the same cells. Design and Methods. We studied six human cell lines (A549, U937, K562/HHT0, K562/HHT10, HL60/S and HL60/MRP1) with different levels of MRPl and MDRI expression. MRPl/P-190 and MDRI/P-170 proteins expression were measured by flow cytometry using MRPl MoAbs and U937 MoAbs, respectively. We analysed the functionality of MRPl/P-190 and MDRI/P-170 by flow cytometry using calcine-AM, CFDA and Rh 123 uptake/efflux in the presence and absence of specific modulators of MRPl/P-190 (2 mM of probenecid) and of MDRI/P-170 (2µM of cyclosporin A/CSA). All the experiments were performed in triplicate. Results: there was a good correlation between MDRI/P170 expression and the modulatory effect of CsA on Rh123 efflux (R=0.9, p<0.001), on Rh 123 uptake (R=0.96, p<0.002) and on calcine-AM uptake (R=0.79, p<0.058). On the other hand, no correlation was found between MDRI/P170 expression and the other functional tests. There was also a good correlation between MRPl expression and the modulatory effect of probenecid on calcine-AM uptake (R=0.87, p<0.05). The correlation between MRPl expression and the modulatory effects of probenecid on calcine efflux (R=0.78) and on CFDA uptake (R=0.88) were significant in a borderline manner. Conclusions. These results demonstrate that the best test to determine MRPl/P-190 activity is calcine-AM uptake/probenecid, while as MDRI/P-170 activity can be measured by calcine-AM uptake ± CsA or Rh123 efflux ± CsA or Rh123 uptake ± CsA and that carboxyfluorescein may not be a substrate for MDR1/P-170. Calcin-AM ± probenecid ± CsA is the sole functional test that can assess the activities of both MRPl/P-190 and MDRI/P-170.

**PO-0967** Characterisation of functional assays of P glycoprotein (Pgp) and mrp transport activity

Poullain S, Wattle E, Cosson A, Fenaux P, Lepelley P
CHU de Lille, France

Multidrug resistance (MDR), caused by overexpression of either Pgp or multidrug resistance associated protein (MRP) is characterised by decreased cellular drug accumulation due to enhanced efflux. In this study, we investigated the specificity and sensitivity of fluorescent probes including rhodamine 123 (R123), doxorubicin (DOX) and calcine-AM (CAM) in order to determine the function of Pgp and MRP using flow cytometry. Verapamil, quinine, bithionine sulphoximine (BSO), genistein and probenecid (an inhibitor of organic anion transport) were used as efflux blockers. Substrates were used together with inhibitors in accumulation and efflux tests on K562/RDR (Pgp+), K562/ADR (MDR+) and their sensitive homologs. Results: Rh 123 is transported almost exclusively by Pgp in short efflux test (t=2h). A significant Rh 123 efflux was observed in GLC4R cells at time t=4h and increased with time. No agents excepting long time exposure to BSO (t=12h) affect MRP mediated export of Rh 123. Rh 123 efflux test/verapamil appears to be specific for Pgp expression. CAM and DOX were substrates for Pgp and NW but CAM appears to be more efficiently pumped out by URP in the efflux test (t=3h). Substrate efflux in K562 had reached plateau level by 1h. Low transport activity, which was detected in GLC4S and K562S with CAM but not with DOX efflux test, was correlated with low level NW expression. Verapamil and quinine modulated efficiently Pgp efflux in K562R. In CAM efflux assay, verapamil modulated Pgp and MRP pumping but quinine was shown to be ineffective in reversing MRP activity. A low level of modulation by BSO was observed in MRP cell line only after 4h exposure. The toxicity of genistein limited its use in vitro especially for long time exposure. Probenecid specifically inhibited MRP mediated efflux in CAM accumulation and efflux tests and provided a specific assay for detection of MRP activity. In conclusion, the independent contribution of Pgp and MRP to MDR phenotype can be discriminated in cells by the Rh 123 efflux test in combination with verapamil and the CAM-probenecid efflux test respectively. Analysis of variation in cellular fluorescence by measuring the rates of substrate influx and efflux together with reversing agents allows the most sensitive test to detect functional activity of Pgp and MRP.

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PO-0968 Comparison of clinical and diagnostic parameters in acute myeloid leukaemia considering p-glycoprotein expression

Machaczka M, Balana A, Rucinska M, Zdziadowska E, Zaluska A, Skotnicki AB
Dept. of Hemaatology, Collegium Med of Jagiellonian University, Krakow, Poland

P-glycoprotein (Pgp) encoded by gene MDR1 is responsible for the classical multidrug resistance (MDR) phenotype. We evaluated Pgp expression in fresh bone marrow samples, obtained prospectively during routinely performed marrow aspiration for diagnostic purposes from 28 patients (pts) with de novo acute myeloid leukaemia (AML) (M0 1; M1 7; M2 8; M3 1; M4 10; M5 1) qualified for intensive chemotherapy. The MDR1 gene product expression was measured by flow cytometry (FACSCalibur; Becton-Dickinson), using a monoclonal antibody UIC2 (Immunotech). Using a cut off of 10% positive blasts, at the moment of diagnosis 46% of pts (13/28) expressed Pgp. Of these 6 presented Pgp+/CD34+/CD33+ phenotype, 4 Pgp+/CD34+ CD33- and 3 Pgp+/CD34- CD33+. The Pgp+ pts had a worse clinical status at the time of diagnosis than the Pgp- pts (average Kamofsky index 60 vs 80). The average bone marrow involvement by myeloblasts was 66% (Pgp+ pts) vs 60% (Pgp- pts). The mean serum lactate dehydrogenase (LDH) level was 1172 vs 912 U/L respectively. All patients were treated with standard 3+7 induction chemotherapy (and +5 days etoposide for M4). In the Pgp+ pts group 54% of pts (7/13) achieved CR after the induction course, 8% (1/12) after two courses. 36% of Pgp+ pts (5/13) died without achieving CR - all in the over 60 age group. In the Pgp- group 67% of pts (10/15) achieved CR after one induction course and 7% (1/15) after two courses. 26% of Pgp- pts (4/15) died without achieving CR - 3 in the over 60 age group. CR rate in the over 60 age group (12 pts) was 20% vs 43% for Pgp+ vs Pgp- pts respectively. CR rate in the up to 60 age group (16 pts) was 64% for Pgp+ and 75% for Pgp-. The mean time to achieve 1.7% leukocytosis after chemotherapy (pts in CR) was 13 days (Pgp+ pts) vs 18 days (Pgp- pts). Number of febrile days, erythrocytes-and platelet transistions and days with a use of haemopoietic growth factors were similar in the Pgp+ and Pgp- CR groups. We conclude that Pgp presence in AML co-exists with other unfavourable factors such as older age, higher serum LDH level or poor clinical status. The expression of Pgp in the elderly is connected with a fatal clinical outcome and AML standard treatment failure, so we suggest considering a different treatment approach.

PO-0969 HIV-protease inhibitors show affinity for the multidrug resistance efflux system

Lucia MB, Rutella S, Runzi C, Cauda R
Department of Infectious Diseases and Center for the flow cytometric study of blood cells, Catholic University, Rome, Italy

The occurrence of multidrug resistance (IMR) is one of the main obstacles in the successful chemotherapeutic treatment of cancer. The classical IMR phenotype is characterised by a reduced ability to accumulate drugs, due to the expression of a drug-resistant, energy-dependent drug-efflux pump. Beside tumour cells, P-gp is expressed in normal cells such as peripheral blood mononuclear cells (PBMC) where it seems to serve multiple functions. In particular it has been shown that AIZ and ddc are recognised by this efflux system. In this study we analysed the effect of the HIV-protease inhibitors (Pis) indinavir, ritonavir and saquinavir on the P-gp-mediated efflux function in PBMC from healthy controls. The ability of the aforementioned Pis to interact with P-gp function was analysed by flow cytometry using rhodamine 123 (rh 123) and daunorubicin (DNR) as P-gp-specific fluorescent dyes. The resistance modification agent (RMA) cyclosporin-A (CsA) was used as positive control. Results show that when PBMC were stained in the presence of rh 123 and DNR increased in a dose-dependent manner as indicated by the shift in fluorescence intensity relative to the controls. Taken together these results suggest that Pis (indinavir, ritonavir and saquinavir) are substrates for the P-gp mediated efflux pump probably because of their lipophilic nature. This finding may have important clinical implications in the long-term treatment of HIV-infected subjects.

PO-0970 Multidrug resistance in CD45- side-scatter gated samples from patients with acute myeloblastic leukaemia

Das-Gupta EP, Paillis M, Russell NH
Nottingham City Hospital and University of Nottingham, UK

We have previously shown low daunorubicin accumulation in cryopreserved samples from AML patients expressing p-glycoprotein, multidrug resistance associated protein or lung resistance protein. Here we address the problem of flow cytometric gating for MDR measurement on fresh AML samples with variable blast counts. The leucocyte common antigen CD45 is expressed at low levels on immature WBCs including leukaemic blasts. We have evaluated the use of CD45 antibody combined with side scatter to gate on leukaemic blasts in a daunorubicin uptake assay on 22 samples consecutively received in our laboratory. Two regions were established; R2 which excludes mature lymphocytes and erythroid cells and R1, which additionally excludes mature myeloid cells. 15/22 (88%) cases (Figure A) the percentage of cells in R1 and R2 differed by less than 20%. Of the remaining 7/22 cases (32%), two were of FAB type M5, in which the majority of leukaemic blasts appeared in R2 (Figure B) and one case was biphenotypic, with blasts spanning the R1/R2 interface. In the remaining four cases, although cells were distributed throughout both R1 and R2, the percentage of 4L cells in R1 agreed with the manual blast count. Mean daunorubicin accumulation was 51% higher in the R2 gate and 22% higher in the total blast population as determined by FSC/SSC characteristics than in the R1 gate, indicating that contaminating mononuclear cells elevate the daunorubicin uptake. Flow cytometric gating on leukaemic blasts is essential for ascertaining incidence of MDR. CD45/SSC gating enables blast determination in the majority of cases, but the M5 phenotype may be an exception to the general rule.

PO-0971 Multidrug resistance in acute lymphocytic leukaemias

Chair and Division of Haematology, Department of Medical and Morphological Research, University Hospital of Udine, Italy

The P-Glycoprotein (Pgp), the lung resistance-related protein (LRP) and the multidrug resistance associated protein (MRP) expressions and the blast cell’s intracellular daunorubicin accumulation were evaluated by flow cytometry in 56 acute lymphoblastic leukaemias (ALL): 36 were at onset and 20 at relapse. By using the monoclonal antibodies MRC-16 (anti Pgp), the LRP-56 (anti LRP) and the MRPm6 (anti MRP), 16/36 (46%) ALL at onset and 14/20 (70%) ALL at relapse were identified as Pgp overexpressing: 6/36 (16%) ALL at onset and 6/20 (30%) at relapse were classified as LRP overexpressing and 2/36 (6%) at onset and 2/20 (10%) were MRP overexpressing. In ALL at onset the more frequent MDR cluster were the Pgp+/LRP-/MRP- (15/36 cases, 45%) and the Pgp+/LRP+/MRP- (11/36 cases, 31 %) followed by the Pgp+/LRP+/MRP+ (5/36 cases, 14%), with an intracellular daunorubicin accumulation, expressed as normalised mean fluorescence index (NMFI) respectively of 349±183, 191±56 and 199±39. Different in ALL at relapse the most frequently observed phenotype was Pgp+/LRP-/MRP- (9/20 cases, 45%), followed by Pgp+/LRP+/MRP- (4/20 cases, 20%) and Pgp+/LRP+/MRP+ (3/20 cases, 15%), with a Daunorubicin NMFI of respectively 213±91, 314±150 and 225±101. Only the Pgp showed a significant negative correlation with the intracellular daunorubicin accumulation both at onset (-0.61), and at relapse (-0.57). These data suggested that as PGP, the LRP is frequently overexpressed. In ALL at onset and at relapse, in contrast the MRP overexpression seems to be a more rare event.

PO-0972 Multidrug resistance phenotype is not Pgp dependent in mantle cell lymphomas

Bernard M, Grenier B, Lamy T, Dauplais C, Le Prisl PY, Faucet R
Laboratoire d’Service d’Hématologie, CHRU Rennes, France

Mantle cell lymphomas (MCL) have a particularly aggressive behaviour and are resistant to different therapeutic regimens. The utility of anthracyclines is still under discussion. Methods. In order to investigate the mechanism involved in chemoresistance, we studied the expression of Pgp and LRP in 26 cases of MCL. These cases all had typical, cytological, and histological characteristics. Immunophenotyping was defined by coexpression of CD19, CD5, strong surface immunoglobulin and lack of CD23 and CD10, (flow cytometry). Overexpression of cyclin D, was assessed by competitive RT-PCR in all cases. Clinical characteristics were: median age 60; male pre-domiance (80%); stage IV (Ann Arbor); 80%; leukaemic phase (65%). Patients did not undergo the same therapeutic procedure and the median of chemotherapeutic regimens was 2.6. Pgp expression was measured by RT-PCR in all cases. Clinical characteristics were: median age 60; male pre-domiance (80%); stage IV (Ann Arbor); 80%; leukaemic phase (65%). Patients did not undergo the same therapeutic procedure and the median of chemotherapeutic regimens was 2.6. Pgp expression was measured by RT-PCR in all cases.

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using a flow cytometric functional test. Briefly, cells were incubated with Rhodamine (Rh) 123 and dye efflux at 37°C was observed with or without Pgp inhibitors (i.e. verapamil). LRP was detected using immunocytochemistry with LRP56 monoclonal antibody (Monosan) and LSAB kit (Dako). Positivity was determined by a cytoplasmic granular pattern in more than 10% of the lymphomatous cells. Results. Three out of 23 cases were positive for Rhodamine 123 assay. Tumoural specificity of the efflux was determined by dual staining with phycocyanin labelled CD19 monoclonal antibody. LRP expression was observed in 11 out of 26 patients: LRP+ patients have a median survival of 20 months whereas the median is not reached in LRP- patients (NS). Conclusions. Lymphomatous Mantle Cell resistance to chemotherapy is not associated with Pgp expression. LRP is potentially involved and further study is underway.

**PO-0973 Induction of apoptosis in Pgp expressing T-lymphoblastic leukaemia cell lines: differentiation and drug combinations against antiestrogens**

Arias A,* Divilansky A,* Hatkelzon L, Nathan I*
*Clinical Biochemistry Department, Haematology Unit, Soroka Medical Center, Ben-Gurion University of the Negev, Beer Sheva, Israel

The effect of non steroidal antiestrogens on apoptosis induction of lymphoblastic leukaemia drug resistant cell lines was studied. Cloniphene, which has a longer dialkyl amino group than tamoxifen and has a higher affinity for estrogen binding sites, was used. In correlation with cloniphene non triphenylethylen antiestrogen, nafoxidine, which possesses a pyrolidino group, was also investigated. The results indicated the following: CCRF-CEM cells were more sensitive to induction of apoptosis by induction of antiestrogens than CCRF-CEM cells and nafoxidine was more potent in influencing both cell lines. Estradiol at 10 nM concentration did not abrogate the activity of antiestrogens on both cell lines. The effect of the addition of verapamil to cloniphene at concentration of 12.5 nM, which enhances the resistance of leukemia cells to cytotoxic drugs, was studied. The results indicate that the combination of both drug resistance reversing agents (verapamil and antiestrogens) with selective agents which are more active in induction of apoptosis in CCRF-CEM cells. In CCRF-CEM cells, addition of vincristine and nafoxidine were sufficient for inducing marked cell death by apoptosis. Genistein, a tyrosine kinase inhibitor, under conditions which did not inhibit the Pgp pump, significantly enhanced apoptosis induced by antiestrogens and nafoxidine in CCRF-CEM cells. The effect of these drugs was also studied in the parental cell line CCRF-CEM. The results imply that the use of antiestrogens in combination with cytotoxic drugs and other apoptosis modulators may be beneficial in overcoming drug resistance due to their multifactorial capabilities to reverse drug resistance and promote apoptosis.

**PO-0974 Aclarubicin induces in vivo the formation of cleavable complexes between DNA and topoisomerase IIα and IIβ**

Biersack H, Hegner B, Eder M, Heil G, Ganster A
Dept. of Haematology and Oncology, Medizinische Hochschule Hannover, Germany

DNA topoisomerase IIα is the primary cellular target for numerous antineoplastic agents which are widely used in clinical practice. The majority of substances acts via the formation of "cleavable complexes" between DNA and topoisomerase IIα. In human cells catabylically active - as well as heterodimeric topoisomerase IIα enzymes exist, consisting of 170 KDa α and 180 KDa β-subunits, which are termed topoisomerase IIα, IIβ and IIαβ. Although the distinct in vitro and in vivo function of the homodimeric and heterodimeric isoforms remain uncertain, topoisomerase IIα is combined as IIαβ heterodimer and IIβ homodimer. Most of the available data, we found, using our assay to detect in vivo cleavage reactions, that aclarubicin is in fact an in vivo cleavable complex forming agent as was shown by direct exposure of K562 cells to fludarabine for 48 hours followed by doxorubicin. The activity of topoisomerase IIα and IIβ is cleaved to a higher extent by conventional cleavage reactions, that aclarubicin is in fact an in vivo cleavable complex forming agent as etoposide or daunorubicin. The antiestrogenic activity of aclarubicin increases not only the formation of cleavable complexes between DNA and topoisomerase IIβ but also enlarges the cleavage formation with topoisomerase IIβ. This suggests that cells, which escape the action of traditional drugs via an overexpression of topoisomerase IIβ and a reduced expression of topoisomerase IIα might still be sensitive to the aclarubicin effect. To answer this question, the expression of topoisomerase IIα isoforms needs to be determined during chemotherapy with cleavable complex forming drugs.

**PO-0975 The activity of P-glycoprotein is characteristic for precursor forms of myeloma plasmocytes**

Brzojek J,* Ciepluch H,* Heilmann A,* Witkowski J*M
*Department of Physio-pathology, Haematology Clinics, Medical University of Gdansk, Poland

The activity of P-glycoprotein affecting the outcome of chemotherapy and involving the activity of P-glycoprotein (Pgp) - an ATP-dependent membrane transporter, capable to extrude substrates from the cytoplasm is a relatively common feature of human myeloma cells. It is not clear whether the development of the Pgp activity is spontaneous or induced by chemotherapy and which is also cells of the plasmacyte forming lineage are the major reposito-ry of this activity. To answer these questions, we have analysed bone marrow (BM) cells from 42 myeloma patients of both sexes, aged 38 to 84 years (average age 63 ± 10.2 years), their in the stage and duration of the disease since diagnosis, in the types and quantities of the monoclonal proteins present in the BM cells. Pgp activity was assessed by using a flow cytometric functional test. Briefly, cells were incubated with phycoerythrin labelled CD19 monoclonal antibody. LRP expression was observed in 11 out of 26 patients: LRP+ patients have a median survival of 20 months whereas the median is not reached in LRP- patients (NS). Conclusions. Lymphomatous Mantle Cell resistance to chemotherapy is not associated with Pgp expression. Pgp is potentially involved and further study is underway.
PO-0977 Functional screening for multidrug resistance in human haematopoietic malignancies

Pérez J, García-López J
Institut de Recerca Oncologica (IRO), Departament de Criobiologia I Teràpia Cellular, L'Hospitalet de Llobregat, Barcelona, Spain

The MDR1 gene product, P-glycoprotein (Pgp) is a Mr 170,000-180,000 energy-dependent transmembrane pump which extrudes a diverse group of unrelated compounds, such as the Vinca alkaloids, anthracyclines, epipodophyllotoxins, taxol, and certain protein synthesis inhibitors resulting in a decreased, and less toxic, intracellular concentration of anticancer agents, representing a major cause for cancer treatment failure. Anthracyclines and the cationic dye rhodamine 123 (Rh123) are P-gp substrates actively effluxed by multidrug resistant cells, allowing functional and phenotypic characterisation of neoplastic cells by Simultaneous staining with antibodies. Analysis of variation in cellular fluorescence by measuring the rates of Rh123 influx and efflux, together with the effect of MDR reversing agents, allows the investigation of drug resistant phenotypes in cancer samples. We have used flow cytometry to investigate in vitro modulation of Pgp-dependent Rh123 fluorescence in human leukaemic cell lines, by measuring the 50% average rate of Rh123 efflux, taking into consideration that variables such as Rh123 cytotoxicity, culture conditions, cell membranes integrity, as well as the effect of specific P-gp modulators, can impair the resolution of the Rh123 efflux measurements. This study also indicates that analysis of Rh123 efflux modulation can be used to adjust the in vitro optimal doses of MDR inhibitors, suggesting that more than one modulator is needed to use P-gp function. Since Rh123 efflux experiments are increasingly being used for the detection of MDR malignant cells, all the above mentioned subjects are relevant for both Rh123 functional efflux and retention assessment, specially for studies on primary leukaemia cells, for the detection of low-P-gp levels of drug resistance, and for the separation of normal from malignant cells in cancer samples on the basis of Rh123 efflux. In summary, the functional detection of P-gp molecules on malignant cells may be not conclusive for the determination of drug resistant phenotypes, thus limiting the usefulness of Rh123-based efflux experiments.

PO-0978 Multidrug resistance-associated P-glycoprotein phenotype and treatment response in B-CLL

†Institute of Immunology, ‡Rudjer Boškovic Institute, †Hospital Merkur, ‡Hospital Dubrava, Zagreb, Croatia

Objective. The relevance of P-glycoprotein (Pgp), a marker of multidrug resistance (MDR), in B-CLL is controversial. The aim of our study was to investigate whether the MDR status at diagnosis correlates with the clinical data (modified Rai, total tumour mass (TTM), total lymphocyte count (TLC)) and therapy outcome. Design and Methods. Study population consisted of 42 patients (25 males, 17 females with a mean age 62±12 yrs). Expression of Pgp was analysed on flow cytometer using two mAbs against specific P-gp molecules, allowing functional and pharmacological assessment, specially for studies on primary leukaemia cells, for the detection of low-P-gp levels of drug resistance, and for the separation of normal from malignant cells in cancer samples on the basis of Rh123 efflux. In summary, the functional detection of P-gp molecules on malignant cells may be not conclusive for the determination of drug resistant phenotypes, thus limiting the usefulness of Rh123-based efflux experiments.

PO-0979 The results of all-trans retinoic acid treatment on bcl2 expression and rhodamine retention in de novo AML

Ankara University Medical School, Ibni Sina Hospital, Department of Haematology, Ankara, Turkey

Retinoids can induce differentiation in myeloblasts and have been used in combination with anthracyclines in AML therapy. ATRA has also been shown to induce downregulation of bcl-2 which may contribute to increased susceptibility to chemotherapy. The effect of ATRA on cellular multidrug resistance has not been studied. In our study we have evaluated the effect of in vivo ATRA use on rhodamine 123 (Rh123) uptake/retention, bcl-2 and surface myeloid antigen expression in patients who received ATRA (45 mg/m2 for 3 days) before taking any chemotherapeutic drugs, and were compared with 8 patients who did not receive ATRA. Bone marrows or fasting peripheral blood samples were taken at diagnosis (day 0) and on day 4 to analyze flow cytometric Rh123 uptake and retention (0, 15, 30, 60 min) and surface CD13, CD33, CD34, HLA-DR and cytoplasmic myeloperoxidase (NTO) and bcl-2 expression. Rh123 uptake/efflux retention were analyzed as percentage, mean fluorescence intensity (MFI) and Kolmogrov-Smirnov (D) value. Other cytoplasmic or surface antigen expressions were evaluated as percentage and intensity. Patients who received ATRA achieved an increase in Rh123 uptake (10/19 patients) or no change (1/19 patients) or a decrease (8/19 patients). When efflux was measured, there was an increase in Rh123 retention (12/19 patients), or a decrease (7/19 patients). Overall Rh123 retention increased (8 patients) or were not effected (10 patients). In the control group, despite an increase in uptake (3/9 patients) and in efflux (4/9 patients), no overall change in retention was observed. ATRA administration resulted with a decrease in bcl-2 expression (10/25 patients), in a increase (8/25 patients), and without change (7/25 patients). In the control group, bcl2 expression increased in 4 patients while decreasing in 3 patients. Surface CD13, CD14, CD33 and cytoplasmic MPO expression did not differ with the use of ATRA, however there was a nonsignificant decrease in CD33 and increase in HLA-DR as a conclusion in vivo use of ATRA increases dye uptake and retention, and decreases bcl-2 expression in a selected group of AML patients.

PO-0980 The effects of hydroxyurea in sickle cell disease: a European registry of benefits and toxicity

European Concerted Action [No. BMH-L-4-96-1659 (912-SSMA)]

A prospective multi-centre register of patients with sickle cell disease, treated with hydroxyurea, is being established under an EU Concerted Action with the object of monitoring clinical effects and toxicity. 163 patients, from 5 countries, have been registered in the pilot phase, predominantly Ss (55 %) and Sb0 (42) genotypes. 34% are of immigrant origin and the majority are Caucasian. The most common reason for initiating therapy was painful crisis (86%). Skin/nail changes were reported in 20%, nausea/vomiting in 15%, headaches in 7%, and hair thinning in 4%. Three pregnancies were reported (1 normal baby, 1 stillbirth, 1 termination). 1 patient was reported as developing a non-haematological malignancy. Severe pancreatitis only occurred once. This pilot phase has demonstrated the value of the approach and register. A World Wide Web site is now under development to open participation to any doctor treating sickle cell patients with hydroxyurea.

PO-0981 MCV based assessment of response to hydroxyurea in patients with sickle cell disease

Al Momen AK
College of Medicine and KKH, King Saud University, Riyadh, Saudi Arabia

The rise in HbF from baseline has been taken as the main laboratory parameter for objective assessment of response to Hydroxyurea (HU). However, there are several setbacks for HbF that make it inconvenient. These include: (1) [HbF] level does not always correlate with the clinical picture. (2) level varies from time to time and from one method to another, (3) methods of HbF measurement are expensive and time consuming, (4) HbF test is not available in many small laboratories and clinics. That is why other simple laboratory parameters, such as MCV, could be much easier, cheaper and more convenient. Objective. To correlates between MCV changes and clinical improvement in sickle cell disease patients who are taking HU for more than one year. Methods. We reviewed the records of 142 adult patients with sickle cell disease who have been on HU for more than three years. Of these 68 were evaluable (37 males and 31 females, aged 21.8 ±4.5 years). Each of these patients had more than four painful episodes/year prior to HU therapy. We divided patient into two groups according to response. Group A = responders (painful episodes 0-1/year). We also calculated the MCV index which is:
Patients who had good response had an MCV index >3. Conclusions. (1) Changes in MCV may be used as a simple and easy laboratory parameter for monitoring response to HU. (2) An MCV index of >3 indicates a good response to HU.

PO-0982 Hereditary pyropoikilocytosis, HbH disease and sickle cell trait presenting as severe sickle cell disease

Al Momenn AK, Al Bahraini AT
College of Medicine and King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia

The combination of sickle cell trait, HbH disease and pyropoikilocytosis is very rare. A 23 years old lady referred to the haematology ward, initially from another city with history of severe sickle cell anaemia with frequent severe painful episodes and hospital admissions (almost every 1-2 weeks). There is no paternal or maternal history of anaemia or sickle cell disease. On physical examination, she looked pale and jaundiced, with no hepatosplenomegaly or bony deformities. Her body weight was 56 kg and her vitals signs were normal. Laboratory investigations were as follows: WBC 4.57 x 10^9/L, RBC 4.99 x 10^12/L, Hb 135 g/L, MCV 35 fl, MCH 11.7 pg, MCHC 36.2 g/L, platelets 635 x 10^9/L, reticulocytes 2%, ESR 3 mm/h. Blood film showed severe pyropoikilocytosis. Total Bilirubin was 28 μmol/L, LDH 387 u/L, HbA 74.1%, HbF 0.7%, HbA2 2%, Hbs 17% and HbH 6.2%, G6PD, iron studies, vitamin B12 and folate were normal. She has a 24 year old brother with similar but less frequent episodes. His laboratory investigations were as follows: WBC 4.97 x 10^9/L, RBC 4.99 x 10^12/L, Hb 135 g/L, MCV 35 fl, MCH 11.7 pg, MCHC 36.2 g/L. No Hbs. Conclusions. From this family we may conclude that the combination of HbH disease, pyropoikilocytosis and sickle cell trait presented as symptomatic sickle cell disease despite very low Hbs level. Her brother who had similar episodes has only HbH disease with pyropoikilocytosis without any Hbs. Conclusions. From this family we may conclude that the combination of HbH disease and pyropoikilocytosis may present with painful episodes similar to severe sickle cell disease.

PO-0983 Optical properties of red cells

Monici M, Agati G, Fusi F, Bartolozzi B, Banchelli F, Bernabei PA*
*IECN-CNRI, CED, Sz. INF.M, Florence, Italy; **DFC, Sz. INF.M, Univ. of Florence, Italy; ***U.O. Ematologia, Az. Osp. Careggi, Univ. of Florence, Italy

Information about cells can be extracted analysing their optical properties. This fact opens the possibility to have new analytical methods both for diagnostics and basic research. In this study, light absorption and natural fluorescence (NF) emission of red cells were investigated and the deriving applications were considered. Red cells from healthy consenting donors were collected, washed and analysed by an epifluorescence microscope coupled with a cooled digital CCD camera, to obtain NF imaging, and with a diode-array multichannel spectral analyser to record absorption and NF emission spectra. The results show that red cell absorption spectra are very similar to the spectrum of haemoglobin, with peaks at about 416, 545 and 580 nm. Thus, light absorption properties of red cells are mainly due to the presence of haemoglobin. NF emission was analysed for excitation wavelengths of 365 nm. Emission was observed at 365 nm excitation, with a 10 times increase in NF intensity. In the same time the absorption spectrum decreased until it practically disappeared. This phenomenon is not easy to explain before of the superimposition of several photochemical processes. Anyway the light absorption decrease indicates a photodegradation of haemoglobin. The great sensitivity of the hemoprotein to light exposition should be taken in account in trans-fusional medicine. NF emission spectra of red cells show a principal peak at 460 nm (blue component), this means that the main component of NF is due to NAD(P)H, similarly to what observed in many other cell populations. However, in red cells, green and red components are more important than in other cells. The compounds responsible for these emissions, together with flavins, are probably involved in haemoglobin synthesis. Analytical techniques based on light absorption and NF properties of red cells could be useful in congenital and acquired haemoglobinopathy diagnostics.

PO-0984 Hereditary haemochromatosis and β-thalassemia association

Chair of Haematology II, University of Bari, Italy

Hereditary haemochromatosis (HH) is an autosomal recessive HLA-linked disorder, characterised by excessive absorption of dietary iron. HH gene (HFE) encodes a major histocompatibility complex class 1-like molecule that requires interaction with β2-microglobulin for normal presentation on the cell surface. Two mutations have been identified in HFE: nt845 G→A (Cys282 Tyr) and nt187 C→G (His63 Asp). For evaluating extragenetic factors influences in phenotypic expression of thalassemia intermedia and thalassemia major, HFE of 40 thalassemia subjects have been studied; 25 subjects of our study were affected with thalassemia major and 15 with thalassemia intermedia. All subjects came from Apulia (a Southern Italy region) and were homozygote or genetic compounds for β globin gene mutations. In thalassemia intermedia major group 3 subjects (12% of population) were heterozygous for nt 187 C→G (H63D) and 3 subjects (12% of population) heterozygous for nt 845 A→G (C282T) mutation. In thalassemia intermedia group 4 subjects (26% of population) were heterozygous for nt 187 C→G (H63 D) mutation; 1 subject was homozygous for nt 187 C→G (H63D). One thalassemic major subject has been found as compound heteryozygote for both mutations. No different clinical behavior have been detected in thalassemia major and thalassemia intermedia subjects with or without HH mutation.

PO-0985 β globin gene mutations in Apulia, Italy

Campanale D, Vitucci A, Pietrapertosa A, Palma A, Tannoia N
Chair of Haematology II, University of Bari, Italy

β-thalassemia is the most frequent hereditary haemolytic anaemia in Apulia (a southern region of Italy). In fact Apulia is third for frequency in Italy after Sardinia and Sicily. Prevalence of β-thalassemia in Apulia is approximately 8%. Molecular analysis of 1704 chromosomes has been carried out in β-thalassaemia carriers in couples at risk for β-thalassaemia. Molecular analysis of globin genes was carried out by A.R.M.S, Reverse Dot Blot and D.G.G.E. We report here our mutations:

<table>
<thead>
<tr>
<th>β* cod.</th>
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<tr>
<td>39</td>
<td>34.0</td>
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<td>61-110</td>
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Analysis of data evidences a large genetic polymorphism although 4 mutations are the most frequent (β* cod. 39, β* 61-110, β* 91-1, β* 91-6) and cover 85% of all the mutations. β-thalassaemia mutation still remains unidentified in 13 out of our β-thalassaemia carriers.

PO-0986 Molecular defects in β-thalassemia subjects of Eastern Sicily preliminary data

Rigoli L, Mec A, Larosa MA, Crisò A, Micieli MR, Ricca M, Barberi I
Department of Pediatrics, University of Messina, Italy

Objective. Molecular analysis was undertaken in 42 Italian patients (East-ern Sicily) with β-thalassaemia major and 8 patients with thalassaemia intermedia to evaluate the correlation between clinical picture and genotype. Design and Methods. Distinction between the two groups was based on MCV-ND and HbA2.
PO-0986 Histidine-rich-glycoprotein in sickle cell disease and homozygous \(\beta\)-thalassaemia: a novel marker of impaired fibrinolysis with prothrombotic effect

Institute of Haematology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

So far, abnormalities in plasma coagulation system and platelet functions have been considered to contribute to vascular occlusions in the tissues and organs of patients with sickle cell disease (SCD) and homozygous \(\beta\)-thalassaemia (\(\beta\)-Th). Few studies have been addressed on the contrary, to the fibrinolytic status/response of these patients. With regard to the fibrinolytic system recent evidence suggests that plasma histidine-rich 3.8 S-glycoprotein (HRGP), a new antithrombotic agent, interacts both with the high-affinity lysine-binding site of plasminogen or with the heparin-antithrombin III complex thus exerting a prothrombotic effect. Owing to its potential role in the regulation of fibrinolysis, we studied HRGP behaviour together with the venous occlusion test (VOT) and some plasma fibrinolytic parameters in 14 Sicilian SCD patient (6 females and 8 males in steady state, aged 12-48 years) and 9 homozygous \(\beta\)-Th subjects. Thirteen healthy volunteers were the controls. HRGP was evaluated by immuno-electrophoresis according to Laurell (Bioclinica Mannheim Italia, Milan). Plasminogen (PLG) was assayed by chromogenic method (Biopool AB, Menarini, Florence), tissue-type plasminogen activators (t-PA and u-PA), plasminogen activator inhibitors (PAI-1 and PAI-2 antigens) were determined by ELISA (Biopool AB). T-PA/PAI-1 ratio was calculated as index of fibrinolytic potential. All plasma determinations were performed before and after VOT. Our results are reported in the table:

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<tr>
<th>Patient's Data</th>
<th>PLG Before After VOT</th>
<th>t-PA u-PA</th>
<th>PAI-1 PAI-2</th>
<th>t-PA/PAI-1 X 10 ratio</th>
<th>u-PA PAI-1</th>
<th>u-PA PAI-2</th>
<th>u-PA PAI-1 X 10 ratio</th>
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<td>PAI-1 PAI-2</td>
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PO-0988 A new group of genetic markers forthalassaemia mutations inside the 5' regulatory region of the \(\alpha\)-globin gene

Papadakis MN, Patrinos GP, Papapanagiotou E, Kollia P, Loukopoulos D, Loutradi-Anagnostou A
Laiko General Hospital, Center for Thalassemia, Unit of Prenatal Diagnosis, University of Athens, School of Medicine, First Department of Internal Medicine, Athens, Greece

The possible role of sequence alterations within the promoter and 5' regulatory region of the \(\alpha\)-globin gene and their correlation with the variability of \(\gamma\)-chain production in thalassemic syndromes is not well defined. In the present study, 30-64-271 (\(\beta\)-thalassaemia and sickle cell anaemia chromosomes were screened for sequence variations inside the 5' regulatory region of the \(\alpha\)-globin gene by a combined PCR-DGGE procedure. Two base substitutions in the \(\alpha\)-globin gene (\(\alpha\)A-3G and \(\alpha\)A-499 T-A) recently described in our laboratory and a 48bp deletion (\(\alpha\)A-225 to -222 AGCA) create four different \(\alpha\)-haplotypes. Each of these \(\alpha\)-haplotypes was linked in cis with 12-18 and 19 \(\beta\)-thalassaemia mutations (with variable phenotypic expression) prevalent in the Hellenic population. The screening procedure applied for this study also revealed two novel base substitutions, 5' to the \(\alpha\)-globin gene (\(\alpha\)A-521 C-A and \(\alpha\)A-500 C-T). Transient expression assay showed that all the above mentioned substitutions act as polymorphisms on the \(\alpha\)-globin gene expression. WF, conclude that \(\alpha\)-Haplotypes may represent genetic markers for the spectrum of thalassaemia mutations among Mediterranean populations. In addition \(\alpha\)-Haplotypes might constitute an important genetic repository upon which mutations leading to thalassaemia and haemoglobinopathies occurred.

PO-0989 Hydroxyurea ameliorates paraparesis due to extramedullary haemopoiesis and dramatically elevates Hb in \(\beta\)-thalassaemia

Goldfarb A, Rund B, Bachmiller EA
Hadassah University Hospital, Ein Kerem, Jerusalem, Israel

Background: Parascal extramedullary haemopoiesis (EMH) causing paraparesis is a rare complication of thalassemia. Treatment of EMH has been based on hyperventilation, surgical decompression, and radiotherapy. Rarely, hydroxyurea (HU) has been successfully used in this setting (K Konstantopoulos, 1992; BR Saxon, 1998) either alone or in combination with hyperventilation, aspirin, and low-dose steroids. We report on a 41 year old Kurdish male patient with paraparesis due to EMH. He is homozygous for -28 (A-C) mutation on Mediterranean haemoglobinopathy II, which has \(\beta\)-thalassaemia intermedia. Follow-up splenectomy at age 10, his baseline Hb was 9.5-16 g/dl, with a foetal Hb of 26% (total Hb: 2.45 g). In 1993 he first developed paraparesis due to EMH (T2-8). MRI demonstrated epidural masses with spinal cord compression and he was irradiated (240 CgY) with improvement. In 1997, paraparesis recurred. Repeated MRI showed recurrence of masses. He was retreated with radiotherapy (100 CgY) to T3-T10 but repeat MRI showed little change in the mass. Several months later, in 1997, weakness recurred with spastic paraparesis and inability to ambulate unassisted. Due to imminent irreversible neurological defects, he was treated urgently with a short course of dexamethasone and radiotherapy (100 CgY to T2-T10). He was judged inoperable, and therefore, HU treatment was initiated (20 mg/kg/day). Clinical improvement was noted and three months after radiation, during hydroxyurea therapy, repeated MRI showed significant regression of EMH, which remains stable during an additional 8-month period. More remarkable were the haematological effects of HU. The Hb rose to 11.0-12.0 g/dl, within 2 months. There was also a rapid and sustained increase in HbF (to 41% within one month after HU was initiated, to a maximum of 68-98%). HBF was measured using alkaline denaturation every 4 weeks. His total HBF rose to a maximum of nearly 10 grams, a fourfold rise over baseline. Concurrently, nucleated cell count fell from 90-100,000 to 24,000 x 10^6/mL. Platelet count is stable at ~400,000. Serum bilirubin decreased by 50%. He remains on HU for one year without adverse effects. Conclusions: We conclude that HU is an effective treatment for EMH which is recurrent after radiation therapy. In addition, HU may markedly elevate total Hb and Hbf levels in this setting.

PO-0990 Pseudoanxanthoma elasticum in thalassaemic patients: a long-term follow up

Cianciulli P, Sorrentino F, Maffei L, Armadori S
Haematology, St. Eugenio Hospital, University Tor Vergata, Rome, Italy

Pseudoanxanthoma elasticum (PXE) is a relatively rare heritable clinical condition affecting elastic fiber rich tissues. It is characterised by redundant skin folds in flexural areas associated with eye and cardiovascular lesions, mainly due to fragmentation and mineralisation of elastic fibers. Hemolyt-
ic congenital anemias are often associated with PHE-like clinical manifestations. The aim of this study is to evaluate the incidence of this condition in a population of thalassemics to examine the complications during a follow up of ten years. Eighty consecutive multi-transfused thalassemic patients (median age 25 years, range 11-59) with homozygous or double heterozygous 3-thalassemia were examined. None of them had a history of gastrointestinal bleeding, recurrent nose bleeds, hypertension or angina. Punch biopsies were taken from the lateral eral side of the neck from 14 patients with yellow xanthoma-like papular and reticular lesions, from 16 sex and age matched thalassemic patients without similar lesions and from 13 clinically unaffected relatives of PHE patients for conventional and electron microscopy. The diagnosis IVS was confirmed in all patients with clinical evidence of PHE-like lesions. Typical alterations were not observed in the dermis of healthy relatives or of 3-thalassemic patients. Size of the skin lesions ranged from 4 mm to 36 cm^2 and 6 mm to 45 cm^2 in pre- and post- follow up, respectively. In 71% the lesions were associated to a patient myocardial infarction. One patient required an emergency subtot al gastrectomy for recurrent, massive gastrointestinal bleeding. Laser therapy was the therapeutic choice for one patient with progressive veinous decreases study indicates a high incidence (17%) of PHE-like lesions in Italian thalassemic patients. Appearance of lesions were correlated with age (p<0.005) and were most frequent in women (71%). Skin lesions appear to be not related to duration of transfusional treatment or iron chelation splenectomy, severity of anaemia, iron overload. In consideration of the disability and sometimes fatal outcome associated with these connective tissue lesions, the management of PHE-like thalassemic patients should include a careful monitoring of potential target organs (skin, eye, gastrointestinal tract, heart).

**PO-0991** Growth in homozygous 3-thalassemia

Ferrara M, Ponte G, Matalase SR, Napolit G, Gangone F, Chiarisello S, Borrelli B, Esposto L. 1 Pediatric Clinic, Department of Pediatrics, II University, Naples, Italy

Hypertensive: regimen and iron chelation in the treatment of thalassemia have improved the survival and the quality of life in thalassemic subjects. Nevertheless side effects related to this management can involve growth. In 44 thalassemic patients in hypertensive and iron dictation therapy (Hb 9.5 ± 1.5 g/dL, DFX s.c. 50 mg/kg/die) with ferritin levels of 2528±1443.42 ng/ml, mean age 13.34 ± 8.3 years (16 M and 28 F), 30 prepuberals (18 F, 12 M, mean age 9.25 ± 8 years) and 14 puberals (10 F, 4 M, mean age 12.42 ± 5.3) with hypochromic anemia treated with subcutaneous DFX therapy started at 13.5±1 years and F 16±1 M, a study of growth with evaluation of height (H), sitting height (SH) and nutritional status, by determination of BMI (Body Mass Index), circumference of arm (MA), waist and hip (WHR), was carried out. The height of SH, DS, respectively 1.14±1.2 and 1.59±1.02 p< 0.05), while the BMI values of HDS, SH DS, were in all thalassaemic subjects significatively low-

**PO-0992** Homozygosis for Constant Spring: the first case described in the West


The hemoglobin Constant Spring is the principal cause of non-deletion 3-thalassemia in South East Asia and Southern China. This is caused by a mutation in the terminal codon of the 3-globin gene which extends the transcription producing an unstable 3-globin RNA that encodes a protein of 172 residues instead of the 141 residues in normal globin. In this work we show the clinical and laboratory data and the molecular identification of homozygosis for Constant Spring in an Argentinian man with parents from Sicilian origin. The molecular studies were carried out by Southern blot with the restriction enzymes Bam HI and Bgl II with the 3 and 3 probes to reject dele-

**PO-0993** 3-thalassemia associated to 3 genes triplication. clinical and molecular study.


Tario Puerta del Mar. Cádiz; #Hosp. Principe de Asturias. Alcalá de Henares; @Clinica Puerta de Hierro. Madrid, Spain

Both, triplication and loss of 3 genes are owing to recombination of frag-

ments that constitute 3 genes. In Spain, the frequency of 3 genes triplica-

tion is 0.005. Subjects with 3 genes triplication are clinical and haemato-

logically normal. Whereas, a heterozygous 3-thalassemia associated to 3 genes triplication can increase the diserythropoesis of the 3-thalassemia, which originates a severe 3-thalassemia picture. Twelve different unrelated families 18 patients) were evaluated because the clinical and haemato-

logical pictures were severer than a heterozygous 3-thalassemia. Seven-

teen subjects with heterozygote 3-thalassemia associated to 3 genes triplic-

ation (except 3) in a chromosome and one heterozygous 3-thalassemia in another chromosome 3 genes triplication in two chromosomes (except except). Sixteen of the patients showed a severe haematological data with a great RDW and in sev-

er of these sixteen, the level of HbF was superior than 5%. Seven patients showed splenomegaly, in two of them was giant with an arite indirect biliru-

bin. In three subjects with the same association and belonging to two differ-

ent families, the clinical data overdue to the 3-thalassemia minor which genotype was (Bgl II) (except). The molecular study to confirm the (except) was carried out by Southern Blot, with the restriction enzymes Bam HI, Bgl II with the 3 probe. All patients showed with restric-

tion enzyme Bam HI a fragment of 18Kb, in twelve of them this fragment was alone. With restriction enzyme Bgl II a fragment of 20Kb was studied by PCR (except). All patients showed with restric-
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**PO-0994** Polyorphism of the enzyme bgl II associated to 3 genes triplication. clinical and molecular study.


Tario Puerta del Mar. Cádiz; #Hosp. Principe de Asturias. Alcalá de Henares; @Clinica Puerta de Hierro. Madrid, Spain

The human 3-globin cluster includes two adult (3a and 3a) and one embryonic (2) gene set added by two pseudogenes (3a2 and 3a3) on the short arm of chromosome 16. Haplovariant zones and DNA polyorphism (SAC, 1 Eco R, Bgl II ...) are within cluster. We present the case of an Argentinian patient from Spanish origin with HbH (3a2a3) and 3a2-a3 assoc-

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Haemoglobinopathies and thalassaemia II

**PO-0995** Hb Johnstown ([109(G11])val→leu]. Second case described and first occurrence identified associated to β-thalassaemia in two Spanish families

Ropero P,* Gonzalez FA,* Sanchez J,* Arrizabalaga B,* Atuxta L,* Villegas A,* Servicios de Haematología y Hemoterapia, *Hospital Clinico San Carlos, Madrid; *Hospital de Cuenca, Bilbao; *Hospital de Galdakao, Vizcaya, Spain

Most abnormal hemoglobins with elevations of oxygen affinity have been divided into two groups, depending on whether or not they associate to a new polymorphism Bgl II restriction endonuclease site (-) in the β-globin gene complex. This polymorphism is absent in two members of the family studied (sister and son) and it has never been found in the Argentinean and Spanish population. Digestion of DNA with Bgl II (5′ probe) of the patient produces two (-specific bands, 26Kb and 13Kb. DNA (α probe) had a shortened Bam HI (10.3Kb) and Eco RI (19Kb) characteristic of haplotypes α-β+. With this end to characterize the β-specific fragment of 26Kb, we have studied the propensosse DNA, her sister (morphological and clinically normal) and her son (genotype αβ+) with several restriction enzymes and probes. The results have demonstrated that in the mother and her son the αβ determinant correspond to the -mutation (Bgl II 13.9 Kb). Bam HI 8, Eco RI 15, Hpa I 13, Hind II 8 and Acc I 15 (IZHR). The abnormal bands persist with restriction enzymes Eco RI and Hpa I (3′HVR probe) and abnormal band appears with Sac I (10 Kb). The size of the Pvu II 3′HVR abnormal allele is 4.1. The size of 26 Kb persists with Bgl I (IZHR) and normal fragments are achieved with Bgl II (3′HVR). The calculate of the fragment is: +5.0 to +10.5Kb=3.7 Kb=26.2 Kb. Whereas the sister and son of patients not have got the 26Kb band. This polymorphism Bgl II (-1) in αL, is associated in cis to deletion α-β-thalassaemia. In the Spanish population, the only polymorphism found with a frequency of 2% is a fragment of 5.2Kb (+5 to 1α gene).

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**PO-0996** Bone mineral density in β-thalassaemic subjects relationships with transfusive regimen and splenectomy

Ferrara M, Ponte G, Matrese SMR, Napoli G, Gangone F, Chiarilli S, Borrelli B, Esposito L, I Pediatric Clinic, Department of Pediatrics, II University, Naples, Italy

Various degrees of bone mineral density alterations, from osteopenia to osteoporosis, are frequently associated with thalassaemic syndromes. These complications can be related to many factors, as hypocalcaemic hypocalcidiipic hypogonadism, iron chelation, iron overload in osteoblasts, vit. D deficit, polymorphisms in the vit. D receptor gene, hypoparathyroidism and bone marrow hypoplasia. In 23 pts with th. major both sex (SM, ISF) aged between 7-35 yrs (mean age 17.6 yrs), 13 splenectomised, transfused at Hb levels >9.5 g/dl, treated with DFX s.c. (50 mg/kg/die) started at 2.5 (2.4 yrs with feritin levels 2528±1443 ng/mL, and in 6 pts aged between 6-21 yrs with th. intermedia, all splenectomised, with feritin levels 941 ng/mL (range 369-3828 ng/mL), BMD (femoral neck and lumbar spine L2-L4) by DEXA (Z-score of normal values), erythroblasts values and the role of the spleen on erythropoiesis have been evaluated. Subjects with th. major were subdivided in 3 groups: in 1th group of 6 pts aged between 7-10 yrs, not splenectomised, DS BMD of lumbar spine were between -0.5 and -1.8 (-1.25±0.536) and DS BMD of femoral neck between -0.3 and -1.3 (-0.73±0.403), without erythroblasts. In 2nd group of 11 pts aged between 10-20 yrs, all splenectomised except 4, DS BMD lumbar spine were not splenectomised between -0.5 and -1.4 (-0.975±0.442) while in splenectomised between -1.8 and -3.1 (-2.1±1±0.302) and DS BMD femoral neck between -0.3 and -1.3 (-0.75±0.435) and -2.3 and -3.8 (-2.6±0.62), respectively. Erythroblasts in non splenectomised were absent while in splenectomised pts were 7946±6853.9. In the last group with subjects over 20 years, all splenectomised, lumbar spine DS BMD were between -2.5 and -3.8 (-3.15±0.489) and femoral between -0.3 and -5.1 (-3.61±1.143) with mean erythroblasts values of 15.001±6.14/19.3. In subjects with th. intermedia aged between 8-21 ys with th. major both sex (SM, ISF) aged between 7-35 yrs, 13 splenectomised, DS BMD lumbar spine were between -0.5 and -1.5 (-1.0±0.7) and -0.5 and -1.1 (-0.8±0.42) respectively with mean erythroblasts values of 30.9±0.270. In the Spanish population, the only polymorphism found with a frequency of 2% is a fragment of 5.2Kb (+5 to α-β gene).

**PO-0997** Splenic function in Omani children with sickle cell disease. Correlation with severity index, hemoglobin phenotype, iron status, and α-thalassaemia trait

Al-Lamki Z, Wali Y, Hussain S, Bererhi H, Ghoghi K, Sultan Qaboos University Hospital, Muscat, Oman

The prevalence of functional asplenia in Omani children with sickle cell disease has not been defined before. The splenic function was studied in 72 Omani patients with SCD (50 homozygous for hemoglobin S (Hb S-S), 11 Hb S-β-thalassemia, 10 Hb S-β-thalassemia major, 5 patients with hemoglobin S-D WL disease and 1 case with Hb S-β-thalassemia major, aged 4.8-16 years, using 99m Tc-labeled tin colloid scintigraphy. They were divided into four groups depending on the result of their colloid uptake. Groups I included 20 patients (27.8%) with normally-visualised spleens (normal splenic function), Group II, 6 patients (8.3%) with slightly decreased uptake (mild hyposplenism), Group III, 41 patients (56.9%) with markedly decreased uptake (severe hyposplenism) and Group IV, 26 (36.1%) patients with none-visualised spleens (functional asplenia). Overall, around 70% of them had preserved splenic function. Except for HbS-β-thalassemia minor patients, the developmental pattern of hyposplenism was not different among the different Hb phenotypes. Factors associated with preservation of splenic function in these patients were DS BMD of larger spleen size (P<0.01), less clinical severity (P<0.05), lower MCV (P<0.01) lower MCH (P<0.01), higher HbF (P<0.001) and presence of α-thalassaemia trait (P<0.05).
PO-0998 Evaluation of a PCR-SSO test for DRB typing
Gomez Arteta E, Gimeno J, Bunuel C, Garcia Ercia JA, Osuna C, Ambro A, Giralt M
Haematology Department, Miquel Selvet Hospital, Zaragoza, Spain
Purpose. To evaluate the implementation of a technique based on PCR-SSO for HLA-DRB typing in a reference hospital. Design and Methods. From September 1996 to September 1997, 389 HLA-DRB typings were performed with a low resolution, molecular biology test, based on PCR-SSO (Roche®). Sample preparation, DNA extraction, PCR based amplification, and hybridization detection were performed according to manufacturer's specifications. The indications for the test: 90 cases (22 families) studied for tentative BMT from a related donor; 57 BM allogenic donors for inclusion in REDMO; 53 patients to include in a register for renal transplantation; 169 rheumatological patients and 20 studied for miscellaneous alterations. In 23 cases HLA-DR and DO typing was performed by a serological test of LCT complement dependent with 2 different plates. Results. In 4 cases (1%) no DRB typing were detected. In 29 patients (5.1%) no DRB1 allels could not be indentified, with some doubts between DR3(DR13/DR13/DR14 in only the cases. In all cases studied DR6 could be indentified. comparing DR3 or DR13 but no DR5 or DR7. In BM allogenic donors the most frequent allels were DR4 (23.6%) and DR7 (18.9%); in rheumatological patients they were DR7 (14.8%) and DR4 (14.2%). In all the cases serologically studied there were no discrepancies and in 1 molecular biology test obtained a better resolution than serology. Conclusions. The implementation of such a molecular biology test for HLA typing is easy and the results can be considered optimals including the economical aspects. The test is useful for a high number of samples assembled for DNA extraction, amplification and detection but not for the initial preparation. Incidentally it can be obtained a better resolution than in serological test, but the definition problems with some alleles are infrequent.

PO-0999 Changes in B cell antigen expression in the elderly
Ginaldi L, De Martinis M, D'Ostilio A, Marini L, Loreto MF, Profeta VF,* Quaglini D
Department of Internal Medicine and Public Health, University of L'Aquila, *Ser. T ASL Teramo, Italy
The involvement of the B cell compartment during senescence plays an important role in the development of the immune dysfunction (e.g. hypergammaglobulinemia, autoantibody production and impaired responses to immunizations). In order to identify specific antigen expression changes on B lymphocytes which may contribute to the immune deficiency in the elderly, we investigated, by triple staining flow cytometry, the level of expression of some constitutive surface markers (HLA-DR, CD19, CD20) and of a series of adhesion molecules (CD49b, CD49d, CD50, CD62L) on B lymphocytes from 23 healthy elderly individuals (82-100 years old, mean age 92) compared to 70 healthy young donors (20-35 years old, mean age 31). Aged donors fulfilled the admission criteria for gerontological studies proposed by the Senorir Protocol. Both percentage and absolute number of B lymphocytes (CD19+ and CD20+ cells) were significantly decreased in elderly compared to young donors. The absolute number of B cells expressing the adhesion molecules CD49b and CD49d was lower in elderly individuals, as well as the percentage value of B cells expressing the adhesion molecules CD50 and CD62L. The percentage and absolute number of B cells coexpressing the CD5 antigen was decreased in elderly subjects. The CD20 expression antigen was increased on B lymphocytes coexpressing the CD49b and CD49d in elderly donors compared to young ones. Also the CD5 expression on B cells from old donors was slightly increased compared to controls. No differences were detected in the expression of CD19, CD50, CD49b, CD49d, CD62L and HLA-DR molecules on B lymphocytes. Quantitative flow cytometry may be of value in the elderly both for clinical and biological studies. The study of antigen density changes on B cells in the elderly may allow a better understanding of the humoral immune defects observed in these subjects and provide insights into the functional defects of the B cell compartment characterizing immunosenescence.

PO-1000 Hotspots of somatic hypermutation in immunoglobulin K genes in follicular lymphoma and multiple myeloma
Kosmas C, Stamatopoulos K, Belessi C, Stavroyianni N, Yataganas X, Loukopoulos D
First Dept. of Medicine, Univ. of Athens, Greece
Antigen selection in B-cell ontogeny is evidenced by non-random distribution of somatic hypermutation (SHM) in immunoglobulin (lg) variable (V) genes, which, furthermore, provides useful information about the ontogenetic assignment of B-cell neoplastic disorders. However, irrespectively of subsequent selection, SHM is targeted to specific hotspots within V genes. In the present study, we analyze the nature of the SHM targeting mechanisms in the clonogenic Igk light chain V genes (Vk) in follicular lymphoma (FL) and multiple myeloma (MM), tumors thought to correspond, respectively, to antigen-selected intra-germinal center (GC) and post-GC stages of B-cell ontogeny. Ten FL and 11 MM Vk sequences previously published by our group (Kosmas et al, Brit J Haematol 1996; Stamatopoulos et al, Brit J Haematol 1997) were analysed for the presence of specific SEM targeting motifs, i.e. the tetranucleotide RGYW and its complementary, WRGY (where R=purine, Y=pyrimidine and W=A or T). In general, SHM of Vk genes was more pronounced in MM than in FL Vk sequences. Comparisons were carried out between: (i) mutation frequencies in each of these motifs, (ii) the incidence of mutations in RGYW or WRGY in complementarity determining region (CDR1) versus (vs.) CDR2 vs. CDR3. In FL, no particular bias was observed in the targeting of SHM at one motif over the other in the whole Vk sequence; significant differences were observed between WRGY targeting in CDR3 vs. CDR2 (p=0.014) and CDR2 vs. CDR1 (p=0.054). In MM, SHM targeting was encountered with a significantly higher incidence in the RGYW vs. the WRGY motif (p=0.026). RDW in CDR1 was targeted at a significantly higher frequency than in CDR2 (p=0.001), whereas comparison between CDR2 and CDR3 gave marginally significant results (p=0.058). In both CDR1 and CDR3 targeting of SHM was more prevalent in RGYW than in WRGY (p=0.003 and 0.08, respectively). In conclusion, targeting of the SHM mechanism at specific motifs in Vk genes of FL and MM might reflect the importance of these mutational hotspots in defining subsequent antigen selection of the neoplastic clone.

PO-1001 Protein-tyrosine phosphorylation, Flow-cytometric visualisation of impaired T cell function in CLL
Kaczmarek P,* Morilla R,* Matuses E,* Catoovsky D
*Department of Haematology, Medical School Wroclaw, Poland; *Department of Academic Haematology, RMH Trust, London, UK
Many disturbances of the T lymphocyte activation cascade occurring in cancer patients have been described. Most of the reports stress the role of the deficient expression of one of the proteins taking part in that process. There is however an increasing bulk of laboratory evidence showing that aberrant activation of these proteins (caused by the insufficient phosphorylation) may account for the decreased T cell activity. The two most important proteins that become phosphorylated upon T lymphocyte stimulation are CD3 and /32-70. We investigated phosphorylation levels in CLL patients at diagnosis and at the stage of residual disease, when compared to normal individuals. The analysed group consisted of 22 previously untreated patients diagnosed with CLL, 15 patients with without detectable residual disease and 15 healthy donors. The results were shown as a ratio of median fluorescence intensity (MFI) of the anti-CD3 stimulated lymphocytes to nonstimulated sample. The ratio was determined separately for CD4 and CD8 cells. A specific monoclonal antibody directed against phosphotyrosine was used. Results Newly diagnosed patients showed a decrease in the phosphorylation (MFI ratio 3.1 in CD4 and 2.46 in CD8) when compared to healthy individuals (MFI ratio 3.64 in CD4 and 2.61 in CD8). Interestingly the third group showed further decrease of tyrosine phosphorylation (MFI ratio 2.58 in CD4 and 2.09 in CD8) that may represent both the persistent immunosuppressive effect of the disease and the influence of the treatment (most of the patients were treated with fludarabine).

PO-1002 CD28 expression on peripheral blood T lymphocytes in different phases of Hodgkin's disease
Boiko D
Institute of Immunology, and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
Objective. A number of phenotypic and functional alterations have been described in T cells of patients in Hodgkin's disease (HD). Recently, research in tumour-induced immunosuppression has centered on understanding the CD28-associated signal transduction machinery and co-stimulating signals. T cell activation requires at least two signals; one provided by engagement of the TCR/CD3 complex, and the second by a costimulatory molecule present on
CD28 expression was found in the CD8+ subpopulation. in patients in CCR as compared to C. The most pronounced decrease of expressing CD28 molecule were significantly lower in HD patients in Aph and gen.

The presence of phenotypically immature lymphocytes in umbilical cord blood is a controversial topic. Moreover, their changes with age have not been systematically evaluated. Design and Methods. In the present study, relative and absolute numbers of CD3+, CD10+CD19+ and CD4+ CD8+ cell subsets were determined in umbilical cord blood from 12 full-term normal newborns, 43 children aged 1-month to 6-years and 10 young adults. The samples were processed by whole blood lysis and monoclonal antibody staining, and cells were analysed by flow cytometry. Results. Immature cells were present in cord blood and progressively declined in both absolute and percentage numbers with age, each according to a particular curve, reaching youth values roughly at the age of 2 to 4 years. The highest values for CD3+ cells were found at birth (43.5 cells per µL; 0.70% of total lymphocytes) and their numbers significantly decreased progressively from 7 to 20 months (p<0.01), and later on at ages older than 2 years (p<0.001). The CD10+CD19+ B-cell precursors were present at birth (30 cells/µL; 0.55%) and slightly increased afterwards, the highest levels being detected in individuals 1 to 20 months old. After this period, they progressively decreased over time until youth (4 cells/µL; 0.16%; p<0.001). Finally, the presence of CD4+CD8+ T-cells was highest at birth (123 cells/µL and 2.47%) and declined with age, reaching significantly lower values at the age of 2 to 3 years (22 cells/µL and 0.61%; p<0.001), and the peripheral blood levels of this cell subset remained in the same range until youth. In addition, T-cell precursors may be subdivided into 2 different subtypes in peripheral blood. There were CD4+ cells with low expression of CD28 (CD4+/CD8+CD28low) and CD8+ cells with low expression of CD4 (CD4+/CD4low). T-cell precursors with simultaneous high expression of both antigens were not present in peripheral blood in any age group. Conclusions. These results demonstrate that phenotype immature cells normally circulate at low levels in peripheral blood, mostly at birth and during infancy, but also during youth.

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PO-1005 Pulsed high-dose dexamethasone therapy in chronic idiopathic thrombocytopenic purpura

Gaman GD, Gaman A, Tanase A, Tanase T
Haematology Clinic, University of Medicine, Craiova, Romania

Methods. Twelve consecutively referred patients who had persistent symptoms of idiopathic thrombocytopenic purpura after 2 or more conventional treatments received high-dose dexamethasone (40 mg given daily on days 1 through 4, of each month for 6 month). Results. The platelet counts rose consistently after 3 cycles of treatment and continued to increase in subsequent treatment cycles. The mean count increased from 18,000 to 240,000/mm³. The platelet counts increased faster in the patients without prior splenectomy (p<0.001) and in the patients who had not received cyclophosphamide before high-dose dexamethasone (p<0.01). Treatment was well tolerated; no patient had to withdraw because of side effects. Conclusions. The rate of response was 100% in all 12 cases with refractory idiopathic thrombocytopenic purpura who was received pulsed therapy with dexamethasone. In 8 patients (73%) the therapy was started with 36-74 month ago. There were two problems: to define the refractory idiopathic thrombocytopenic purpura (different by author) and the duration of therapy.

PO-1006 Peripheral blood progenitor cell mobilisation in patients with rheumatic diseases: preliminary experiences

Jantunen E, Myllýkangas-Luostarinen R, Mahlamäki E*, Kaipiainen-Seppänen O, Nousiainen T
Department of Medicine and Clinical Chemistry, Kuopio University Hospital, Kuopio, Finland

High-dose therapy supported by peripheral blood progenitor cell transplantation has been recently suggested as an experimental treatment for severe or therapy-resistant autoimmune diseases including a variety of rheumatic diseases. Only limited data is available on the efficacy and feasibility of progenitor cell mobilisation in these patients. Two patients with rheumatic diseases have recently mobilised with high-dose cyclophosphamide (CY) (4 g/m²) followed by granulocyte colony-stimulating factor (G-CSF, filgrastim 5 µg/kg/d). The patients were 64 and 55 year old, had suffered several years from rheumatic diseases (mixed connective tissue disease, Wegener’s granulomatosis) and had been previously treated with several anti-rheumatic drugs including CY. Both patients experienced a neutropenic fever after the mobilisation. The first patient mobilised well with a peak B-CD34 of 36·10⁶/L. With two apheresis, a total of 4·0·10⁶/kg CD34+ cells were collected, but after CD34-selection, only 1·1·10⁶/kg CD34+ cells were available. Thereafter, the patient was re-mobilised with G-CSF alone (5 µg/kg/d). B-CD34 peaked at only 5·10⁶/L.
With two apheresis, only 0.8·10^11 kg CD34-positive cells could be harvested. The other patient failed to mobilise with CYG-CSF (peak B-CD34 only 2·10^10 kg) and is going to be mobilised with G-CSF (10 µg/kg) alone. These preliminary observations suggest that at least a subset of patients with thrombocytopenia may be difficult to mobilise with current methods. Stem cell dysfunction either caused by therapeutic disease or its previous treatment might explain these difficulties. Analysis of BMFIT EULAR registry data may give more information on the efficacy and feasibility of various mobilisation methods in these patients. Finally, a randomised study comparing G-CSF vs. CYG-CSF might be warranted.

**PO-1007** Detection of autoreactivity using an in vitro skin explant model in a patient with Evans syndrome and in patients with systemic autoimmune diseases. 

*Hromadníková L, Sedlářek P, Vavincová P, Čermáková M, Štechová K, Starý J, Fialová M, Dickinton AM, Vavinc L.* 2nd Department of Paediatrics, University Hospital Motol, Prague, Czech Republic; *University Department of Haematology, Royal Victoria Infirmary, Newcastle-upon-Tyne, UK*

A 20-year-old male with an 8-year history of AIHA and ITP (Evans syndrome) was admitted for autologous peripheral blood stem cell transplantation. In spite of being treated with immunosuppressive therapy, his being in haematological remission, severe autoreactivity in his peripheral blood was detected using an in vitro skin explant model. When sensitised lymphocytes from an autologous mixed lymphocyte culture (MLC) were cocultured with patient skin explant severe histopathological changes were observed. Similar histopathological changes were observed in the skin explant 7 months after reactivation of VZV. The patient was re-tested again one year post-transplant while on immunosuppressive therapy. Platelet counts at this time were within the normal range. Only mild histopathological changes were observed in this skin explant co-cultured with in autologous MLC sensitised lymphocytes. Patient skin explants cultured in medium alone supplemented with inactivated autologous serum were without histopathological changes. When we investigated skin explants of healthy individuals with autologous lymphocytes from an autologous MLC, no histopathological changes were observed. We have also extended this study to test autoreactivity using the skin explant model in different autoimmune disorders (SLE, RA, etc.). We conclude that an in vitro skin explant model may be an useful tool to monitor the grade of activity of pathological autoimmune cell clones in patients in clinical remission or relapse. Moreover it may be able to determine the efficacy of the immunosuppressive therapy and the grade of achieved remission. This is the first report of the novel use of the skin explant model for studying autoimmune diseases.

**PO-1008** Evans syndrome - a case series of 4 patients. 

*Jabaji Y, Timr P, Šmečka V.* Dept of Paediatrics, Regional Hospital C. Budíjkovice, Czech Republic

**Objective.** To explore the role of pulsed high-dose dexamethasone (HD DM) in the management of children with Evans syndrome. **Design and Methods.** From 1989 to 1996 four boys aged 1 to 12 and 11 to 12 yrs were diagnosed with Evans syndrome and successes so far followed up at our Dept. All had polyspecific warm IgA, AbC3, D3. One pt had in addition granulocyte Ab, and another granulocyte as well as platelet Ab. The initial presentation and course were variable, but all experienced recurrent episodes of the disease with thrombocytopenia becoming more pronounced or more frequent over time. A number of IST were applied. However, the quality and duration of response varied greatly in the individual pts and between them. We tried HD DM given 24 mg/m² /d (max. 40 mg). 4-6 wks for 4 cycles overall. This regimen was always launched at relapse, but further continued irrespective of the disease status. Results. HD DM was quite well tolerated by all pts and could be delivered on an outpatient basis. This regimen was significantly superior to other regimens in 3-4 wks or 12 ≥ 0.25-0.5 months, respectively. In another pt it was comparable to or marginally better than other IS modalities (CR: 8 vs 3-5 months ). This pt deteriorated in terms of AIHA immediately after the first cycle of HD DM, but he could be salvaged with one dose of HD IVIG w/o problems thereafter. The response was excellent immediately after each cycle of HD DM in the fourth boy. However, it lasted for 2 wks only (≥ 0.25-0.8 months on other IS), and he should be rescued by HD IVIG. Conclusions. HD DM may be of benefit for some children with Evans syndrome who tolerate it quite well. It is a cost-effective regimen that can be delivered on an outpatient basis, and should be smoother and less toxic than many other therapeutic options, e.g. cytotoxic drugs or CSA. However, only a large-scale randomised study may better define the role of HD DM in the management of this disease.
the use of ATG pretransplantation and 3) a rigorous depletion of T-cells from the graft by CD34 selection (CellPro). In three patients (m, 30 y; m, 35 y; f, 46 y) the autografted stem cells had been depleted. The grafts contained a mean of 2.04 × 10^6/kg (1.85–2.14) WBC, 1.23 × 10^6/kg (1.18–1.27) CD34+ cells, 0.42 × 10^6/kg (0.33–0.59) CD3+, 16.8 ± 13.2 kg (8.05–25.9) GM-CSF and 5.06 ± 10 kg (2.36–8.43) IFU. Engraftment as defined by a WBC > 1.0 × 10^9/L and a platelet count of > 50 × 10^9/L was achieved after respectively 12, 19 and 33 days. Post-transplantation toxicity consisted of moderately severe enytemia, grade 1–2 mucositis and slightly elevated serum liver tests. Severe infections were not seen and there were no relapses or changes on the EDSS in the immedisse posttransplantation period. At the meeting the 6 months follow up of these three patients will be presented.

**PO-1012** T-cell depleted autologous stem cell transplantation for multiple sclerosis


Objective. To analyze the short-term outcome of patients with multiple sclerosis (MS) who received an autologous stem cell transplantation (ASCT) as part of a prospective phase II trial. Methods. Blood stem cells were mobilized with Cy (3 g/m²) and G-CSF (15 g/kg). The graft was T-cell depleted by CD34+ selection (Isolex 300 or Clinimacs). Conditioning regimen included BCNU (300 mg/m²) and ATG (Imerexus; 60 mg/kg in 4 days) and ATG (Imerexus; 60 mg/kg in 4 days). Patients were assisted in LAF rooms, received oral ciprofloxacin, fluconazole, acyclovir, inhaled pentamidine, and G-CSF. Results. Main characteristics and incidences in the first 3 patients were:

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Conclusions. Tolerance and toxicity of ASCT in MS seem to be similar to that observed in patients with haematological malignancies and a similar performance score. Evaluation of treatment results requires further follow-up.

**PO-1013** Cellular and serological studies in chronic autoimmune thrombocytopenic purpura (CATP)


An expansion of CD5 + B-cells has been previously reported in patients suffering from chronic autoimmune thrombocytopenic purpura (ATP). However clinical-biological implications of such feature are not completely understood. With this background we carried out cellular and serological studies in 21 consecutive ATP patients followed-up in a single institution. At the time of immunological studies, 11 patients had active disease (i.e. platelet count lower than 50 × 10^9/L with no therapy) and 8 patients had stable disease (i.e. platelet count lower than 100 but higher than 50 × 10^9/L). As far as treatment is concerned, 11 patients were untreated, 7 were receiving low doses of corticosteroids, 2 underwent splenectomy and 2 were given Ig anti-D. When immunological data of our patients were compared, with those of 11 healthy controls selected for statistical purpose, an increased percentage number of CD5+ B-cells could be demonstrated (2.52 ± 0.27% vs 0.85 ± 0.15%, p < 0.02). Furthermore, the expansion of CD5 + B-cells did not correlate with either platelet count (r = -0.054 p = n.s.) nor disease status (i.e. active versus stable disease) (2.08 ± 1.5% vs 2.73 ± 2.3%, p = n.s.). In order to provide information lacking in the literature, sera taken at the time of cellular studies and stored at -20°C were analysed with immunoassay tests (ELISA) for the presence of soluble CD4 (sCD4), sCD8, sCD25, sCD3, sCD23. Serum levels of soluble CD4 and CD25 were not significantly different than those of healthy controls (p = 0.680 and p = 0.142, respectively). Interestingly, the percentages of circulating T-cells significantly lower than those of normals (589.1 ± 222.3 vs. 785.3 ± 253.1; p < 0.01). In keeping with results obtained in other autoimmune conditions (i.e. rheumatoid arthritis) increased levels of sCD25 could be found (p = 0.033). When correlation between serological and cellular findings were attempted, we were able to demonstrate a close correlation between the absolute number of CD5+ B-cells and serum levels of sCD25 (r = 0.802; p < 0.01). These data, similar in many aspects to those of CLL differ, however, for the levels of sCD23 which in our set of patients was significantly lower than in normal (p < 0.025). In conclusion, decreased levels of sCD25 can reflect the functional defect of CD5+ B-cells in the context of unbalanced immune response. sCD4 is a useful marker of activity in ATP closely correlated with B cell abnormalities. Longitudinal studies are warranted in order to understand the clinical implications of these findings.

**PO-1014** Increased familial development of tumors in patients with Fas apoptosis pathway deficiency without Fas gene mutations

Ranenoghi U, Merletti F, Migliaretti G, Di Franco D, Gambarduto C, Cerchion R, Bermond S, Bonissoni S, Dianzani U*. Dept. of Paediatrics and Dept. of Human Oncology, University of Torino, Dept. of Medical Sciences, University of Eastern Piedmont, Italy

Fas/Apo-1 is a transmembrane molecule which binds Fas, a trigger proposed in programmed cell death. Fas is involved in lymphocyte-mediated cytotoxicity. Patients with the autoimmune lymphoproliferative syndrome (ALPS) carry loss-of-function mutations of the Fas gene and develop autoimmune phenomena and non-malignant lymphoproliferation. Many molecular pathways are heterozygous for Fas mutations, however, their heterozygous parents are generally healthy. We identified 7 unrelated patients with the ALPS-like clinical pattern whose T-cells displayed reduced Fas capacity to induce cell death, but no Fas gene mutations. We suggested that amino acid alterations in other molecules involved in Fas signaling. A genetic component is supported by the observation that T cells from 7/7 mothers and 5/7 fathers were resistant to Fas-induced cell death. Fresh tumors and tumour cell lines are often resistant to Fas induced cell death, since they downregulate Fas expression or secrete soluble forms of Fas. These alterations may protect neoplastic cells from immune surveillance. Since patient families carry resistance to Fas-induced cell death, as a familial trait, we assessed the possibility that they are predisposed to cancer development by comparing the observed cases of cancer in these families with the frequency expected based on the Poisson distribution. The maternal family line displayed higher mortality for cancer than expected, with 9 observed deaths vs 4.67 expected and a SMR of 1.846 (90% CI: 1.07-3.19). The trend was similar in males (observed/expected: 6/3.24; SMR: 1.85; 90% CI: 0.94-3.62) and females (observed/expected: 3/1.63; SMR: 1.84; 90% CI:0.71-4.76). No increased frequency was found in the paternal family line. Cancer incidence in the maternal family line showed an observed/expected ratio of 9.5/5.7 (SMR 1.62; 90% CI: 0.93-2.79) in males. No increased frequency was found in females of maternal family line, as individual related through matrilineal (mitochondrial) inheritance, and in the paternal family line. These data support the possibility that genetic alterations of the Fas signaling pathway may be a novel genetic factor predisposing to cancer development and suggest that the trait is controlled by the paternal line.

**PO-1015** Results of splenectomy for idiopathic thrombocytopenic purpura. A single center experience

Vaza MJ, M烂reno JA, Aramua A, Solis C, Palomera L, Escarlin A*, Iturbe T, Caller L, Guiti駸rez M.*Department of Haematology and Digestive Surgery; University Clinical Hospital, Zaragoza, Spain

Objective. The aim of this study is to evaluate results of splenectomy, surgical and infectious risks and to determine prognostic factors for splenectomy in idiopathic thrombocytopenic purpura (ITP). Design and Methods. From 1990 to 1996, 24 patients underwent splenectomy for ITP. There were 10 males and 14 females. The mean ± SD age of the patients was 40±19 years (range 14-75). At the time of diagnosis, 79% presented bleeding diatheses with a mean platelet count of 17 ± 15 × 10^9/L and 21% nonestabished etiology of ITP with a platelet count of 26 ± 18 × 10^9/L. Our patients responded initially to steroid treatment in 83.3% (n=20), two of them became resistant, and 4 patients were steroid-
resistant at the beginning. The indications for splenectomy were refractoriness to medical treatment in 6 cases (25%), steroid-dependence in 16 cases (66%) and contraindication to long-term treatment in 2 cases (8%). The mean duration of thrombocytopenia before splenectomy in refractory ITP was 6±10 months and in steroid-dependence ITP 31±85 months. In all the cases, the splenectomy was performed by left subcostal incision, except in 1 case by laparoscopic splenectomy; we found splenomegaly in 5 cases (20.8%) and 8 cases accessory spleens (33.3%). There was no operative mortality, but two patients (8.3%) suffered from a postoperative complication: pancreatic fistula and subphrenic abscess. Results. The first platelet count early postoperative was 275±248×10^9/L for patients with long-term complete responses, although it was 123±210×10^9/L for patients recidive post-splenectomy. The mean time of follow-up was 14.1 months (2-48). At this moment, 18 of the patients are in complete remission (75%) and 6 in failure (25%); of them only one present severe refractory thrombocytopenia (2×10^9/L) at all therapy. Discussion. 1. Splenectomy appears to be the best treatment in chronic ITP when a durable response to corticotherapy cannot be achieved; in our series, early platelet count is a good predictive factor of response; 3. the morbidity-mortality of our study has been minimum.

Poster Discussions  Granulocytes and monocytes

PO-1016  Granulocyte and monocyte ingestion abilities in leviameiosis treated children with acute leukemia

together with primary disorder of immunity in patients (pts) with malignant diseases, aggressive polychemotherapy provokes significant secondary immunosuppression. The aim of this study was to determine the influence of immunomodulating drug Lervamisole (Le) on phagocytic functions in immunsuppressed patients. We studied 10 normal individuals (WBC 7.12±1.54×10^9/L; EOS 3.5±1.5%, 0.108-0.396×10^9/L), 7 with mild eosinophilia (WBC 11.285±5.602×10^9/L; EOS 8.1±2.2%, 0.546-1.3×10^9/L), 5 with moderate eosinophilia (WBC 18.389.984×10^9/L; EOS 20.5±6.8%, 2.08-6.602×10^9/L) and 4 with hypereosinophilic syndrome (HES) (WBC 26.8±19.181±10^9/L; EOS 33.2±12.03%, 6.34-45.12×10^9/L). The number and the morphology of the EOS in each cell fraction were estimated by cytofluorimetric analysis. In normal individuals EOS were obtained from layers V-IX (98.5±1.08%) and only 1.55±0.08% were “hypodense” (layers II-IV), in patients with HES the EOS distribution among the various gradients was quite different. 64.4±11.1% were “hypodense” (layers II-IV) while 36±1% had normal density. The range of “hypodense” EOS varied from 25.7±6.7% for patients with mild eosinophilia to 47.8±12.1% for patients with moderate one. “Hypo- dense” EOS had morphological abnormalities (degranulation, vacuolation, small dense nucleus) and their number was associated with the extent of eosinophilia. The increased number of “hypodense” EOS in patients with marked eosinophilia might be related to eosinophil activation and release of eosinophilic enzymes. All our patients presented CNS complications, circulatory disorders and respiratory dysfunction that improved dramatically after normalisation of EOS by corticosteroid or hydroxyurea administration.

Our study supports the presence of “hypodense” EOS in the peripheral blood and their association with the extent of eosinophilia and the patients' clinical manifestations.

PO-1017  Cardioprotective action of magnolol in a rat model of myocardial ischemia/reperfusion injury

Lee Y-M, Yen M-H

The evidence has shown that various antioxidants afford cardioprotection in myocardial ischemia/reperfusion. Magnolol, an active component extracted from the Chinese medicinal herb Magnolia officinalis, possesses antioxidant and free radical scavenging activities. The cardioprotective action of magnolol was evaluated in an open-chest anaesthetised rat model of myocardial ischemia/reperfusion injury. The results demonstrated that pretreatment of magnolol (0.2 and 0.5 µg/kg, i.v. bolus) at 10 min before occlusion significantly suppressed the occurrence of ventricular fibrillation and mortality when compared to the untreated control. Magnolol also caused a significant reduction in infarct size when compared with the control group. Furthermore, magnolol (0.2 µg/kg) significantly suppressed reduced the myeloperoxidase activity, an index of neutrophil infiltration in the ischaemic myocardium, as compared with that of the control group. In vivo, magnolol (5, 20 and 50 µM) significantly suppressed human neutrophil migration in a dose-dependent manner. It is concluded that magnolol can suppress ischaemia-induced vascular amythiasms and attenuate the infarct size resulting from ischaemia/reperfusion injury. The pronounced cardioprotective activity of magnolol is likely mediated via suppressing neutrophil infiltration in the ischaemic myocardium.

PO-1018  Variability in eosinophil density in relation to their concentration in peripheral blood


First Department of Internal Medicine, University of Athens Medical School, Laiko Hospital, Athens, Greece

Eosinophilia is associated with clinical manifestations due to granule-proteins related injuries, especially when it is persistent and extensive. We studied the distribution of eosinophil (EOS), according to their density, in normal individuals and in patients with eosinophilia. We fractionated peripheral blood leukocytes (WBC) in nine layers according to their densities (18-25%, I-IX), after centrifugation at 1200g for 45 min in 20°C, using multiple discontinuous gradients of metrizamide. We studied 10 normal individuals (WBC 7.12±1.54×10^9/L; EOS 3.5±1.5%, 0.108-0.396×10^9/L), 7 with mild eosinophilia (WBC 11.285±5.602×10^9/L; EOS 8.1±2.2%, 0.546-1.3×10^9/L), 5 with moderate eosinophilia (WBC 18.389.984×10^9/L; EOS 20.5±6.8%, 2.08-6.602×10^9/L) and 4 with hypereosinophilic syndrome (HES) (WBC 26.8±19.181±10^9/L; EOS 33.2±12.03%, 6.34-45.12×10^9/L). The number and the morphology of the EOS in each cell fraction were estimated by cytofluorimetric analysis. In normal individuals EOS were obtained from layers V-IX (98.5±1.08%) and only 1.55±0.08% were “hypodense” (layers II-IV), in patients with HES the EOS distribution among the various gradients was quite different. 64.4±11.1% were “hypodense” (layers II-IV) while 36±1% had normal density. The range of “hypodense” EOS varied from 25.7±6.7% for patients with mild eosinophilia to 47.8±12.1% for patients with moderate one. “Hypo- dense” EOS had morphological abnormalities (degranulation, vacuolation, small dense nucleus) and their number was associated with the extent of eosinophilia. The increased number of “hypodense” EOS in patients with marked eosinophilia might be related to eosinophil activation and release of eosinophilic enzymes. All our patients presented CNS complications, circulatory disorders and respiratory dysfunction that improved dramatically after normalisation of EOS by corticosteroid or hydroxyurea administration.

Our study supports the presence of “hypodense” EOS in the peripheral blood and their association with the extent of eosinophilia and the patients' clinical manifestations.

Infections of HIV patients are mainly associated with abnormalities of cell immunity including PMN (neutrophenia) and/or PMN dysfunction. The purpose of this study is firstly to investigate transduction mechanisms in HIV patients and secondly to study the role of G-CSF on an eventual transduction defect objectivated in these patients. PMN were isolated in parallel from 6 normal controls (HC) and AIDS patients (CD4<200/L). Transduction mechanisms were studied thanks to chemiluminescence (CL) emission with 4 stimulants: 5: cerulesia (PMN/yeast 1/20), FMLP (1 µM, NaF (20 mM stimulating a G-protein (G-prob) and PMA (25 µM) acting directly on the intracellular protein kinase (CIPK) with or without corresponding inhibitors. These last are respectively genisten (1 µM inhibitor of a yeastine kinase for yeast and FMLP), okadac acid (1 µM) for G-prot, and finally staurosporine (0.1 µM) for PKG. Moreover, some, including we of PMN were perfomed with G-CSF (4000 U/ml) during 10 minutes. Complement receptors to C3b, C3bi are not coupled to a G-prot and are mainly activated by yeast. On the opposite, others PMN Rec are dependent on a G-prot to activate a PLC.

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* Significant difference between witness and inhibitors (p<0.05). T=lag-time in seconds.
Significant (p<0.05) decrease of fmlp mediated-CL and increase of the lag-time with NaF - with a rebound phenomenon of emitted CL - were found in AIDS. No CL decrease was observed with PMA and yeast, path-way independent of G-prot. In presence of G-CSF, improvement of fmlp CL in HIV patients and significant (p<0.05) increase of NaF CL in AIDS and the rebound phenomenon of emitted CL on fmlp CL in HIV patients. In conclusion, a reversible inhibition of a G-prot seems to be present in PMN from AIDS patients. G-CSF in vitro correct this defect and it could act via a G-prot.

PO-1020 Two case studies of chronic idiopathic neutropenia preceding acute myeloid leukaemia

Division of Haematology, Department of Medicine, Institute of Legal Medicine, Institute of Pathology, Karl-Franzens-University, Graz, Austria

Chronic idiopathic neutropenia is a disease of unknown etiology charac-
terised by reduced blood neutrophil counts for prolonged periods of time.
Although the course of the disease may be aggravated by severe infections it is DCL reference method was performed according to the NCCLS rec-
not been reported to date. We present two patients with chronic idiopathic-
netropenia who showed disease progression to AML. The diagnosis of chronic idiopathic neutropenia was confirmed by extensive clinical and
and predictive negative value (PNV) were obtained for whole results and
method and were calculated true positive (TP), true negative (TN), false pos-
ommendations. The results of the analyzers were compared with reference
values. The ADVIA 120 evaluation data showed a decrease (36%) of manual review (TN). The FP were 35% and the TP were 28%. No FN were detected. The PNV was 100% and the whole flag PPV was 51.1%. The PPV for each type of flag were: 37% for LS, 54.5% for AL, 55.5% for NRBC and 77% for IG. Conclu-
for each type of flag: left shift (LS), immature granulocytes (IG), atypical
lymphocytes (AL) and nucleated red blood cells (NRBC). Results. The ADVIA 120 evaluation data showed a decrease (36%) of manual review (TN). The FP were 35% and the TP were 28%. No FN were detected. The PNV was 100% and the whole flag PPV was 51.1%. The PPV for each type of flag were: 37% for LS, 54.5% for AL, 55.5% for NRBC and 77% for IG. Conclusions. The evaluation of DLC ADVIA 120 suggests an improvement of flags because the manual review decrease in 36% without more FN.

PO-1022 Effects of metabolic inhibitors on lectin-induced degranulation of human neutrophils

Grudnikov IV, Timoshenko AV
Department of Biophysics, Belarusian State University, Minsk, Belarus

Objective. The degranulation of neutrophils can be triggered by some lectins which occupy a functionally active population of membrane glycoreceptors. The respective signaling mechanisms leading to the release of granule enzymes remain unclear. To evaluate an involvement of different intracel-
ular signaling pathways in realisation of lectin-induced degranulation of
neutrophils, the selective inhibitors were tested with respect to affecting
lysozyme release from cells. Methods. Neutrophils were isolated from leuka-
cyte-rich blood plasma by centrifugation through Histopaque-1077 and
suspended in phosphate-buffered saline solution, pH 7.35. To induce the
degranulation of neutrophils the cell suspension (3×10^6 cells/mL) was
incubated for 20 min at 37°C with α-NeuAc [2→6]Gal/GaINAc-specific
agglutinin from Sambucus nigra (SNA) at a final concentration of 100 µg/mL. Lysozyme activity in cell-free supernatants was assayed by mea-
surements of the rate of lysis of Micrococcus lysodeikticus. To test the effi-
ciency of the used inhibitors, they were added at concentrations in a range of 10-100 mM to cells 5 min prior to lectin. Results. Astablocid (phospholipase A2 inhibitor), indoethacin (cyclooxygenase inhibitor), neemycin sulfate (phospholipase C inhibitor), trifluoperazine (calmodulin antagonist) protein kinase C inhibitor), Nymethylmaleimide (sulfhydryl reagent) were found to reduce SNA-induced lysozyme release from neutrophils on 25-45%. The treatment of cells with bisindolylmaleimide (protein kinase C inhibitor), H-8 (inhibitor of various protein kinase), PD 98059 (MAP kinase inhibitor), and methoxyverapamil (a Ca^2+ channel blocker) did not affect the release of lysozyme. Remarkably, MBK66 (inhibitor of lectin-activating protein) was shown to activate the degranulation response. Con-
clusions. The results demonstrate a different potency of signaling inhibitors to affect SNA-induced release of lysozyme from human neutrophils and sug-
that only selective intracellular pathways may lead to trigger the degranulation response.

PO-1023 Autoimmune neutropenia of infancy

Dept. of Haematology-Oncology, University Children’s Hospital, Skopje, FYROM; Dept. of Immunology and Blood Transfusion, Clinical Center Rebro, Zagreb, Croatia

This report describes the clinical and laboratory findings and the long-term
history of autoimmune neutropenia of infancy (ANI) in a defined childhood
population in FYROM. Between ‘95 to Jan. ‘99 only one child presented
with ANI and serological evidence of antigranulocyte antibodies. The infant
was well until 2 months of age when he began having an increased rate of
infections, particularly otitis media, several upper respiratory tract infec-
tions, stomatitis and perianal abscesses. He did not require immunosup-
pressive therapy to induce remission. The routine antibiotic therapy ade-
quately controlled all infectious episodes. His WBC count ranged from 700
to 6000/µL, and his absolute neutrophil count (ANC) ranged from 14 to
3380/µL with otherwise normal haematological findings. Antineutrophil
antibodies were detected by using the granulocyte microagglutination and
granulocyte immunofluorescence assays. Spontaneous remission occurred
after 10 months. The patient was followed for 28 months. Conclusions. ANI is a condition which rests on a serological diagnosis. In our case it was fol-
lowed by a chronic benign course. The ANC normalised after 10 months. Symptomatic treatment of the infections was sufficient.

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Mice for both the p16INK4 (212.7±64.7 vs. 426.7±141.7; n=3) and p21WAF1/CIP1 KO mice which provides a measure of progenitor replication. The numbers of cells per CFU-GM colony per day were counted using the system of the femur of adult male Balb/c mice, and were stained according to May-Grünwald-Giemsa, so as to maximize comparison to the well-documented morphological aspects of bone marrow cells in humans. Differential counts were performed on 400 white cells. Reference values and morphological aspects of the different developmental stages of the different cell lineages were studied in normal conditions or in a mouse model for eosinophilia - typical features of allergic asthma were induced after sensitization with and repeated airway exposure to ovalbumin. Most striking in comparison to human myeloepoiesis is the formation of an annular nucleus which first becomes apparent in some myelocytes. Most myelocytes appear doughnut-shaped and out of this annular nucleus lobes are formed as maturation into polymorphonuclear neutrophils (PMN) occurs. However, the annular nucleus may not be entirely lost even in the most mature PMN. The myeloid progenitor cells contain only a few azurophilic granules filling the cytoplasm and a small nucleus. The polymorphonuclear granule is more pronounced in PMN with annular nucleus. Contrary to earlier reports, the presence of an annular nucleus is not restricted to eosinophils. These cells are generally larger than neutrophils, and their relatively less mature nuclei may or may not appear annular. The small acidophilic granules pack the cytoplasm within this structure resulting in a small pale blue cytoplasmic border. In conditions of marrow eosinophilia the increase in annular and segmented eosinophils is more pronounced than that of eosinophilic myelocytes and metamyelocytes. Mast cells but not basophils were rarely present. Monocyte had irregular nuclei and vacuolated basophilic cytoplasm. Small mature and a few large granular lymphocytes containing azurophil granules were observed. Monocytes were easily recognized; active megalocyte-epoiesis was present with a varying amount of cytoplasm.
PO-1028 Evaluation of emotional and behavioral effects of acute leukemia diagnosis and treatment in children in Brazil: preliminary results

Daudt LE, Fogliatto L, Astigarraga C, Michalowski MB, Ketzer C, Rohde LA, Silla LM
Hospital de Clínicas de Porto Alegre, Serviço de Haematologia e Serviço de Psiquiatria de Infância e Adolescência, Porto Alegre, RS, Brazil

Objective. Diagnosis and treatment of malignancies in childhood, as acute leukemia, can have a great impact in children’s lives leading to significant behavioral and emotional changes. This study aims to evaluate the prevalence of emotional and behavioral symptoms in children with diagnosis of acute leukemia that have been exposed to chemotherapy. Methods. Three groups, formed by children aged 3-14 years old were evaluated by an instrument of psychopathology evaluation validated in Brazil - Child behavior Check List (CBCL). Group 1 – children with acute leukemia in treatment for a minimum period of 4 months and a maximum period of 36 months; Group 2 – children with blood dyscrasias in treatment for a minimum period of 4 months and a maximum period of 36 months; Group 3 – children that have been followed by the Pediatrics ambulatory at the Hospital de Clínicas de Porto Alegre. Results. Until the present moment 53 children were evaluated (Group 1: 12, Group 2: 12, Group 3: 29). The acute leukemia children group shows a significant increase in internalisation and externalisation symptoms when compared to the blood dyscrasias children group (p<0.05). However, there were no significant differences in the internalisation and externalisation symptoms between children with acute leukemia and children evaluated at the Pediatrics outpatients. Conclusions. The present results indicate that leukemia diagnosis and treatment cause significant emotional and behavioral changes in children. We emphasize the need to increase the number of patients evaluated to verification of our hypotheses.

PO-1029 Quality of life in surviving patients with diagnosis AMI. ALL in the years 1984-95

Edsberg H, Thronæs M, Lamvik I
Trondheim University Hospital, Department of Haematology, Trondheim, Norway

Patients. In the IV Norwegian health region (population 625,000) a total number of 252 patients (+16 years of age) with AMI or ALL were diagnosed in the 12 year period 1984-95. One hundred and fifty of those were started on intensive cytostatic treatment in order to induce remission. Thirty-two patients (the study group) were alive in 1998, following a median observation time of 7 years. Ten of the surviving patients had been transplanted. The patients were asked to respond to the same standard questionnaire with a comparable population within the same age groups. In the study group, how-ever, there were no significant differences in the internalisation and externalisation symptoms between children with acute leukemia and children evaluated at the Pediatrics outpatients. Conclusions. The present results indicate that leukemia diagnosis and treatment cause significant emotional and behavioral changes in children. We emphasize the need to increase the number of patients evaluated to verification of our hypotheses.

PO-1030 Quality of life evaluation in elderly patients affected by aggressive non Hodgkin’s lymphoma: an interim report from IIL

Marti F, + Bertini M, + Mozzana R, + Avanzini P, + Bertè R, + Grasso F, + Ponzini G, + Pollici S, ++ Pizzuto M, ++ De Paoli A, ++ Reppold I, ++ Ematologia Reggio Emilia, ++ Ematologia Ospedaliera Torino, + Medicina Gallerate (VA), + Medicina Piacenza; Oncologia Aosta; Medicina Rho (MI); + Ematologia Catanzaro; + Ematologia Pordenone; + Medicina Legnago (MI) for the Italian Lymphoma IIL, Italy

Objective. The evaluation of quality of life (QOL) was one of the end points of a study which randomized elderly patients to receive two treatments differ-ently scheduled: a traditional regimen (mini-COP) administered every 21 (or 28) days versus a weekly regimen (F-VEBEC). We considered QOL an impor-tant parameter in the search of the golden standard treatment for elderly patients affected by aggressive lymphomas. In fact a complete recovery of a good state of health is not so usual such in younger patients. Design and Methods. We utilised the EORTC QLQ-C30 (version 2) questionnaire. It is a 30-item questionnaire already experimented in other clinical trials in oncol-ogy. The QLQ-C30 incorporates nine multi-item scales: five functional scales (physical, role, cognitive, emotional and social); three symptoms scales (fatigue, pain and nausea/vomiting) and a global health and QOL life scale. The questionnaire was administered at diagnosis, during treatment and at the end of it. The time required to complete the questionnaire was approxi-mately 15 minutes. Results. From June 1996 to November 1998 206 patients affected by diffuse large cell lymphomas were enrolled in the study. At the moment 105 questionnaires (51%) were filled out, but only 62 (30%) were evaluable. The main cause of missing data was the bad compliance of the cutting physician, but also the high rate of patients which not complet-ed the treatment (progressive disease or toxic death) must be considered. Although the sample is too restricted to have a statistical significance the data agree with: role, cognitive and emotional functioning) were improved after the treatment while physical and social functioning were unchanged. The symptoms decreased. Finally, at the end of treatment the patient assess their global health status and QOL a little better. Conclusions. The main problem of this study was the difficulty of obtaining accurate answers from elderly patients and the lack of practice of physicians in assessing QOL. Nev-ertheless QLQ-C30 seems to confirm its validity also in elderly patients. The improvement of global health status and quality of life after chemotherapy strengthens the intent to treat also elderly patients affected by aggressive lymphoma. The study is ongoing and the results have not yet been analysed separately between the two regimens.

PO-1031 Functional status and quality-of-life benefits of epoetin-alpha independent of disease response in the treatment of patients with haematological malignancies

Denovellis GD, for PR0 CRIT Study Group
Dana-Farber Cancer Institute, Boston, USA

Objective. To assess functional status and haematological response in anemic patients (pts) with haematologic malignancies (HM) treated with chemotherapy (CT) and concurrent epoetin-α, and to correlate these out-comes with disease response. Design. Open-label multicentre trial of anemic (Hb ≤11 g/dl) CT pts. Pts received epoetin-α 10,000 international units (IU) SQ TMI, increased to 20,000 IU if necessary, based on Hb response at 4 weeks, for a maximum of 16 weeks. Activity, energy levels, and overall quality-of-life (QOL) scores were evaluated by the pt-reported survey instrument, linear analog scale assessment (LASA). Results. This study enrolled 2,370 pts including 515 with nonmyeloid HM.

Change in Hb

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<td>baseline to final activity</td>
<td></td>
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<tr>
<td>Activity</td>
<td>CR (n=64)</td>
<td>-34.3±17°</td>
<td>14.0±25°</td>
<td>21.8±25°</td>
</tr>
<tr>
<td>PR (n=118)</td>
<td>6.6±31</td>
<td>10.2±28</td>
<td>17.1±27</td>
<td>29.5±26°</td>
</tr>
<tr>
<td>SD (n=109)</td>
<td>2.1±21</td>
<td>5.7±24</td>
<td>16.9±24°</td>
<td>25.1±24°</td>
</tr>
<tr>
<td>PO (n=57)</td>
<td>7.5±20</td>
<td>-1.4±26</td>
<td>1.6±24</td>
<td>11.7±23</td>
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<tr>
<td>PO (n=57)</td>
<td>-34.3±17*</td>
<td>14.0±25°</td>
<td>21.8±25°</td>
<td>27.3±32°</td>
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<tr>
<td>PR (n=118)</td>
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<td>PO (n=57)</td>
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<td>25.1±24°</td>
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<tr>
<td>PO (n=57)</td>
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<td>-1.4±26</td>
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<td>11.7±23</td>
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<tr>
<td>Energy</td>
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<tr>
<td>SD (n=109)</td>
<td>2.4±32</td>
<td>8.1±25</td>
<td>18.3±25°</td>
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<tr>
<td>PO (n=57)</td>
<td>1.2±31</td>
<td>6.2±20</td>
<td>6.9±19</td>
<td>3.3±13</td>
</tr>
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</table>

Conclusions. Overall QOL

| CR (n=63) | -38.8±32 | 16.7±25° | 19.8±28° | 23.7±30° |
| PR (n=118) | 1.3±31 | 8.9±31 | 12.6±26° | 21.4±22° |
| SD (n=109) | 0.1±28 | 9.0±23° | 16.9±24° | 24.2±23° |
| PO (n=57) | 1.9±32 | -3.0±24 | 4.9±20 | -17.0±19 | 0.76

For P < 0.5; * < 0.1; CR-complete response; PR = partial response; SD = stable disease; PO = progressive disease; r = correlation coefficient.

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Conclusions. Epoetin-α therapy improved activity, energy, and overall QOL in CT pts with HM, independent of disease response. The subset of pts with PD failed to show an increase in LASSA scores. Greater HB increase were associated with greater improvements in functional status. Results support judicious use of epoetin-α to maximise functional status of anemic CT pts.

PO-1032 Post-traumatic stress symptoms in patients undergoing autologous stem cell transplantation

Wettergren L, Langius A, Björkholm M, Bjorvell H

Objective. The aim of this study was to prospectively evaluate the prevalence of post-traumatic stress symptoms (PTSS) in patients with haematological malignancies to compare the patients’ prevalence 2 for part time in decision making in treatment decisions (ASCt). The findings were related to sense of coherence and quality of life aspects. Design and Methods. Twenty patients were evaluated before ASCT, at a first follow-up, 2-6 months after ASCT (n=14) and at a second follow-up, 6 months after follow-up 1 (n=12). Four standardised instruments were used: Impact of Event Scale (IES), Hospital Anxiety and Depression scale (HAD), EORTC Quality-of-Life questionnaire (QLQ-C30) and the Sense of Coherence (SOC) scale. Results. The mean values on the intrusion and avoidance subscales of the IES were higher compared to other studied samples. The mean scores declined from before ASCT to follow-up 1 and 2, though only statistically significant (p<0.05) in the intrusion subscale between ASCT and the follow-up. The intrusion subscale correlated positively to the anxiety and depression subscales in the HAD scale and emotional function in the QLQ-C30 but not to sense of coherence and physical dimensions. Conclusions. The high levels of PTSS and their relation to emotional distress emphasises the importance of psychosocial care for this group of patients.

PO-1033 Patients’ preference for involvement in treatment decisions in palliative oncology

Schrötzek C, Porzolt P, Bergmann L

Depts. of *Haematology and Oncology and °Psychotherapy, University of Ulm, Ulm, Germany

Objective. This study was performed to investigate whether physicians of in-patients did know their patients’ preferences for participation in decisions regarding their treatment. Design and Methods. We used a self-administered questionnaire (categories: active, collaborative or passive role) to evaluate patients’ preference for involvement in treatment decisions. A group of patients with advanced cancer and a palliative treatment goal was compared to patients with chronic non-neoplastic disease. Simultaneously, the senior house officers on the wards were asked with a standardised questionnaire how they thought the patients wanted to be involved in treatment decisions. Weighted K-coefficient was calculated to estimate the agreement between patient preference as stated by patients themselves and rated by the caring physicians. Results. Fifty-nine in-patients with cancer (mean age 58.6 years) and eighty-six patients with chronic non-neoplastic disease (mean age 58.2 years) were included in the study. Most of the patients with cancer, namely 72.8%, preferred a collaborative role with the physician in the decision making process. Of the patients with chronic non-neoplastic disease about half, namely 48.9%, preferred a collaborative role. Extent of agreement between patient preference as stated by the physicians’ assessment was not significantly different between the two groups. Overall, complete agreement was achieved in only 52 cases (35.9%). K-coefficient was 0.1 indicating merely chance agreement. Conclusions. These data suggest that physicians may not know the patients’ preference for involvement in treatment decisions. The knowledge about patient’s preference might enhance communication and ultimately patient satisfaction.

PO-1034 Prospective study on the quality of life of acute leukaemia patients treated chemotherapy

Sretenović M, Rolović Z, Berger D, Petrović M, Boikovic D, Čolović M

Institute of Haematology, Clinical Centre of Serbia, Belgrade, Yugoslavia

Objective. The aim of this study was to describe the changes in health-related quality of life in acute leukaemia patients and to establish possible prognostic value of Performance status (PS) for the chemotherapy success. Design and Methods. Longitudinal, prospective research included 82 adults. Difficulties due to illness and toxicity of treatment, PS and patient’s experience of quality of life (EQL) were investigated using: List of symp-

toms, Kamofsky Performance Status Scale (KPS), Acute Toxicity Scale (WHO) and The EQL Questionnaire (EQLQ) which measured patient’s perception of health (PHQ), perception of his life (PL), his attitude toward the future (OF), estimation of his social (ES) and family relationships (EF). Results. The patients in complete remission (CR) did not have higher initial KPS (F1=9.2; p=0.15) than non-responders. The KPS at the end of the induction therapy was more strongly related to the survival (r=0.49; p=0.00) and the disease-free survival (r=0.45; p=0.01) than the KPS at the other points of evaluation. The most significant correlations among PS and EQL dimensions were found at the beginning of treatment, during the induction therapy and relapse. The global EQL and PHQ proved more strongly related to F to (in 5/7 and 4/7 points of evaluation, respectively, than the other EQL components. PHQ was the most changeable, while EF and ES were the most stable EQL components during the follow-up. The EQL turned out to be more improved (t=2.3; p=0.03) than F (t=1.99; p=0.06) in the patients with the longest mean survival (OS). Conclusions. The prognostic value of the initial KPS for to receive university education, 11.8% - to COPS was related to OS and disease-free survival temporarily. KPS was susceptible to the transient occurrence and changed faster than EQLQ results, but changed less when different phases of the treatment were compared. PS and EQL components were related mostly at the points of the most serious health problems and the lowest KPS.

PO-1035 Quality of life and medico-social problems of the children with malignancy in remission

Borodina I, Geludkova O, Boukhny A, Lasareva I, Rusanova M

Rumyantsev A

Research Institute of Pediatric Haematology, Moscow, Russia

We have studied 180 families having children with malignancy in remission from 1 to 11 years. The most frequent late complications, requiring correction, are disorders of CNS (20%), vascular disorders, disorders of endocrine system (5%), late anthracycline cardiac abnormalities (1.3%). Up to illness 83.6% of children visited children’s establishments. After illness 10% of children began to be trained during intensive chemotherapy at home, and 0.9% - at school: on supporting therapy - 27.3% and 6.4% after ending the treatment - 12.7% and 34.6% accordingly. The children are trained at home after ending the treatment for the following reasons: in 31.8% of cases the doctor does not permit, 23.8% of children don’t want to learn at school by themselves, 38.1% of the parents do not let children go at school for the reason of fear of the infections and overload. In future 56.6% of children think, that they will have problems connected to education. Children don’t want to complete the school, 25.4% - professional school, 1.3% do not want to learn, 3.9% - are at a loss to answer. The children which are learning at school consider that in 59.6% of cases healthy children in the class display to them friendly attention, in 32% - are indifferent, in 6.4% - are indulgent, and 2% - negative. Only 6.6% of the children have lost old friends after the illness and have not yet got in new school, 5.2% completely have replaced a circle of dialogue, 47.3% the former friends were saved, 37% have saved the old friends and have got new, and 3.9% can’t give answer. Only 11.8% of the parents think, that the child will not have problems connected to transferred illnesses in the future, 26.3% of the parents think, that the children experience difficulties with returning to the normal life, 36.8% - that most difficult for a child in the future will be creation of a family, 19.7% - that their child will have problems with gain of a trade, problem with school - 19.7%, 25% - complex problems with the physician and 9.2% - that their child will have problems in dialogue with other children, and 10.5% suffer from complex. 14.5% of the parents don’t see problems for the future family life of children, 38.2% of the parents think that the chronic diseases will hinder their children, 32.9% are afraid for health of future grandchildren, 8% are afraid that their child can not provide family his own in the future because of the physical inability.

PO-1036 Comparison of quality of life of non-Hodgkin’s lymphoma and Hodgkin’s disease patients

Novik AA, Ionova TI, Maximov AG, Pozvuz AS, Konovalenko AL

St. Petersburg Lymphoma Study Group, St. Petersburg, Russia

It is widely accepted that quality of life (QL) assessment presents important information for the process of treatment evaluation. Taking it into account the objective of our research was to compare QL of patients with high grade (HG) Non-Hodgkin’s lymphoma (NHL), low grade (LG) NHL and Hodgkin’s disease (HD) before treatment and to assess the influence of conventional chemotherapy (CT) on QL parameters. ED-3, QL-C30 was used for QL assessment. The questionnaire was administered before and after treatment. The patients (all of them IAB-V IV stages) were treated by 6 or 8 cycles of CT: HG NHL-ChOP, LG NHL-COP and HD-COP-ABV (if after
PO-1038

β2-microglobulin in cerebrospinal fluid in patients with multiple myeloma

Leznichenko IF, Bessmeltsev SS, Abdukkadyrov KM, Bilinov MN
Russian Research Institute of Haematology and Transfusiology, St. Petersburg, Russia

β2-microglobulin (β2-M) was determined in the cerebrospinal fluid (CSF) of patients(pts) with multiple myeloma (MM) in order to assess its value as a marker in diagnosis of neuroleukaemia (NL). β2-M was determined before chemotherapy. In 7 pts without NL it was 0.6±0.3 mg/L, in 7 pts with NL was 1.6±1.3 mg/L. 

In 1 pt NL was diagnosed. Period of observation was from 14 to 49 months before chemotherapy. Under study were 15 pts in different stages of MM:

- 6 were in 2A stage,
- 6- in 3A stage,
- 1- in 3B stage and
- 1 with nonsecretory myeloma.

As a factor of unfavorable prognosis of MM.

PO-1039

Acute porphyrias in Russia

Pustovit Ya-S, Pivnik AV, Karpova IV
Dept of Haematology and Intensive Care, Haematology Research Centre of the Russian Academy of Medical Sciences, Moscow, Russia

In the Russian Haematological Centre in the period from 1992 to 1998 26 cases of proven acute porphyrias were observed. In this group 21 patients had acute intermittent porphyria (89%), and 2 patients with inborn coproporphyrias (8%) and 1 patient with porphyria variegata (4%). These were 23 women (88%) and 3 men (12%). In accordance to the classification they belonged to the hepatic porphyrias group. At the peak of the disease, the following were the prominent symptoms: acute abdominal pain (96%), vomiting (67%), constipation (64%), different mental disorders (47%), peripheral polyneuropathy (83%). Most of them also had red urine (40%), arterial hypertension (51%), tachycardia (54%), photosensitivity in the form of skin pain syndrome (21%) and fever (33%). In most cases patients had to be placed in the intensive care unit. Eight patients (31%) for a long period of time were on mechanical ventilation. Pathogenic therapy included the following: infusion of Normosang (60%), Sandostatin (7%), concentrated glucose 20-40% in big volumes (97%), suppression of the reproduction system in women using different oral contraception, prophylactic antibiotic therapy and symptomatic therapy. Laboratory tests were carried out in phases: 1) Watson-Schwarts’s qualitative screening test, 2) Quantitative levels of porphyrins, PBG, delta-ALA in urine. II. Porphyrin levels in stool. III. Activity of phoporphobilinogen deaminase in red blood cells in AIP patients. IV. DNA-lyase of porphyrins. Patients were from different parts of Russia (Moscow, St. Petersburg, the Volga region, Siberia and the Far East) and the former Soviet Union (Uzbekistan, Belarus). Conclusions. Acute porphyrias is a group of little-known diseases widely spread everywhere in the former Soviet Union. Our observation does not allow us to judge the spread of different nosological forms in different geographical regions. Good laboratory diagnostics, timely and correct pathogenic treatment provides a high percentage of survival in patients with this hereditary pathology and provides them with a relatively good quality of life in future.

PO-1040

An experimental evaluation of the micro 21, a computerised blood film morphology system

Rogers B, Britto-Babapulle F
Dept of Haematology, Royal Berkshire Hospital, Reading, UK

The Micro 21 (Intelligent Medical Imaging Inc, Palm Beach Gardens, Florida, USA) is a computerised blood cell morphology system which captures digital images of cells from stained blood films and presents them for review on a VDU. WBC images are automatically categorised on the basis of cellular shape, size, colour, density and texture. The images can be manually reclassified as necessary. The aim of this study was to assess the ability of the system to demonstrate low numbers of abnormal cells. Dilutions of turkey erythrocytes (TE) which are nucleated and large enough to be counted as WBCs were prepared in isotonic saline and added to 3 human whole blood samples with endogenous WBC counts of 1.1, 5.4 and 17.4 x10^9/L such that TE were present in the 3 samples at concentrations
ranging through 10 dilutions from 4.72 to 0.008×10^10/L, the concentrations of endogenous WBCs remaining constant. Counts of TE cells suspended in saline were performed on a Bayer H^2^ cell counter. Standardised blood films were carefully made from the samples containing TE using a measured volume of blood. Following staining (Wright's stain) these films were scanned on the Micro 21 and the number of TE counted in approximately 130 nucleated cell images compared with the expected number calculated from the dilution factor. In order to evaluate the reproducibility of the Micro 21 in demonstrating cells at low concentration, the films representing the 6 lowest TE concentrations (0.250-0.008×10^10/L) were scanned in triplicate. The use of a semi-automated film maker provided with the Micro 21 was shown to lead to slightly reduced sensitivity in the detection of low numbers of TE compared to the standardised manual films used in this study. Using manual films, the Micro 21 located TE in each of the triplicate scans on the sample with an elevated WBC count down to a level of 0.10×10^10/L on the normal sample to 0.02×10^10/L and on the leucopenic sample, to 0.012×10^10/L. Our study suggests that the Micro 21 may be used in the early detection of leukemic blasts or signs of marrow recovery following chemotherapy or transplant.

PO-1041 Study of methods collection and red cells depletion of cord blood
Abdulkadirov KM, Romanenko NA, Starkov NN, Balachova VA, Selzter AV
Department of Clin. Haematology, Russian Institute of Haematology, St. Petersburg, Russia

We have compared two methods of cord blood (CB) collection and two methods of CB sedimentation. Method #1 blood bag; Method #2 blood bag syringe. Collection method 1 (the umbilical vein was entered with the transfixion set needle) resulted in a volume of 71.9±7.7 mL UCB (range, 18-241 mL), with a cells count of 1.45±0.20×10^6 cells/mL (range, 6.17-30.1×10^6/mL and viability of 99.5±0.5%, n=42). The bacterial contamination was not found. Method #2 (the umbilical vein was entered with the transfixion set needle and the umbilical artery was punctured with the syringe needle) yielded a volume of 86.1±46.1 mL (range, 32-134 mL), a cell count of 13.0±6.9×10^6 cells/mL and viability of 99.0±1%, n=8. However, we have obtained the bacterial contamination in this study. Using manual films, the Micro 21 located TE in each of the triplicate scans on the sample with an elevated WBC count down to a level of 0.10×10^10/L, on the normal sample to 0.02×10^10/L and on the leucopenic sample, to 0.012×10^10/L. Our study suggests that the Micro 21 may be used in the early detection of leukemic blasts or signs of marrow recovery following chemotherapy or transplant.

PO-1042 PVB19 infection in patients with aplastic crises of haematological diseases
Yarzhyshinka OE, Fevraleva IS, Novokon VN, Kravchenko SK, Lazarev IE, Logina IV, Mamilaeva ZH, Pinvik AV
National Center for Haematology, Moscow, Russia

Parvovirus B19 is transmitted by air and with the haemotransfusions of blood components. We investigated in dynamics the sera of 76 haematological patients, 156 samples on presence of parvovirus B19 DNA by a modified PCR method (nested-PCR) and determined a level of anti-parvovirus-B 19 antibodies IgM and IgG by the EUSA method. DNA of parvovirus B 19 was detected in 26% of cases, in 30% of them there was a persistent virus, that was connected to absence of the specific immune response, which in a half of the patients was suppressed with polychemotherapy. In the other half of the patients, the absence of the specific immune response is not clear: Immunosuppressive treatment was not conducted, the patients received only haemotransfusions. In one case persistence of a virus is marked with a high level of anti-parvovirus antibodies IgG. Thus, in patients with various haematological diseases concern to group of risk in the relation of parvovirus B19 infection and its persistence. The PCR method in our modification allows to conduct screening examination of the patients and donors.

PO-1043 Ultrasound guided fine needle biopsy of the spleen in suspected haematological malignancies: high clinical efficacy and safety in a multicenter Italian study
I Divisione di Medicina Interna - Ematologia, Ospedale Civile, Piacenza and The Multicenter Focal Spleen Lesion Study Group, Italy

Purpose. To obtain more information on technical problems, clinical efficacy and safety of Ultrasound Guided Fine Needle Biopsy (UG FNB) of the spleen in a large series of patients with suspected haematological malignancies. Design and Methods. We collected the experience with UG FNB of the spleen in patients with suspected haematological malignancies from eight Italian Clinical Centers that utilised this technique for at least ten years. A Collection schedule was sent to all Centers to collect all information about technique, results and complications of UG FNB of the spleen. Results. We analysed 398 biopsy procedures both on focal splenic lesions and on splenic parenchyma. The overall accuracy was 90.9% for the whole series, 84.9% for cytologic sampling, 88.3% for histochimical sampling and 90.3% for the double biopsy. Tissue core biopsy was better in patients with suspected lymphoma (90.9 vs. 68.5% for cytology). Complication rate was low (less than 1% for major complication and 5.2% for all complications). Conclusions. UG FNB of the spleen is a very effective diagnostic procedure with low (but not negligible) risk. In overall indications, aspiration cytology and core needle biopsy show similar diagnostic yield, except for the diagnosis of splenic lymphoma, in which core needle biopsy showed better results.

PO-1044 Study of bone marrow involvement by magnetic resonance imaging (MRI) in type 1 Gaucher's disease (GD)
Roca M, Giraldo P, Garcia-Mur C, Perez-Calvo JI, Giralt M
Departments Radiology and Haematology, M i g u e l S e r v e t Hospital Zaragoza, Spain

Purpose. To evaluate bone marrow involvement in GD by MRI, to determine the full extent of bone disease by a predictive score according the different MRI patterns and to compare MRI score with the clinical Severity Score Index (SSI) in order to establish a link between both systems. Design and Methods. Sixteen GD type 1 adult patients were studied. TL and T2 WI were performed in spine, pelvis and femora. Three infiltration patterns were established: Homogeneous (H), non-homogeneous (NH) and normal (N), assessing progressive values for each RM pattern (N; 0; NH: 1-3; H: 4). The total MRI score in each patient was calculated by addition of every value in each evaluated bone area. The SSI (Zimram, 1992) was applied according age at the diagnosis, skeletal and haematological complications and visceral involvement. Both systems have been compared by non parametric Spearman correlation test. Results. Mean age: 39 years (range 13-68), male/female: 6/10. The MRI pattern distribution was: All patients have some degree of bone abnormality. In spine 13/16 patients (81%) showed bone marrow involvement (patterns: H: 38.4%, NH diffuse 46.1%, and NH mottled 15.3%). Pelvis was infiltrated in 12/16 (75%) and normal in 4 (25%) (patterns: NH diffuse 58.3%, NH mottled 33.3% and NH mottled 8.3%). In femur 8/16 (50%) showed some degree of abnormalities (patterns: NH diffuse 25% NH mottled 37.5% and NH reticular 37.5%). Osteous complications: bone infarcts (2 patients), vertebral collapse (2 patients), avascular necrosis (4 patients), and bone pain crisis (2 patients) with posterior MRI normal pattern. The mean MRI score was 7.2 (range: 0-15) and SSI 9.1 (range: 2-22). The correlation coefficient (r) between MRI and SSI score was 0.98 showing high correlation. Hypothesis contrast test (t test 7.4) confirmed this value. Remarks. MRI is a very useful method to evaluate bone marrow infiltration in GD. The quantitative full extent of bone disease is one of the most important parameters to evaluate the severity of disease in GD.
PO-1045 Linearity and reportable range of haematology analysers

Laboratoire d’hématologie, Hôpital Paul Brousse, Villejuif, France

Introduction. According to good laboratory practice, each haematology laboratory has to ensure the range and the accuracy of the parameters reported. Accuracy of the haematology analysers is usually controlled with low, normal and high commercial haematology controls. As the commercial calibrated samples are not very high or low, it is necessary to check the result linearity. Therefore, linearity combined with separate calibration can be used to establish the range of highest and lowest patient values that can be accurately reported. That’s why we attempted to verify the linear performance of our haematology instruments. For this purpose, we were one of the first French hospital laboratory to use linearity kits (CBC-LINE™ Hyceil R&D Systems). These kits provide a map of measuring linearity of haematology analysers for white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB) and platelets (PLT) parameter determinations. Methods. We used a full range and a low range linearity kit. Each kit contains six levels of control material of known concentration of the previously described four parameters. The full range kit contains separate cell concentrates for RBCs, HGB, WBCs and PLTs (in order to decrease the potential of interference between elevated cell concentrations). After calibrating our haematologic analysers (H²-Technicon, NE 1500-Sysmex) we tested all levels of concentration from the lowest to the highest ones. We ran each vial four times, gently inverting the vial 10 times between runs. Statistical study was done separately by us and by the manufacturer. Results. This work documents the linearity of our two instruments: we noticed a strong positive relationship between the expected results and the obtained results for RBC, HGB, WBC and PLT parameters \( r^2 = 0.99 \). Also, this work allows us to determine the range which our instruments are accurate (for example: 11.10^13/µL to 893.10^13/µL for platelets on H² analyser). This implies that all results included within these linearity limits can be reported with a guarantee of reliability. The extension of the highest linearity value could be feasible with an extended range kit, which is not commercialised in France. In addition to the linearity verification, this work gives us some information about analytical performances of our instruments: relative accuracy, analytical sensitivity and repeatability. Conclusions. Verification of the linearity of our instruments is required before HLA class II molecular typing and introduction of CB data to the Belgian NMPD. A total of 872 units were stored in the bank. The number of fully validated CB units for allogeneic transplantation is 617 (70.8%) and 39 (4.5%) for related CB transplantation. 150 samples (17.2%) were in quarantine before validation by a second viral control and 66 units (7.5%) were on hold for administrative problems like the absence of a second virology and/or the pediatric certificate. This loss samples (7.5%) due to a low compliance has been decreased by multiplication of the informative methods as conferences, pamphlets, collaboration with ONE. In case of successful collection, a personal phone contact and an informative letter have to be used to remind again the importance of the 4 months control. Up to now, 47 CB have been requested for preliminary search, 4 familial CB transplantations have been performed and the bank provided 4 non related CB.

PO-1046 Metabolic peculiarities of blood cells in rats with hereditary stress-induced hypertension

Belil GE, Romanova TP, Proshina OV
Medical University, Saratov, Russia

The aim of the present work was the comparative study of some metabolitic parameters in erythrocytes and neutrophils of non-inbred white male rats (group 1) and rats with genetically determined stress-induced hypertension (group 2). Average arterial pressure was 125±4 and 162±8 mm Hg in rats of the 1st and the 2nd group, respectively (p<0.001). Animals of the 2nd group had of the heart weight enlarged and the increase of average diameter of cardiomyocytes. Their erythrocytes had the diene conjugates (DC) level 2.3 times higher (p<0.001) and triene conjugates (TC) level 1.6 times lower (p<0.01) than those in animals of the 1st group. Malonic dialdehyde (MDA) content was equal in erythrocytes of both groups (p=NS). In hypertensive rats red blood cells catalase activity was decreased by 23% (p<0.001) at the background of the increase of superoxide dismutase activity by 24% (p<0.001); in blood plasma DC level was elevated more than 3-fold (p<0.001) whereas MDA content was decreased by 24% (p<0.001) and TC was not determined. In neutrophils of the 2nd group rats glycogen content, ATPase and myeloperoxidase activities were less, but succinate dehydrogenase activity was higher than in animals of the 1st group. These results demonstrate that blood pressure elevation in animals with hereditary stress-induced hypertension is accompanied by alteration of lipid peroxidation processes and enzymatic link of antioxidant system in erythrocytes as well as by metabolic changes in neutrophils.
Haematology-in-Focus Symposia

HIF-1048 Molecular mechanisms of immunogenic and anergising
T cell activation

Boussiotis VA, Freeman GJ, Berezovskaya A, Grass I, Nadler LM
Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA

The ability of tumors to downregulate the function of the immune system is mediated by several mechanisms, one of which is the functional inactivation of helper T lymphocytes a process termed anergy. Induction of anergy results in the absence of CD4 + T cell help and inability of the host to generate tumour-specific CTLs. Generation of tumour-specific cells for immunotherapy requires a significant clonal expansion of such cells in vitro, prior to their adoptive transfer to the patient. Therefore, understanding the molecular mechanisms that control antigen-specific receptor and cell cycle progression in anergic T cells will allow the reversal of the anergic defect and the clonal expansion of tumour-specific T cells. For optimal vaccination T cells require two signals. The first which gives specificity to the immune response is mediated via T cell receptor (TCR) by antigenic peptide presented by MHC on the surface of antigen presenting cells. The second signal is neither antigen specific nor MHC restricted and is termed costimulation. In the absence of costimulation, TCR cross-linking by antigen leads to the state of anergy. Among the costimulatory pathways, CD80 (B7-1):CD28 appears to have a unique role, since it is both necessary and sufficient to prevent the induction of anergy. Anergic cells are incapable of activating IL-2 gene transcription when restimulated with antigen even in the presence of costimulation. Biochemically, anergy is characterised by hypophosphorylation of TCRζ chain, activation of protein tyrosine kinase fyn, loss of activation of ikk, ZAP-70, Ras, ERK, JNK and defective transactivation of the IL-2 enhancer elements AP-1 and NFAT. Interestingly, an energising stimulation activates a novel signal pathway which results in activation of Rap1, a GTP-binding protein that functions as a negative regulator of IL-2 transcription. Activated Rap1 appears to interfere with IL-2 gene transcription by two mechanisms: by blocking activation of the MAP kinase cascade and by differentially regulating activation of IL-2 transcription factors. Recently we identified that an active mechanism for direct blockade of clonal expansion is present in anergic cells. Anergic T cells fail to progress past the G1 restriction point of the cell cycle and activate cyclin-dependent kinase (cdk)1 and 2, synthesise evelin E and hypophosphorylate pRB. The cell cycle is arrested at the G1 restriction point and activation of cdk4 is also defective. These results effect from the increased expression of p27kip1 edk inhibitor, which is secondary to the increase of intracellular cyclic AMP and the absence of CD28-mediated dephosphorylation of p27kip1 in the ubiquitin-proteasome pathway. Forced expression of p27kip1 in T cells recapitulated the anergic defect and inhibited response to antigen even in the presence of costimulation. The ability of p27kip1 to block T cell clonal expansion and antagonize TCR-mediated IL-2 transcription suggests that P27kip1 may represent a potential target for therapeutic approaches for the modification of T cell immune response.

HIF-1049 Development of T cell-mediated immunity for the treatment
of acute lymphoblastic leukaemia

Cardoso AA
Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA

Despite the important successes attained in the treatment of childhood acute lymphoblastic leukaemia (ALL), significant difficulties remain to be resolved. Novel therapeutic strategies are necessary to increase both treatment efficacy and cure rates while also associated with protective therapy. One obvious treatment modality that will likely lead to the attainment of these goals is immunotherapy. Leukaemia cells are poor allogeneic antigen-presenting cells (APC) but can be modified to become efficient APC. One of the methodologies applied is the cross-linking of the CD40 molecule expressed on ALL cells by its ligand CD40L, which considerably increases their immunogenicity. Hence, we have developed a methodology that allows the generation of patient-derived leukaemia-specific autologous T cell lines, from the bone marrow of patients with B cell leukaemia, which can efficiently lyse the leukaemia cells. These results demonstrate the existence of anti-leukaemia T cells in these patients' T cell repertoire, so the development of leukaemia cell vaccination strategies using modified APC-competent leukaemia cells and/or adoptive transfer strategies using ex vivo generated anti-leukaemia T cells can be envisioned. Effective tumour cell vaccination will likely require that tumour cell vaccines migrate to the sites where relevant T cells are located, and, ideally, be capable of attracting circulating T cells. We have observed that leukaemia cells do express the chemokine receptor CCR4, and migrate through endothelium in response to its specific ligand SDF-1, which is produced by BM stroma derived from ALL patients. Interestingly, we also observed that CD40-stimulated ALL cells produce a chemokine that induces transendothelial migration of both autologous and syngeneic T cell lines. To be successful in adoptive immunotherapy, anti-tumour T cells must be able to migrate through endothelium, home to the BM and, most importantly, lyse the leukaemia cells in their leukaemia-permissive environment. Ex vivo generated anti-leukaemia T cell lines are capable of dothelial migration through both vascular and BM endothelium while their cytolytic competence. Importantly, these anti-leukaemia T cells are capable of lysing the leukaemia cells in the presence of autologous bone marrow stroma without significant damage to the stromal cells. The demonstration that anti-leukaemia-reactive T cells exist in ALL patients and that leukaemia cells can be modified to become efficient APC suggests that immunotherapy may be an important complementary strategy for the treatment of ALL.

HIF-1050 Generation of leukaemia-reactive CD4+ and CD8+ T cell lines

Marti W AF, Willemsen R, Falkenburg JH F
Dept. of Haematology, Leiden University Medical Center, The Netherlands

Introduction. Treatment of haematological malignancies with allogeneic stem cell transplantation (alloSCT) may be accompanied by a graft-versus-leukaemia (GVL) effect. In case of a relapse after alloSCT patients can be treated with donor lymphocyte infusions (DLI) to boost the GVL effect. However, DLI is frequently complicated by graft-versus-host disease (GVHD). Treatment of a relapse of leukaemia with leukaemia-reactive T cells may lead to complete remissions without causing GVHD. Therefore, we generated leukaemia-reactive T cells in vitro and analysed them using functional assays. Design and Methods. T cell lines were generated by restimulating with leukaemia cells. T cell clones were generated by limiting dilution assays. After 3 to 4 weeks T cells were tested in the 51Cr-release assay (1Cr-Ra) against PHA-and EBV blasts from donor and patient and patient leukaemia cells and in the progenitor cell growth inhibition assay (PIA) for recognition of leukemic or normal haematopoietic progenitor cells (HPC). Results. Both CD4+ and CD8+ T cell clones were obtained which were cytotoxic for patient cells but not for donor cells in the 1Cr-Ra. Panel- and blocking studies were done to analyze the respective HLA restriction elements. When tested in the PIA the T cell clones inhibited growth of patient leukaemia progenitor cells in a dose dependent way. Furthermore, a dose dependent growth inhibition of normal patient HPC but not donor HPC was observed. These results suggest that in vitro cultured donor-derived CD4+ and CD8+ T cells specifically recognize both normal and malignant patient-derived lymphohematopoietic cells and may be used for in vivo therapy. Using the same culture protocol leukaemia-reactive T cell lines can be generated under Good Manufacturing Practice (GMP) conditions and administered to relapsed patients after alloSCT. Conclusions. These results show that it is possible to generate leukaemia-reactive T cells in vitro which may be used for treatment of relapsed leukaemia patients after alloSCT when cultured under GMP conditions.

HIF-1051 The immune system of chronic myeloid leukaemia patients in complete remission is activated and functionally competent


Little is known with regard to the immune status of chronic myelogenous leukaemia (CML) patients after treatment induced remission. This aspect is of relevance towards understanding whether the immune compartment may play a role in controlling the disease and whether it can be ‘utilised’ for specific immunotherapeutic strategies. We studied the immune-phenotype of circulating lymphocytes, the capacity to produce TNFα and IFNγ, as well as the cytotoxic activity against the NK susceptible and NK resistant cell lines K562 and Raji Daudi in 12 CML patients in complete...
remission after treatment with interferon-α (IFN) or hydroxyurea (HU). Peripheral blood mononuclear cells (PBMC) were incubated with monoclonal antibodies against CD3, CD4, CD8, CD16, CD56, CD122, CD23, CD12, CD20 and intracellular TNFα and IFN-γ cytokines. The overall mean percent of CD3+ lymphocytes was 47.8±1.6%; CD4 and CD8 were 36.8±1.0% and 19.6±3.5%, respectively, with a CD4/CD8 ratio of 1.85±1.41%. The NK associated antigens CD16 and CD56 were expressed in 9.5±4.2% and 16.5±8.8% of PBMC, respectively. No notable differences were observed between IFN and HU treated patients. Interestingly, in all patients an increased proportion of CD4/CD25 positive T cells was recorded, with a clear mean expression of 20.9±8.6%. Spontaneous NK function was 23.6±1.4%; this was associated with a high IL2 generated LAK activity, with an overall killing of 60.4±22.7%. In the 9 patients evaluated (6 treated with IFN and 3 with HU), 14.0±8.2% of CD4+, 10.8±4.6% of CD8+ and 5.6±3.0% of CD56+ lymphocytes showed intracellular production of TNFα. 5.3±2.7% of CD4+, 12.1±7.5% of CD8+ and 5.6±3.4% of CD56+ cells produced intracellular IFNγ. These results indicate that in CMV patients complete remission after treatment, the host immune compartment appears phenotypically activated and functionally competent, suggesting that immune mediated strategies in this phase of the disease may be worthy of further investigation.

**HIF-1052 Investigate of the immune response to CMV infection through the release of IFNγ as a measure of immune constitution post-BMT**

Morte C, Dodi IA, Morgan C, Pay AL, Fallan P, Madrigal JA

The Anthony Nolan Research Institute. The Royal Free Hospital, Hampstead, London, UK

Despite the use of anti-viral drugs CMV infection post-allogeneic bone marrow transplantation still represents an unresolved issue. It is generally accepted that anti-CMV responses are mediated by both cytotoxic T cells and NK cells, and that the activation of both populations leads to the production of IFNγ. Using the Elispot technique we have been able to measure quantitatively the number of cells producing IFNγ in these two populations and to assess the response of the two different cell populations when challenged either with peptides from pp65 or CMV-infected fibroblasts. We previously showed that CTL clones can be generated in vitro from CMV positive healthy donors using different pp65 peptides. In HLA-A2, CMV positive donors the anti-CMV response was predominantly T cell mediated. The majority of donors responded to the immunodominant peptide (NLVPMVATV) with the exception of one individual who showed no response. One possibility is that such individuals use a different spectrum of peptides or an alternative HLA restriction element, which we are currently investigating. In HLA-A2, CMV negative donors the response was only mediated by NK cells. IFNγ production was seen when NK cells were co-cultured with autologous CMV-infected fibroblasts but not when uninfected fibroblasts were used. Furthermore we were able to demonstrate that this production is dependent on the presence of an MHC class II accessory cell. The fact that in CMV positive donors we have not been able to detect anti-CMV NK activity, may indicate that the increased level of specific anti viral T cell response serves to down regulate their NK responses. These findings may have relevance in the transplant setting with NK cells being one of the first cell types to appear in the periphery. We are currently addressing these questions in BMT recipients.

**HIF-1053 Abstract not published**

**HIF-1054 Clonality studies in high-risk myelodysplastic syndromes and secondary acute myeloid leukaemia treated with intensive chemotherapy followed by stem cell transplantation**


Department of Haematology, University Hospital Gasthuisberg, Leuven, Belgium

The myelodysplastic syndromes (MDS) are a group of clonal disorders of a haematopoietic progenitor cell with an inauspicious prognosis. Currently, only allogeneic transplantation offers potential cure for this disease. Intensive chemotherapy followed by autologous stem cell support (auto-TX) is an interesting alternative therapeutic approach for high-risk MDS patients not eligible for an allo-TX. Unfortunately, little is known about the clonality of the marrow and blood during complete haematological remission in MDS. Therefore, we have studied the clonal nature of mature cells and progenitors in remission marrow and blood and autologous. This was performed by sorting purified mature myeloid (CD16+ 14−, CD4+4) and lymphoid (CD3+, CD19+) cells and immature (CD34+ 38+) and committed (CD34+ 38+) progenitors for clonality analysis using X-linked polymorphisms in the HUMARA and FKG alleles. Nine female patients (median age: 60y; range: 34-73) with high-risk MDS (RAEB, RAEB-T, CMML) were treated at our institution with intensive chemotherapy (ICE NOVA, Daun-Ara-C, high-dose Ara-C, FLAG). Monoclonality was documented in the mature myeloid cells and marrow progenitors at diagnosis. Five out of 9 patients obtained a polyclonal remission (PC), whereas in 3/9 the haematological remission status was monoclonal (MC) and 1 patient rapidly progressed towards SAML. 5/9 patients subsequently underwent an allo-TX (PBPC and/or BM) with documented PC in 3 analysed autographs. These 3 patients recovered with PC haematopoiesis. One of these three pts. remains in PC remission 36 months post auto-TX, one out of 3 relapsed 5 months after auto-TX with chromoresistant disease and the third relapsed after 28 months. However, with intensive chemotherapy we could re-institute a second polyclonal CR currently lasting for 36 months. We conclude from this study that intensive chemotherapy can restore PC haematopoiesis in high-risk MDS patients. Subsequent transplantation with PC haematopoietic progenitors might be a promising treatment strategy to increase disease-free survival.

**HIF-1055 Hypercellular marrows with peripheral cytopenias may be the result of an inflammatory process**

Raza A

Rush Cancer Institute, Chicago, USA

Chemotherapy, growth factors and cytoprotective therapies have only provided palliation in MDS while curative therapies have proved elusive largely due to a lack of understanding regarding the basic pathology underlying the cytopenias. On the basis of circumstantial evidence it has been postulated that the myelodysplastic states may represent an ongoing inflammatory process in the stromal cells of the bone marrow resulting in a dysregulated cytokine milieu. Such a changed landscape could provide a selective growth advantage to a haematopoietic progenitor which is more rapidly proliferating than its counterparts resulting eventually in a monoclonal hematopoietic state. Apoptotic death of the progeny could result either directly from excessive proliferation or from the action of pro-inflammatory/ pro-apoptotic cytokines present in the vicinity. Cytopenias would ensue as a consequence of innocent bystander death. Monoclonality predisposes to malignancy accounting for the evolution of a blastic population and also explaining the existence of cytogenetic anomalies affecting a proportion of cells in an otherwise monoclonal marrow. Evidence suggestive of MDS being an inflammatory process such as myelosuppression, high levels of pro-inflammatory cytokines, excessive apoptosis, a prominent and active monocytic/macrophage system with accompanying increased levels of M-CSF, lowered serum cholesterol levels as well as immune-regulatory dysfunction will be presented. Biochemical and cell effecter studies in these marrows provide compelling evidence to consider a possible viral insult amongst the initiating events in MDS and the case for cytomegalovirus as a distinct possibility will be discussed.

**HIF-14 British Journal of Haematology**

Research Trust Symposium “Secondary acute leukaemias” 249
HIF-1056 Allogeneic stem cell transplantation (SCT) for myelodysplastic syndromes (MDS) and secondary acute myeloid leukemias


Allogeneic SCT from an HLA-identical sibling donor is a curative treatment option for a young patient with MDS. Age and lack of sibling donors limit this application. Alternative stem cell sources have been more recently, such as unrelated donors, nonidentical family members or autologous transplants. We analysed 1,378 transplants reported to the EBM'T to evaluate the outcome of the varying procedures according to the known risk factors. The 3-year disease-free survival (DFS) was 36 percent for 885 patients transplanted with stem cells from matched siblings. The relapse risk at three years was 36 percent. In the multivariate analysis age and stage of disease had an independent prognostic significance for DFS, survival and treatment-related mortality. Patients transplanted in an early stage of the disease had a significantly lower risk of relapse compared to patients transplanted in more advanced stages. The 3-year DFS was 25 percent for the 1,398 patients with voluntary unrelated donors, 28 percent for the 91 patients with alternative family donors, and 33 percent for the 126 patients autografted in first CR. The nonrelapse mortality was 58 percent for patients with unrelated donors, 66 percent for patients with nonidentical family donors, and 25 percent for autografted patients. The relapse rate of 18 percent was relatively low for patients with nonidentical family donors, 41 percent for patients with unrelated donors and 55 percent for patients treated with autologous SCT. Both allogeneic SCT and autologous SCT have emerged as treatment options for patients with MDS. About one third of the patients transplanted with stem cells from histocompatible siblings and about one quarter of the patients with stem cells from other sources may be free of disease for three years or longer.

HIF-1057 Prognostic factors in multiple myeloma: contribution to clinical management

San Miguel JF, Almeida J, García-Sanz R, González M, Orfao A
Servicio de Hematología, Hospital Universitario de Salamanca, Spain

The main reason to study prognostic factors in MM should be the identification of risk groups in order to facilitate the treatment decision-making process. Prognostic factors can be classified into three major subgroups: 1) characteristics of the malignant clone (morphology, immunophenotype, cytogenetics, DNA content, proliferative activity, oncogenes, multidrug resistance); 2) host factors (age, clinical performance, immune status number of CD4 & NK cells, idiootype reactive T cells); 3) features that reflect the tumour burden and disease complications (BM infiltration-percentage & pattern, circulating clonal cells-PC & B lymphocytes, clinical stage, anaemia, renal function, bone lesions, hypercalcemia, protein dysfunction, levels of BP-M, IL-6, CRP ...). Many of these factors are inter-related, and therefore of limited value. Using multivariate analysis, several groups have found that the best combination of variables to predict outcome is BP-M and the proliferative activity of PC. In our experience age and performance status also improve the prognostic assessment. Cytogenetics are emerging as one of the most important factors. The presence of either partial or complete deletions of chromosome 13 or abnormalities involving 11q have an adverse influence. By contrast, trisomies of 7, 11 and 13 as well as DNA-hypersploidy are associated with a favorable outcome. Molecular changes such as trisomies of 7, 11 and 15 as well as DNA-hypersploidy of chromosome 13 or abnormalities involving 11q have an adverse influence. Using multivariate analysis, several groups have found that the best combination of variables to predict outcome is BP-M and the proliferative activity of PC. In our experience age and performance status also improve the prognostic assessment. Cytogenetics are emerging as one of the most important factors. The presence of either partial or complete deletions of chromosome 13 or abnormalities involving 11q have an adverse influence. By contrast, trisomies of 7, 11 and 13 as well as DNA-hypersploidy are associated with a favorable outcome. Molecular changes such as trisomies of 7, 11 and 15 as well as DNA-hypersploidy of chromosome 13 or abnormalities involving 11q have an adverse influence.

HIF-1058 Syndecan-1: a new independent prognostic marker in multiple myeloma

Seidel C,* Sundan A,* Hjorth M,* Turesson I,* Dahl IMS,* Abildgaard N,* Wang A* Baretz M* for the Nordic Myeloma Study Group
*The Institute of Cancer Research and Molecular Biology, Norwegian University of Science and Technology, Trondheim, Norway; *Department of Medicine, Lidkping Hospital, Lidkping, Sweden; *Department of Medicine, Malmö University Hospital, Malmö, Sweden; *Department of Haematology, University Hospital, Trondheim, Norway.

Syndecan-1 is a member of a family of integral membrane heparan sulphate proteoglycans. In the bone marrow of myeloma patients, syndecan-1 is reported to be expressed on myeloma cells only, and it is also expressed on malignant plasma cells in peripheral blood. Previous studies have shown that syndecan-1 is shed from the surface of myeloma cells in culture and into human serum. In this study we have analysed serum levels of shed syndecan-1 in a large, well-characterised population of myeloma patients, to determine its relation to prognosis, and other variables at diagnosis. Serum samples taken at diagnosis from 114 myeloma patients were analysed by ELISA and by a competitive radioimmunoassay. Syndecan-1 was elevated in 74% of patients as compared with 28 healthy controls (median 643 U/mL and 148 U/mL, respectively, p<0.001). Syndecan-1 in serum correlated to serum creatinine, secretion of urinary M-component, soluble interleukin-6 receptor, C-terminal telopeptide of type I collagen (ICTP), β2-microglobulin, percentage of plasma cells in the bone marrow, stage and serum M-component concentration. In a multiple regression model creatinine and the percentage of plasma cells in the marrow were the best predictors of syndecan-1 (r²=0.18). In order to evaluate syndecan-1 as a prognostic marker in multiple myeloma, it was entered into a multivariate Cox regression model. It was not available for this analysis. Syndecan-1 was found to be a powerful independent prognostic variable, along with World Health Organisation (WHO) performance status and allumina corrected serum creatinine. In a recent world-wide overview, the Nordic Myeloma Study Group (NMSG), Department of Medicine (Haematology Section), University of Lund, Sweden

The Nordic Myeloma Study Group (NMSG), Department of Medicine (Haematology Section), University of Lund, Sweden

Multiple myeloma is a haematological malignancy for which no curative therapy is at present available. In Scandinavian countries half of the patients are over the age of 70 at the time of diagnosis. The main aim of standard therapy given to a myeloma patient should therefore be to prolong the progression-free survival and the quality of life with as few side effects as possible. Before starting therapy all patients should be carefully and thoroughly evaluated with regard to three questions: which patients should be treated? when should therapy be started? and what intensity of therapy should be chosen? For most elderly patients a combination of intermittent oral melphalan and prednisone (IMP) is still the best choice. During the last 20 years a number of randomised studies have compared the effect of different combination chemotherapy regimens vs IMP, but have not been able to show a definite advantage for any of these. The same was the finding in a recent world-wide overview. α-interferon has in several studies been shown - and recently also in an overview of more than 4000 patients - to prolong progression-free survival with a median of 6 months, but not the overall survival. Studies of the quality of life of interferon-treated patients, using the EORTC QLQ-C30 questionnaire, has verified the negative sideeffects of interferon therapy, at least during the first 6 months of therapy, and when overall survival is used as the end-point this therapy seems not to be cost-effective. Recently high-dose therapy with ABMT or peripheral stem cell support (PBSC) has been introduced as a therapeutic alternative for younger patients with myeloma. The NMSG has in a population-based study of intensive therapy (VAD x 3, cyclophosphamide, melphalan and PBSC), given to myeloma patients below the age of 60, found a significant improvement in overall survival when compared to historical controls (60 vs 44 months). Since it seems possible to control the toxicity of such a regimen seem quite well, this approach may be considered also for selected patients above this age.

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HIF-15 Multiple myeloma

250
**HIF-1061 Arsenic compounds induce apoptosis in multiple myeloma (MM), activate pro-caspase-3 but do not affect BCL2 family members**


Memorial Sloan-Kettering Cancer Center, New York, NY and University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

Arsenic compounds are currently used to treat patients with MM. We have shown that arsenic compounds induce apoptosis in MM cell lines and that this effect is mediated by the activation of pro-caspase-3 but not by the activation of BCL2 family members.

**HIF-1063 Proteins involved in the regulation of cellular iron homeostasis**


Howard Hughes Medical Institute, Children's Hospital and Harvard Medical School, Boston Massachusetts, USA

Iron is an essential nutrient and is potentially toxic, thus its homeostasis must be tightly regulated. Very little is known on how iron is secreted, stored and mobilized in vivo. In this study, we have investigated the roles of the transferrin receptor and the hemochromatosis proteins in the regulation of cellular iron homeostasis.

**HIF-1062 Mammalian iron metabolism: insights from animal models**

Levy JK, Montross LK, Andrews N.C

Howard Hughes Medical Institute, Children’s Hospital and Harvard Medical School, Boston Massachusetts, USA

Objective. To study the roles of the transferrin receptor and the hemochromatosis protein (HFE) in iron homeostasis. Design and Methods. Mutations were made in the murine transferrin receptor gene (Tfr) and the murine Hfe gene by homologous recombination in embryonic stem (ES) cells. The Tfr mutation removed exons encoding most of the cytoplasmic domain, all of the transmembrane domain and part of the extracellular domain of the transferrin receptor. Two Hfe mutations were made. One disrupted two exons encoding the central portion of the protein, to generate a null allele. The second mutation resulted in a single amino acid substitution, Cys282Tyr (C282Y), identical to the mutation found in human patients with hereditary hemochromatosis. Correctly targeted ES cell clones for each of these mutations were used to generate novel mouse strains carrying the mutations in their germines. Mice homozygous and heterozygous for each mutation were analyzed. Tissue iron (liver and spleen), serum transferrin saturation and red blood cell indices were measured for liver and offspring. Results. Tfr-/- animals died in utero, before embryonic days 9.5 and 12.5. They appeared to die from severe anemia. However, they also had increased apoptotic cell death in the developing nervous system. Mutant animals with all other genotypes survived. Tfr+/- mice had hypochromic, microcytic anemias, normal transferrin saturations and decreased tissue iron in both liver and spleen. Hfe-/- and Hfe C282Y/C282Y animals showed liver iron loading, increased transferrin saturation and splenic iron deposition. The phenotype was more severe in Hfe-/- than Hfe C282Y/C282Y animals. Erythropoiesis was unaffected by Hfe mutations. Animals homozygous for either of the Hfe mutations had mild increases in liver iron stores. Conclusions. Mutations in both Tfr and Hfe alter iron homeostasis in mice. The patterns of abnormal iron metabolism differ. These results place new constraints upon models for the pathogenesis of hemochromatosis.
The Netherlands
Pavia Medical School and IRCCS Policlinico S. Matteo, Pavia, Italy
Stoelwinder B,* Groenen-Döpp YAM,# Bos HJ,* Werre JM*
red cells of different age and on vesicles derived from old red cells.
bodies to different band 3 domains were used to explore their presence on
was not the only explanation. Therefore, we turned to a study in which anti-
ment of anticomplement activity
...per se, by loss of CD55 and CD59 in vesicles,
gene may be a leukaemia susceptibility gene. The male-specificity of this
dimorphism of HH, the leukaemia data supported our hypothesis the HH
born data showed that there is no genetic basis for the phenotypic sexual
diseases. As a marker of cell age the HbA1c-percentage was used. Results:
1) In fractions I to V the HbA1c-% increased from 3.7±0.54 to 6.7±0.59, p<0.016; 2) only antigenicity to antibody K10 was shown on old and not
on young cells. The same antigenicity was shown on vesicles (see table).

HIF-1064 Erythropoiesis, erythropoietin and iron
Cazzola M
Department of Internal Medicine and Medical Oncology, University of
Pavia Medical School and IRCCS Policlinico S. Matteo, Pavia, Italy

Congenital anemias due to ineffective erythropoiesis can be associated with
excessive iron absorption and progressive iron loading. Life-threatening iron
overload may occur in non-transfused patients with thalassemia inter-
media, congenital erythrocytopenic anemia type II (CDA II), and X-linked
congenital sideroblastic anemia (XLSA). The mechanism by which the erythroid marrow expansion induces a positive iron balance is unknown.
Recent observations suggest that HFE, an MCH-related protein which is
mutated in genetic hemochromatosis, may physiologically regulate iron
homeostasis by interacting with transferrin receptor (TfR) and thereby
inhibiting iron absorption. A soluble form of TfR is present in human plas-
a and the erythroid marrow is its main source. Erythropoietin not only
expands erythropoiesis but also increases the expression of TfR in individ-
ual erythroid cells. We studied 28 patients with thalassemia intermedia,
CDA II or XLSA whose Hb ranged from 6.6 to 12.5 g/dL. Soluble TfR lev-
els ranged from 3 to 12 times normal. As judged by serum ferritin, iron load
was closely related to the patient's age (r = 0.72, p < 0.0001) and the sol-
uble TfR level (r = 0.52, p < 0.005). Multiple regression analysis showed that 86% (p < 0.0001) of the variation in serum ferritin was explained by age (representing the duration of exposure to the risk) and by changes in soluble TfR (the putative determinant of iron loading). We also studied the effect of coinhentance of the genetic hemochromatosis HFE mutant allele C282Y in a separate group of XLSA patients. There was a significant high-
er frequency of the C282Y mutation in 18 unrelated XLSA hemozymytes than that found in the normal population. The relationship between iron overload and TfR levels suggests that the large amounts of soluble recep-
tor generated by erythroid marrow can impair regulation of iron home-
ostasis in patients with congenital anemias. Coinhentance of the HFE
mutant allele C282Y may be considered an additional risk factor for devel-
opment of iron overload in these patients.

HIF-1065 Domains of band 3 associated with senescent antigens can
be demonstrated on vesicles derived from old red cells
Bosman GJCGM,* Wilkieens FLA,* Bartholomeus IJP,* Roerdinkholder-
Stotewinder B,† Groenen-Döpp YAM,* Bos HJ,* Werre JM*#
*Blood Bank Geldersche Rivieren, †Biochemistry Dept. FMW KUN,
*Clinical Chemistry Laboratory Ziekenhuis Rijnstate, Arnhem/Nijmegen,
The Netherlands

Introduction. Death of red cells (RBC) can be explained by complement
dependent lysis followed by phagocytosis. During this process altered band
3 and naturally occurring immunoglobulins act as respectively senescent
antigen and senescent antibodies. Previous studies showed that a decrease
of anticomplement activity per se, by loss of CD55 and CD59 in vesicles,
was not the only explanation. Therefore, we turned to a study in which anti-
bodies to different band 3 domains were used to explore their presence on
red cells of different age and on vesicles derived from old red cells. Meth-
ods. RBC were fractionated into five fractions of different cell age (I-V)
by a combination of counterflow centrifugation and a density fractionation
technique (Percoll). Vesicles were isolated from whole blood by a cen-
trifugation procedure. Immunoblotting was performed with 9 different anti-
body directed to different band 3 domains of the erythrocyte membrane: (i)
Antibody Directed to Presence in Presence in

<table>
<thead>
<tr>
<th>Antibody code</th>
<th>Directed to band 3 epitope</th>
<th>Presence in fraction I-V</th>
<th>Presence in vesicles</th>
</tr>
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<tbody>
<tr>
<td>BII-136</td>
<td>25-35</td>
<td>++</td>
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<tr>
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<td>390-550</td>
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</tr>
<tr>
<td>HF12</td>
<td>840-911</td>
<td>+++</td>
<td>+</td>
</tr>
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*Domains 560 to 580 and 810 to 830 show senescent antigenicity.

Conclusions. This study shows that band 3 domains associated with senes-
cent antigen(s) can be lost from old red cells by the process of vesicula-
tion. Whether more anticomplement activity than senescent antigen activ-
ity is lost from (old) red cells (by vesiculation) has still to be determined,
as this could be an important part of the RBC death process.

HIF-1066 Frequency of HFE C282Y mutation in haemopoietic
malignancies
Dorak MT, Poynton CH, Burnett AK, Worwood M
Dept of Haematology, University of Wales College of Medicine, Cardiff,
UK

We have previously postulated that the gene causing hereditary haemoch-
matosis (HH) could be a leukaemia susceptibility gene (Dorak et al. Immunol
Cell Biol 1994; 72:435). Following the cloning of the HFE gene and
identification of its common C282Y mutation in HH, we examined the
frequency of this mutation in 116 patients with childhood acute lym-
phoblastic leukaemia (ALL), 110 adult patients with Hodgkin's disease
(HD), and 380 local newborns by PCR-RFLP. The frequency of chromo-
somes carrying the C282Y mutation (gene frequency) in newborns was
6.3% with no difference between males and females. The corresponding
frequencies in ALL and HD were 9.1% and 5.9%, respectively. The sex-spe-
cific frequencies in ALL were 12.7% in males and 4.7% in females
(p<0.04). In HD, there was a slight but nonsignificant increase in males
(6.9% vs 4.8%). The male-specific frequency of C282Y mutation in child-
hood ALL was higher than the control frequency (12.7% vs 6.3%, p<0.02;
odds ratio=2.2; 95% confidence interval =1.24-4.1). Only one newborn and
one leukaemic patient were homozygous for the mutation. The frequencies of
the possession of at least one copy of the mutation were also different
between male leukaemics and controls (23.8% vs 12.4%, p= 0.015).
These molecular results support previous observations that in heterozy-
ous carriers of the HH gene, the risk of haemopoietic malignancies is
increased in males (Nelson et al. Cancer 1995; 76:875). Whilst the new-
born data showed that there is no genetic basis for the phenotypic sexual
dimorphism of HH, the leukaemia data supported our hypothesis the HH
gene may be a leukaemia susceptibility gene. The male-specificity of this
association resembles that of the homogamous HLA-DRB4*01 association
(p=3.6 X 10-5) in the same group of patients.
HIF17 Differentiation and apoptosis therapies

HIF1067 ATRA followed by chemotherapy vs ATRA plus CT, and the role of therapy in newly diagnosed acute promyelocytic leukaemia (APL): results of APL 93 trial


In a first randomised trial (APL 91 trial) we showed that the combination of anthracycline-Ara-C chemotherapy (CT) gave better event free survival (EFS), fewer relapses and better survival than CT alone in newly diagnosed APL. We present here results of the APL 93 trial involving 93 European centres. The aim of this trial, open from April 1993 to October 1998, was to test the chronology of ATRA and CT or both for maintenance. Trial design. Pts with initial WBC <5000/mm³ were randomised between ATRA (45 mg/m²/d, 15 days/3 months, continuous 6 mp) and ATRA + methotrexate (15 mg/m²/week), or both.

Results. The first 500 pts from 93 centres included in APL93 trial before 1/1/98 are analysed here at the reference date of 1/1/2000. One hundred and twenty-five (125) pts (12.5%) achieved CR/CRh in the ATRA group, 128/500 (25.6%) in the ATRA+CT group and 127/500 (25.4%) in the CT group. For survival, the difference was significant for chemotherapy (p=0.001). For EFS, the difference was significant for chemotherapy (p=0.033). An adverse effect was found between ATRA and chemotherapy. Similar results were observed for EFS (p=0.05 and p=0.001). For survival, the difference was significant for chemotherapy (p=0.02), but not for ATRA (p=0.29). Only 3 of the 24 pts who were maintained with ATRA and chemotherapy in the high WBC group relapsed. Conclusions. Induction treatment combining ATRA and CT can consistently yield CR rates >90% on large multicentre bases. Our results also show, in terms of relapse, a benefit for ATRA+CT vs ATRA alone and for maintenance treatment combining intermittent ATRA and continuous 6 MP+ MTX, especially in pts with high WBC count at diagnosis.

HIF1068 ATRA and G-CSF in the treatment of t(11;17) PLZF-RAR α positive acute promyelocytic leukaemia


The combined use of retinoic acid and chemotherapy has led to an important improvement in cure rates in acute promyelocytic leukaemia. Retinoic acid forces terminal maturation of the malignant cells and this application represents the first generally accepted differentiation based therapy in leukaemia. Unfortunately, similar approaches have failed in other types of haematological malignancies suggesting that the applicability is limited to this specific subgroup of patients. This has been endorsed by the notorious lack of response in acute promyelocytic leukaemia bearing the variant t(11;17) translocation. Based on the reported synergetic effects of retinoic acid and the haematopoietic growth factor G-CSF, we studied maturation of t(11;17) positive leukaemia cells using several combinations of retinoic acid and growth factors. In cultures with retinoic acid or G-CSF the leukaemic cells did not differentiate into mature granulocytes, but striking granulocytic differentiation occurred with the combination of both agents. At relapse, the patient was treated with retinoic acid and G-CSF prior to re-induction chemotherapy. With retinoic acid and G-CSF treatment alone, complete granulocytic maturation of the leukaemic cells occurred in vivo, followed by a complete cytogenetic and haematological remission. Bone marrow and blood became negative in fish analysis and semi-quantitative PCR showed a profound reduction of PLZF-RARα fusion transcripts. This shows that (t11;17) positive leukaemia cells are not intrinsically resistant to retinoic acid, provided that the proper co-stimulus is given. These observations may encourage the investigation of combined use of ATRA and haematomatopic growth factors in other types of leukaemia.

HIF1069 Arsenic treatment in human haematological malignancies

Dregan L, Dombeert H, Department of Haematology, Hôpital St Louis, Paris, France

Arsenic was proposed in the 19th century for the treatment of haematological malignancies. The drug was given orally until the toxic dose was reached. Chinese researchers from Mandchouria, have proposed trioxide of arsenic (as arsenic sulphate) for the treatment of acute promyelocytic leukaemia. They started trials in 1971 and two teams have treated more than 140 patients with 10 mg of As2O3 daily until complete remission. The results were 75% of CR in the novö patients and 50% of CR in relapsed patients. The treatment was applied from 1996 in Shanghai (Rui Jin Hospital), and was applied in 1997 in New York (Memorial Sloan Kettering Cancer Center) and in 1998 in Paris (St Louis Hospital). Results in relapsed patients (Shanghai, New York and Paris) showed more than 90% of CR without any dramatic adverse effect. From these data the Shanghai team started to treat de novo patients obtained a similar complete remission rate while a liver adverse effect (fulminant hepatic failure) was observed. In Paris, according to the outcome obtained in mice transplantated with human t11;17 APL cells, a trial was launched ATRA and As2O3. The question of stopping the treatment at time of CR (Chinese criteria) or after bone marrow remission (New York) is discussed in relation with the bone marrow toxicity of arsenic (dysmyelopoiesis) which could delay the normalisation of blood. Several animal and in vivo studies were performed and demonstrated the efficacy of As2O3 on cell lines and fresh cells from patients either in myeloid malignancies (chronic myeloid leukaemia) or in lymphoid maligancies (myeloma, HLTV acute Thymoplastic leukaemia, chronic lymphocytic leukaemia, Sezary syndrome). For these reasons trials started in Paris for chronic myeloid leukaemia, myeloma, HTLV1 ATL and chronic B lymphoid malignancies using As2O3. Results will be presented during the EHA meeting by the investigators. In conclusion, arsenic is an old drug being newly used for various haematological malignancies inducing apoptosis of malignant cells.

HIF1070 The phosphatidylinositol 3'-kinase inhibitor LY294002 increases chlorambucil- and radiation-induced apoptosis of B-CLL cells


Binding of cytokines, including interleukin (IL)-3 and 4, to their cell-surface receptors activates receptor-associated protein tyrosine kinases (PTKs). In turn, these PTKs activate phosphatidylinositol (PI) 3'-kinase, an enzyme that phosphorylates lipids in the 3-position of the inositol moiety. The consequent activation of protein kinase B following binding of these lipids plays an important role in mediating the anti-apoptotic function of cytokines. The cytoprotective action of protein kinase B is attributable in part to the phosphorylation and inactivation of BAD, a pro-apoptotic member of the BCL-2 protein family. Here we have investigated the ability of IL-4 and autologous plasma to protect B-CLL cells from apoptosis induction following treatment with chlorambucil or γ radiation and the ability of the PI 3-kinase inhibitor LY294002 to reverse this protection. We incubated purified B-CLL cells from twelve patients in RPMI 1640 medium supplemented with 50% foetal calf serum (basal medium). The cells underwent slow apoptotic death when deprived of the cytokine. Addition of LY294002 (25 to 250 µM) resulted in a dose-dependent increase in apoptosis induction. Addition of interleukin-4 (IL-4, 1-10 ng mL⁻¹) or the substitution of autologous plasma (5 to 50%) for foetal calf serum resulted in a dose responsive inhibition of both basal and LY294002-induced apoptosis. Chlorambucil (5 to 50 µg mL⁻¹) or γ radiation (3 to 20 Gy) induced dose-dependent apoptosis when BCLL cells were cultured in basal medium. IL-4 or autologous plasma dramatically decreased cell killing by either cytotoxic treatment. Addition of LY294002 to cultures containing IL-4 or plasma strikingly augmented apoptosis induction by chlorambucil or radiation. Cell killing by IL-4 and cytotoxic agents was supra-additive, suggesting a synergistic action. Herbimycin A (RMA), a selective PTK inhibitor, also synergised with the cytotoxic agents in inducing apoptosis. In conclusion, the observations here suggest that IL-4 and autologous plas-
ma oppose the induction of BCLL cell apoptosis via activation of a pathway comprising JNKs and PI 3'-kinase. Because plasma cytokines may present an obstacle between treatment and development of bone-marrow transplants, successful so far, (prophylactic) treatment with donor granulocytes. Cure can only be obtained with bone-marrow transplants, because attempts at gene therapy have been unsuccessful so far.

HIF-1071 VP-16 facilitation by diamide & protoporphyrin IX in leukemia: towards post-genomic apoptosis sensitisation
Fennell DA, Cotter FE
Molecular Haematology Unit, UCL, London, UK
Objective. Resistance to programmed cell death (apoptosis) remains a significant problem limiting the efficacy of cytotoxic drugs and radiotherapy. Dysregulation of apoptosis regulatory genes such as Bcl-2 and Bcl-x, endow cancer cells with a survival advantage when stimulated to undergo apoptosis. We have therefore sought to overcome apoptosis by pharmacologically modulating the downstream actions of these anti-apoptosis proteins. Results. SEMK2 is a highly-resistant infant biphagocytic leukemia cell line which expresses Bcl-2 and Bcl-xL at high levels and fails to undergo significant apoptosis (<20%) when treated with VP-16 (etoposide) over a 6 log range of concentrations as measured by collapse of mitochondrial membrane potential (ΔΨm) using DIOC(3-13) propidium iodide (PI) cytofluorimetry or caspase dependent (Z.DEVD.fmk inhibitable) cell surface phosphatidylserine (PS) expression (measured using fluoresceine isothiocyanate conjugated Annexum VI PI). In the presence of a sub-threshold concentration of 1 μM diazenediaenecarboxylic acid bis (5 N,N-iodide (PI)) cytofluorimetry or caspase dependent (Z.DEVD.fmk inhibitable) cell surface phosphatidylserine (PS) expression (measured using fluoresceine isothiocyanate conjugated Annexum VI PI). In the presence of a sub-threshold concentration of 1 μM diazenediaenecarboxylic acid bis (5 N,N-iodide (PI)), a biotilcrosslinking agent which has been shown to collapse ΔΨm, facilitation of VP-16 concentration dependent ΔΨm collapse and PS expression was observed in intact cells for 24 and 48 hours respectively. The median effective concentration Λ was reduced by approximtely 2 log-fold. Similar effects were observed with a sub-threshold concentration (100 μM) of protoporphrin IX (PPIX), an agonist of the peripheral benzodiazepine receptor and putative component of the mitochondrial permeability transition pore complex (PTPC). Direct dissolution of ΔΨm by the protophorone mCCCP produced a change in Λ of similar magnitude to VP16 in the presence of diamide and PPIX. Conclusions. Pharmacological modulation of mitochondrial PTPC presents an attractive and novel therapeutic approach for achieving apoptosis sensitisation by functionally antagonising the action of Bcl-2 like anti-apoptosis proteins.

HIF-1073 Gaucher disease: clinical picture, molecular diagnosis, and therapy
Zimran A
Shaare Zedek Medical Center, Jerusalem, Israel
Gaucher disease, the most prevalent lysosomal storage disorder, is due to an inherited deficiency of the enzyme β-glucocerebrosidase. There are 3 clinical forms: type I is characterised by the absence of neurologic involvement and has a predilection in Ashkenazi Jews, whereas type II, the acute form and type III, the subacute form, are both neuroopathic and panethnic. The large number of mutations detected so far in the glucocerebrosidase gene explains, in part the great phenotypic heterogeneity, which is in fact a hallmark of the disorder. With a population of over 400 patients in our clinic, we have had the opportunity to evaluate both common (i.e. hepatosplenomegaly, hyperpension and bony complications) and less frequently seen manifestations (i.e. lung involvement, cardiac lesions, immunological abnormalities, growth parameters in children). While the gold standard for the definitive diagnosis is based on enzymatic assay, this is an imperfect tool for the detection of heterozygotes, and it cannot predict prognosis. Hence, molecular analysis is used both as an adjunct for ascertaining of carrier status as well as for approximation of disease severity. PCR-based methodology has allowed large scale screening for Gaucher disease, which is problematic where not geared to at-risk populations. Enzyme replacement therapy has revolutionised the management of symptomatic patients. Therefore Gaucher disease has become a model for metabolic disorders and their treatment modalities, both at the level of basic research and at the level of society and ethical ramifications of emerging medical technologies. Varying regimens have been successful in affecting the haematological and visceral features of the disease; on the other hand the effect on bones and lungs is less predictable. Nonetheless, the high cost and the need for repeated intravenous infusions highlight the need for new therapeutic approaches and modalities, among which substrate inhibition and gene transfer are currently being investigated.

HIF-1074 Diseases associated with neutrophil hyperactivity: the double-edged sword phenomenon
Matzner Y
Hadassah Medical Organization, Jerusalem, Israel
While playing a major role in host defense by eradicating infectious agents, neutrophils are also involved in the pathology of various inflammatory conditions. The systemic inflammatory host response to an inciting event is termed systemic inflammatory response syndrome (SIRS). It is attributed not only to the release of neutrophil granule content and reactive O2 species, but also to the production of both proinflammatory (TNF-α, IL-1α, IL-1β, IL-6, IL-8, IFN-γ) and growth factors like G-CSF, GM-CSF, IL-3) and anti-inflammatory cytokines (i.e. TGF-β, as well as their antagonists (i.e. IL-1 receptor antagonist). Such an uncontrolled inflammatory response is evident in severe sepsis, sepsis, sepsis, sepsis, reperfusion injury, cardiopulmonary bypass, etc. Therapeutic attempts with antiinflammatory agents, anti TNF-α and O2-dismutase have been largely unsuccessful but hepatitis sering and serine protease inhibitors (traysol) may limit inflammation induced damage. Uncontrolled inflammation also contributes to the clinical picture in certain non-haematologic disorders such as autoimmune diseases, asthma, psoriasis, Sweet syndrome, Behçet syndrome and familial Mediterranean fever (FMF). Anti-inflammatory agents may modify these conditions. The most beneficial effect was observed in FMF where C5a IL-8 inhibitor deficiency, that results from mutation in a transcription factor encoding gene (NMFV), was described as the cause of the inflammatory attacks. In these cases, continuous colchicine therapy suppresses neutrophil motility toward serosal areas prone to uncontrolled inflammation due to unopposed C5a/IL-8 release. More potent anti-inflammatory agents are currently being developed.

HIF-1075 Intravenous immunoglobulin blocks nitric oxide synthesis and activation-induced apoptosis in THP-1 macrophage cells
Williams MA, Rhoades CJ, Lewis A, salmon KC, Kelsey SM
Dept of Haematology, St Bartholomew’s and The Royal London School of Medicine and Dentistry, Queen Mary and Westfield College, London, UK
Intravenous immunoglobulin (IVig) down-regulates pro-inflammatory cytokine secretion but promotes the release of anti-inflammatory mediators such as IL-10. By contrast cytokines such as GM-CSF and IFN-γ prime and activate macrophages. We have studied the effects of GM-CSF, IFN-γ and IVig on activation of and nitric oxide (NO) production by the human macrophage cell-line THP-1 in response to LPS, GM-CSF and IFN-γ-induced NO production by macrophages but their predominant effect was to prime...
macrophages for further generation of NO in response to LPS. The priming effect was mediated via up-regulation of inducible nitric oxide synthase (iNOS) expression. IFN-γ activated NF-κB (a known transcription factor for iNOS) and AP-1 (Jun-fos, a putative transcription factor for NO). However, only AP-1 was activated by GM-CSF. Hyperactivation of macrophages by LPS reduced cell proliferation and enhanced the degree of apoptosis, an effect that was further augmented by IFN-γ (but not GM-CSF) priming. This is consistent with the finding that monocytic apoptosis is increased in septic patients (Williams et al., Infect Dis 1998; 178:1921-33). IVIg almost completely blocked activation-induced secretion of NO, whereas it only partly inhibited iNOS protein expression. IVIg also inhibited LPS-induced apoptosis, but not proliferation arrest. The activation of NF-κB and AP-1 in response to all agents was attenuated by co-culture with IVIg. The data suggest that the predominant effect of the cytokines GM-CSF and IFN-γ is to prime macrophages for activation-induced NO release by inducing iNOS synthesis. The effect of IFN-γ may be mediated via the transcription factors NF-κB and AP-1, whereas that of GMCSF is probably mediated through activation of AP-1 alone and is NF-κB independent. We conclude that IVIg may attenuate iNOS synthesis by inhibiting the activation of the transcription factors NF-κB and AP-1. However, we believe that the predominant effect of IVIg on NO production is more likely to be a direct inhibition of iNOS enzyme activity. IVIg also inhibits activation-induced macrophage apoptosis that is probably mediated in part by the down-regulation of NO production.

HIF-1076 Treatment with prednisolone reduces the expression of VLA-4 and ICAM-1 on circulating monocytes in healthy men

Dettke M, Dreer M, Rohrbach M, Leitner G, Höcker P Department of Clinical Transfusion Medicine, AKH Wien, Vienna, Austria

Treatment with high dose glucocorticoids induces a temporary decrease in the activation of circulating monocytes which may be related to an alteration in the expression of monocyte adhesion molecules (AM). To test this hypothesis, we conducted a randomised, placebo-controlled trial to determine the effect of a 3 day course of prednisolone (PRED) on the expression of the AM VLA-4 (CD49d), ICAM-1 (CD54) and LFA-3 (CD58) on circulating monocytes. Altogether 15 volunteers randomised into 3 groups participated in our study (2 treatment groups, 1 control group; 5 individuals/group). Once a day the treatment groups received a single i.v. infusion of PRED, either at a dose of 2.5 mg/kg or 10 mg/kg b.w, while the control group received placebo (NAC i.v.). The expression of AM was monitored before treatment, during the 3-day treatment course, and followed up for 2 days after the last PRED administration. The expression of AM was determined by dual color FACS analysis. While in the control group the expression of monocyte AM remained constant (individual variation of AM expression ≤15%), in both treatment groups the administration of PRED induced a profound decrease in the basel expression of CD54 and CD49-d on circulating monocytes. Compared to the expression at base line, at the end of the 3 day treatment course the level of CD54 showed a decrease by -35%±10% while CD49-d was reduced by -55% (for both AM p<0.05). This reduced expression of both AM was still detectable 2 days after PRED treatment (CD49-d: -17%±4%; CD49-d: -42%±8%). In contrast to the diminished expression of CD54 and CD49-d, the level of monocyte CD58 showed no alteration during or after PRED treatment. Our data indicate a profound reduction in the expression of selective monocyte AM during PRED treatment. Since CD54 and CD49-d participate in various cell-cell contact mechanisms relevant for monocyte activation, our data may help to explain the clinical benefit of PRED and possibly other glucocorticoids in the treatment of inflammatory disorders.

SS13 Stem cells and haematopoiesis

SS1077 Laminins containing α5 chain are expressed in bone marrow and are adhesive to multipotent haematopoietic FDCP-mix cells

Gu Y, Ekblom M Department of Cell and Molecular Biology, Biomedical Center, Uppsala Sweden and Department of Medicine, University Hospital, Uppsala, Sweden

Objective. Laminins are extracellular matrix glycoproteins which by cell adhesive interactions influence phenotype and functions of many types of cells. Laminins are heteromers composed of five α, three β and γ polypeptides. So far five α, three β and two γ polypeptide chains, and 11 variants of laminins have been proposed. Laminins interact in vitro with mature blood cells and malignant haematopoietic cells. However, most studies have been performed with laminin-1 (α2β1γ11) and its expression in bone marrow is unclear. We have therefore determined the nature of the laminin isoforms in the bone marrow. Methods and Results. Using an antisem which reacts with most laminin α chains we found laminins widely expressed in mouse bone marrow. However, laminin α1 chain, and consequently laminin-1 was not expressed. Laminin β2 chain was not either found in the bone marrow. Instead, laminin α2, α4 and α5 polypeptides were expressed, each with a specific distribution. Laminin α2, α4 and α5 polypeptides were localised in the walls of the arteriole, α5 chain was in addition expressed in the subendothelial basement membranes of the sinusoids, and laminin α4 chain was also expressed in the sinusoidal spaces. Northern blot analysis revealed expression of laminin α2, α4 and α5 chains in long-term bone marrow cultures, indicating upregulation of laminin α1 chain expression in vitro. Laminins containing α5 chain, in contrast to laminin-1, were adhesive to multipotent haematopoietic FDCP-mix cells. Integrin α6 and β1 chains mediated this adhesion, as shown by antibody perturbation experiments. Conclusions. Laminin-2 (α2β1γ1), laminin-8 (α4β1γ1) and laminin-10 (α5β1γ1) are expressed in bone marrow. Laminin-10 was adhesive for multipotent haematopoietic cells, and this could be mediated by integrin α6β1.

SS1078 Molecular approach of the c-kit stem cell factor interactions during human embryonic haematopoiesis


Objective. Receptor tyrosine kinases (RTKs) mediate cellular responses to the extracellular signals involved in the regulation of proliferation and differentiation. Ligand binding initiates a cascade of reactions such as receptor dimerisation and autophosphorylation at specific tyrosine residues. The c-kit proto-oncogene encodes a RTK for stem cell factor (SCF) and both play a critical role in the growth and differentiation of haematopoietic stem cells (HSCs). Design and Methods. We have investigated the expression of the c-kit and SCF genes and corresponding proteins in haematopoietic tissues during human embryogenesis. The analysis was performed using RT-PCR amplification on the mRNAs from human embryonic yolk sac (YS), aorta-gonads-mesonephros (AGM) region and early embryonic liver at the different stages of gestation (d24 to d65). The amplification products were separated by gel electrophoresis, transferred into Nytlon membranes than hybridised with the labelled specific probe. Quantitative Phosphofomager analysis of the hybridisation signals was normalised to β-actin gene. Results. We have observed the expression of c-kit gene in all studied tissues. In contrast the expression of SCF gene was only observed in AGM region (d24) and in liver (d28 to d34) at the high level. These results were confirmed using RNase Protection Assay (RPA). Moreover, immunochemical staining with fluorescent anti-c-kit and SCF antibodies demonstrated continuous expression of c-kit protein whereas the presence of SCF protein were observed in embryonic liver only between d34 and d36. Conclusions. This temporal and spatial restricted expression of SCF, corresponding to the beginning of human haematopoiesis in AGM region and the colonisation of the liver, indicate that this factor may be important to molecular characterisation of HSCs in AGM and liver. Moreover SCF gene may constitute the molecular and/or phenotypic marker for the migration of AGM progenitors to liver. Finally our results strongly suggest that the biological functions of c-kit receptor is restricted to early stages of human embryonic haematopoiesis.
SS-1079 Cytokine receptor profile of CD34+ cells in AML and B-lineage ALL and in their normal bone marrow counterparts

De Waelie M, Renmans W, Van der Gucht K, Schots R, Van Riet P
Depts of Laboratory Haematology and of Clinical Haematology, AZ-VUB, Brussels, Belgium

The survival, proliferation and differentiation of haematopoietic cells is at least partially regulated by cytokines. Some of them act on primitive precursors, others on multipotent or on committed precursors. Cytokines exert their biological functions through interactions with membrane receptors. We now studied the cytokine receptor profile of myeloid and B-lymphoid CD34+ cell subsets in normal bone marrow and compared the results with those obtained on CD34+ cells in AML and B-lineage ALL. Bone marrow aspirates of 6 healthy adults and of 11 AML and 10 B-precursor ALL patients were analyzed. Mononuclear cells were labeled with a CD34 antibody and with antibodies directed against c-kit, G-CSFR, IL-3R, IL-6R and IL-7R. The labeled samples were examined with flow cytometry. In normal bone marrow the myeloid and B-lymphoid CD34+ subsets were distinguished by their forward scatter. c-kit, G-CSFR and IL-6R were mainly found on the myeloid CD34+ subset. IL-7R was mainly present on the B-lymphoid CD34+ cells but the reactivity was low. IL-3R was found on both CD34+ subsets. Similar but more pronounced differences were found between AML and B-lineage ALL. CD34+ cell subsets in B-precursor ALL showed an upregulation of IL-3R and of IL-7R in comparison with B-lymphoid CD34+ cells in normal bone marrow. In AML CD34+ cells only a higher expression of IL-3R was found in comparison with their normal counterparts. In conclusion, myeloid and B-lymphoid CD34+ cells in normal bone marrow have a different profile of cytokine receptors. These differences are more pronounced in acute leukaemia and allow to distinguish AML and B-lineage ALL. Whether abnormal expression of cytokine receptors may be helpful for the detection of minimal residual disease remains to be determined.

SS-1080 Mobilisation of CD34+ haematopoietic progenitor cells in vitro by VLA-4 directed antisense oligonucleotides

Kroonenwett B, Lichterfeld M, Zöller M, Haas R
German Cancer Research Center, Heidelberg and Dept. of Internal Medicine V, University of Heidelberg, Heidelberg, Germany

The β1 integrin very late antigen-4 (VLA-4) plays a central role in mobilisation and homing of CD34+ haematopoietic stem cells since treatment of primates and mice with VLA-4-specific monoclonal antibodies resulted in peripheralisation of progenitor cells. In this study, we examined antisense oligonucleotides (ODN) directed against the mRNA of the α4 chain of VLA-4 as inhibitor of VLA-4 expression to reduce the adhesive properties of CD34+ cells. Antisense ODN were transfected into immunomagnetically enriched CD34+ cells from leukaemia patients after G-CSF-supported cytotoxic chemotherapy using the cationic lipid DOTAP. As assessed by dual-color immunofluorescence analysis in 6 experiments, a significant antisense-mediated downregulation of the VLA-4 expression of 29% (SD: 13.1%; p<0.01) was measured after 4 days in suspension culture. The specific antisense effect was confirmed by Western and Northern blot analysis. Downregulation of VLA-4 expression on CD34+ cells resulted in an inhibition of the colony-forming capacity in a semi-solid culture assay of 39% (SD: 12.8%; p<0.01) as well as in an significant inhibition of adhesion to IL-1β-stimulated endothelial cells of 29% (SD: 19%; p<0.05). This indicates a functional role of VLA-4 not only for adhesion but also for proliferation of CD34+ cells. Using Detergent-type long-term bone marrow nulei cultures as in vitro model for stem cell mobilization, a population of 0.8% (SD: 0.6%; p<0.05) of CD34+ cells was found in the supernatant of the cultures after transfection of the antisense ODN while no CD34+ cells could be detected before transfection and in the supernatants of control ODN-transfected cultures. This was reflected by an 30-fold increase of colony-forming cells in the supernatant of antisense-treated cultures compared to control cultures. There is evidence that systemic administration of α4-specific antisense ODN in mice showed an increased number of peripheral mononuclear cells expressing the stem cell antigen but the specificity has to be further clarified. In conclusion, VLA-4-directed antisense ODN are useful tools for studies on function of VLA-4 on CD34+ cells. In addition, treatment with VLA-4 antisense ODN could be useful to improve stem cell mobilisation in vivo especially in a subgroup of patients or healthy donors with inefficient mobilisation of CD34+ cells for autologous or allogeneic transplantation.

SS-1081 Differences in quality of CD34+ peripheral blood stem cells in whole blood transplants versus leucapheresis material

de Boer F, Dräger AM, van Hapener MJAM, Jonkhoff AR, Pinedo HM, Ossenkoppele GJ, Schuurhuys GJ
Departments of Haematology and "Medical Oncology, University Hospital Vrije Universiteit Amsterdam, The Netherlands

Background and goal. In a previous study we showed the occurrence of apoptosis as well as a loss of expression of L-selectin (CD62L), reported to be relevant for stem cell homing, on CD34+ cells after cryopreservation of leucapheresis material (1), indicating a loss of quality of CD34+ cells. In whole blood transplantation, 1 liter of unprocessed GCSF mobilised blood preserved for 1-3 days at 4°C is used. For both transplantation modalities cases of delayed platelet recovery are observed. Compared to transplantation with thawed cells cultured and 5-10 times less CD34+ cells are reinjected with whole blood, which nevertheless result in similar recovery. Our goal was to study whether differences in quality of CD34+ cells present sent in these transplants can account for this. Design and Methods. Patient group A: relapsed, resistant or high risk NHL receiving a myeloablative regimen consisting of BCNU, etoposide, ARA-C and melphalan (BEAM) followed by transplantation of frozen-thawed leucapheresis product. Patient group B: relapsed, resistant NHL who received a comparable (3 days) regimen (BAM) followed by transplantation with 1 liter of unprocessed whole blood. Apoptosis analysis: FACS analysis using Syto61® FSC in combination with 7-AAD (3) and CD34 APC. Recovery of marker expression: incubation in RPMI FC5% 10° C 37° C 5% CO2, 2 hours. Results. The mean percentage of non-apoptotic (Syto61® (7+7AAD)) CD34+ cells in transplants was 48% (range 27-71%, n=5) in thawed leucapheresis samples vs. 91% (range 88-95%, n=4) in day3 whole blood samples. The mean CD62L expression on non-apoptotic CD34+ cells in whole blood after 3 days was 52% which increased up to 80% (day 0 value 76%) upon incubation. In contrast, CD62L expression in thawed leucapheresis material was only 35% (fresh >70%), but no recovery of expression was seen. In addition c-kit, another marker possibly relevant for transplantation was less expressed on the leucapheresis material: 82% vs 45%. Conclusions. 4°C preservation of mobilised whole blood maintains the quality of the CD34+ cells better than a leucapheresis procedure followed by freeze/thawing; this might account for the successful transplantation with lower total numbers of CD34+ cells.

1. de Boer, Bone Marrow Transplant 1998; 22:1103.
2. Ossenkoppele, Bone Marrow Transplant 1996; 18:2058.

SS-1082 High extent of ex vivo expansion of bone marrow and cord blood LTC-IC and functional mature cells: application to large scale clinical applications

INSERM U417, Hôpital Saint-Antoine, Paris; Service d’Hematologie Biologique, Hôpital Trousseau, Paris, France

Considerable efforts are currently being made to define culture conditions which enable optimal expansion of haematopoietic cells. However, the majority of investigations to date have not been applied to cord blood expansion using clinical culture systems. The aim of this study was to evaluate the ex vivo expansion of normal CD34+ cells in gas-permeable polypropylene bags suitable for clinical use. Cells were cultured for 14 days in serum free medium supplemented with SCF, IL-3, IL-1, Flt3-1, G-CSF,MGDF or Epo. The bags supported the expansion of haematopoietic cells allowing mean expansions of up to 2193 fold for total nucleated cells, 140 fold for CFU-GM and 6 fold for LTC-IC. Increasing the initial cell concentration from 5x103 to 1.104 CD34+ cells/mL induced the production of granulocytic cells with terminal differentiation while simultaneously decreasing the overall extent of expansion of the white blood cells produced. The percentage of phagocytic cells was 30±2% in expanded fractions initiated at 5x101, 103 or 105 cells/mL and 45±6% in cultured cells obtained from starting fractions containing 5x103 cells/mL, as compared to 58±4% in normal controls. Similarly the expanded cells produced H2O2, although in lesser quantities than control cells. We investigated the possibility of freezing expanded cells. Total cell recovery was 45±4%, while recoveries of progenitors and stem cells ranged from 65 to 90%. These experimental conditions have been applied to cord blood expansion using a specific combination of Fl3, MGDF, SCF and G-CSF feeding to expansion rates of 900 fold for total cells, 97 fold for CFU-GM, 23 fold for BFU-e, 8 fold for LTC-IC and 8 fold for E-LTC-IC. This suggests the feasibility of expansion of all haematopoietic compartments in the context of cord blood and bone marrow transplantation, in clinically suitable conditions.
SS14 Cytokines

SS1083 Hailey cell adhesion to bone marrow fibroblasts via VL-4 / VCAM-1 is regulated by TGF-β
Internal Medicine I, Division Haematology, L. Boltzmann Inst. for Cytokine Res, University of Vienna, Vienna, Austria

In bone marrow (BM) of patients with hairy cell leukaemia (HCL), the hairy cells (HC) are associated with fibroblastoid cells and surrounded with fine reticulin meshwork. Such selective tissue localisation may be attributed to the adhesive and migratory properties of the HC and allow the cells to receive signals for survival and proliferation. We have recently reported that BM of HCL patients contain high levels of transforming growth factor-β (TGF-β) as compared to healthy donors (ND) (24.3±7.8 vs. 4.9±6.7 ng/mL). This cytokine plays an important role in regulating cell-cell and cell-matrix adhesion. Therefore, we investigated the in vitro interaction between the HC and BM fibroblasts (BMF) and the influence of TGF-β on this process. Co-cultures of peripheral blood leukocytes or purified HC and BMF demonstrated a striking adhesive property of the HC to BMF. Within few minutes, HC were observed to adhere to and migrate beneath the fibroblasts. This was followed by fragmentation of the adherent cells and formed cell survival and proliferation. Under the same culture conditions, cells from HD remained in suspension. Adhesion assays revealed that TGF-β further increases the adhesion of HC while neutralising anti-TGF-β antibodies inhibits it. Preincubation of the HC with antibodies against the α4 chain of VL-4 integrin (CD49d) or preincubating the BMF with anti-VCAM-1 (CD106) Ab inhibited the adhesion of HC. This inhibition could be augmented by anti-TGF-β Ab and reduced by TGF-β. FACs analysis and immunofluorescence revealed high expression of VL-4 on the HC. We conclude that the adhesion of the hairy cells to bone marrow fibroblasts through binding of α4-VLA-4 integrin to its ligand VCAM-1 may represent a key step in marrow infiltration with the malignant cells and that TGF-β may play an important role in this process.

SS1084 Effects of lenogastim and filgrastim on neutrophil chemotaxis in patients undergoing chemotherapy
Azzara A, Carulli G, Rizzuti-Gullaci A, Cecconi N, Capochiani E, Petriini M
Unit of Haematology, Department of Oncology, University of Pisa, Italy

Recently we reported the inhibiting effects of Filgrastim (F) on neutrophil mobility in 7 patients with non-Hodgkin’s lymphoma undergoing chemotherapy. The chemotactic chamber method with microscope filters and a very sensitive computer assisted image processing system – capable of giving several parameters about the kinetics of cell migration were used. That study showed that random motility (RM), was reduced from 73.9±7.6 mm to 54.7±5.2 mm (p=0.039) (n.v. 120-160), and the chemotactic wave, which for this function is typically detectable beyond the first plane, was not observed in patients treated with F. Administration of F induced a more pronounced reticulin meshwork. Such selective tissue localisation may be attributed to the adhesive and migratory properties of the HC and allow the cells to receive signals for survival and proliferation. We have recently reported that BM of HCL patients contain high levels of transforming growth factor-β (TGF-β) as compared to healthy donors (ND) (24.3±7.8 vs. 4.9±6.7 ng/mL). This cytokine plays an important role in regulating cell-cell and cell-matrix adhesion. Therefore, we investigated the in vitro interaction between the HC and BM fibroblasts (BMF) and the influence of TGF-β on this process. Co-cultures of peripheral blood leukocytes or purified HC and BMF demonstrated a striking adhesive property of the HC to BMF. Within few minutes, HC were observed to adhere to and migrate beneath the fibroblasts. This was followed by fragmentation of the adherent cells and formed cell survival and proliferation. Under the same culture conditions, cells from HD remained in suspension. Adhesion assays revealed that TGF-β further increases the adhesion of HC while neutralising anti-TGF-β antibodies inhibits it. Preincubation of the HC with antibodies against the α4 chain of VL-4 integrin (CD49d) or preincubating the BMF with anti-VCAM-1 (CD106) Ab inhibited the adhesion of HC. This inhibition could be augmented by anti-TGF-β Ab and reduced by TGF-β. FACs analysis and immunofluorescence revealed high expression of VL-4 on the HC. We conclude that the adhesion of the hairy cells to bone marrow fibroblasts through binding of α4-VLA-4 integrin to its ligand VCAM-1 may represent a key step in marrow infiltration with the malignant cells and that TGF-β may play an important role in this process.

SS1085 Effect of megakaryocyte growth and differentiation factor (MGDF) on globins and GATA family transcription factors. Differences between bone marrow and cord blood CD34+ cells
Division of Haematology, Policlinico Careggi, Florence; *Institute of Histology, University of Bologna; *Institute of Obstetric and Gynecology, University of Bologna, Italy

MGDF has been shown to enhance expression of β- and γ-globin chains in the erythro-megakaryocytic cell line NB4 (Bonsi et al. Br Haematol 1997). We cultured CD 34+ cells from bone marrow (BM) and cord blood (CB) in the presence of IL-3, SCF and Epo with and without MGDF. Its presence increased the number of GPA+ cells collected at any time (7, 11, 14 days) from BM, but not CB-derived cultures. Using semiquantitative RT-PCR analysis β-globin mRNA resulted similarly expressed in BM- and CB-derived cells and not influenced by MGDF that, on the contrary, increased expression of γ-globin in day 7 and day 14 BM and CB cultures respectively. Finally γ-globin mRNA was clearly increased by MGDF, although only in BM-derived cells. These data confirm that in combination with other growth factors MGDF not only displays a cooperative activity in the proliferation, but also a role in the expression of erythroid specific proteins such as globins. In this light we also studied the possible changes in the expression of transcription factors. Among GATA members, we observed that zinc-finger protein GATA-2 mRNA was not influenced by MGDF, while GATA-1 decreased progressively over the time of culture, and again MGDF was not effective either the cells were from BM or CB. On the contrary levels of GATA-3 and leucin-finger NF-E2 mRNAs were clearly increased in the presence of MGDF, although only in BM-derived cells. Roles of GATA-1 and -2 in erythropoiesis are well known; we also observed that GATA-3 could possibly participate to- and mean of MGDF function in these events. As the activity of MGDF was especially evident in BM cells and marginal in CB ones, this results may depend on different expression and/or affinity of c-mpl in BM and in CB CD34+ progenitors.

SS1086 Myeloblastin is involved in ex vivo expansion of primitive haematopoietic stem cells
Kobari L, Giarratana MC, Barrett C, Lutz P, Cayre Y, Douay L
INSERM U417, Hôpital Saint-Antoine, Paris; Service d’Hématologie Biologique, Hôpital Trousseau, Paris, France

Myeloblastin (mbn) is a serine protease involved in the control of normal haematopoietic stem cell (HSC) proliferation and in the leukemic transformation of haematopoietic cells. Our group has shown that inhibition of its expression by oligonucleotides antisense block HSC in G0/G1 phase. The aim of the present work was to study the role of mbn in the context of cord blood primitive HSC ex vivo expansion. Methods. AC133+ cord blood cells were treated by oligonucleotides sense, antisense and scrambled, in presence of SCF. After 3 days cells were washed and seeded in ex vivo serum free, stroma free expansion conditions, in presence of FLs-1 (100 ng/mL), MGDF (100 ng/mL) + SCF (100 ng/mL) + G-CSF (10 ng/mL). After 14 days of liquid culture, we evaluated the consequences of the inhibition of the expression of mbn on the level of expansion of LTC-IC and L-ETCs. Results. After 3 days of incubation with oligonucleotides, a significant difference was observed between the frequency of LTC-ICs in cells populations treated by anti-sense (1/6) vs non treated (1/13) vs scrambled (1/16), i.e 2 and 2.5 fold more LTC-IC in antisense anti-mbn treated cells. Similarly, after 14 days expansion, the frequency of LTC-IC, and E-LTC-ICs was superior in the antisense incubated cells vs scrambled (1/69 vs 1/794 vs 1/907 and 1/689 vs 1/1616 vs 1/1812, respectively). Indeed we observed 6.5 and 8.6 fold more LTC-ICs and 1.5 and 2 fold more E-LTC-IC in the cell population incubated with the antisense oligonucleotides. Our results show that treatment of AC133+ cells (which recognize 4% of CD34+ cells) with anti-sense anti-mbn allows enrichment of a quiescent population and a subsequent increase in LTC-IC and E-LTC-IC ex vivo expansion. Conclusions. Our data suggest that myeloblastin is a gene involved in the process of HSC into proliferation. Its expression is hopefully a new marker of a HSC subpopulation ready to enter into cycle. The understanding of such molecular mechanisms which control HSC expansion will allow a new approach of ex vivo manipulation of haematopoiesis.

SS1087 Cytokine-induced expansion of human CD34+ and CD34+ CD41+ marrow cells cultured on normal osteoblasts
Ahmed N, Khoekher MA, Hassan HT
Division of Biomedical Sciences, School of Health Sciences, University of Wolverhampton, England, UK

Thrombocytopenia remains a significant cause of morbidity in cancer patients undergoing allogeneic BMT that consumes millions of health care resources and prolongs hospitalizations. Stem cell mobilization has been used to overcome this problem. The autologous CD34+ mobilized stem cell harvest has been shown to reduce the time to neutrophil and platelet engraftment [1, 2]. The efficacy of mobilization regimens is dependent on the degree of hematopoietic stem cell mobilization. Therefore, understanding the mechanism of haematopoietic stem cell mobilization by cytokines is important. In this study, we examined the cytokine expression of human bone marrow stromal cells (BMSCs). We used six cytokines: GM-CSF, G-CSF, IL-3, IL-6, SCF, and TNF-α. We observed that TNF-α and IL-3 were the most powerful cytokines in stimulating BMSC proliferation. The results showed that TNF-α and IL-3 induced the greatest proliferation of BMSCs among the six cytokines tested. Our data demonstrated that the optimal concentrations of TNF-α and IL-3 for BMSC proliferation were 10 ng/mL and 100 ng/mL, respectively. The results of this study provide a better understanding of the cytokine expression of BMSCs and may help in the future development of novel therapeutic strategies for stem cell mobilization.
SS15 – Chronic lymphocytic leukaemia - Clinical

SS15 Chronic lymphocytic leukaemia - Clinical

SS1089 Allotransplants and autotransplants in chronic lymphocytic leukaemia (CLL)


A report of CLL Subcommittee on behalf of EBMT Chronic Leukaemias Working Party

Unité de greffe de cellules souches hématopoïétiques Hôtel Edouard Herriot, Lyon, France

CLL in younger patients could be an active disease with bad prognostic criteria taking into account Binet classification, bone marrow histology white cell doubling time, p2 micoglobulin and CD 23 levels and cytogenetic study (11p-;17p-). The therapeutic strategy for these patients include allo and autotransplants. We analysed data from the European Blood and Marrow Transplant Registry (EBMT) on the outcome of 360 transplants: 187 allologeneic and 193 autologous haematopoietic stem cells transplants in patients with CLL treated between 1983 and 1998. For this indication number of transplants performed in Europe is rapidly increasing (autotransplants: from <990 to >1993: 62%). Median age at transplant and median interval from diagnosis to transplant was 43 years (21-57) and 42 months (4-162) in allogroup and 50 years (29-66) and 38 months (2-215) in autogroup. Prior to conditioning, 60% of patients in allogroup and 93% in autogroup were in response (CR and PR). Eighty-five percent in allogroup and 72% in autogroup received peripheral blood progenitor cells (PBPC). For autotransplants, a negative cell selection was performed in 37% and in a positive CD34 cell selection in 13% of cases. We observed 10% and 3% of graft failure after allotransplantation and autograft respectively. In allogroup, 86% were transplanted from HLA-identical sibling and 5% from unrelated donors. Seventy-two percent of patients received cyclosporine and methotrexate and 19% a T-depleted marrow as Graft-versus-host disease (GVHD) prophylaxis. Thirty-nine percent of patients developed a grade 2-4 acute GVHD and 19% an extensive CGVHD. After transplant, Eighty-nine autotransplanted and 28 autotransplanted patients died. Three-year survival probability is 76% for auto group and 45% for allogroup. Risk of relapse at 3 years is 43% for auto group and 16% for allogroup. Transplant-related mortality is 13% and 50% for autotransplanted and allografted patients respectively. In Europe, increase of allogeneic and autologous transplants number for CLL oblige to evaluate this therapeutic strategy in this disease as it was performed in multiple myeloma.

SS1088 SCID-repopulating cells from human umbilical cord blood are maintained after 12 days culture in IL-3, IL-5, SCF, FLT-3-lTPO


Dept of Haematology/Oncology, University of Tübingen, Germany

Long-term repopulating haematopoietic stem cells are mostly not maintained by current culture techniques for human haematopoietic cells. Therefore, ex vivo expansion strategies have resulted in the loss of long-term repopulation capacity, of the grafts, and xenograft-mediated haematopoiesis was undetectable in the recipient animals after several weeks. We have tested the engraftment capacities of either uncultured or cultured human umbilical cord blood (CB) cells in NOD/SCID mice. CD34^+LIN^- cells from fresh CB were enriched at a purity of >98.5% using two consecutive MACS immunomagnetic separation steps (pos: CD34; neg: anti-B, T, NK mAb). 1.5 x 10^5 CD34+LIN- cells in either the TPO+ or the TPO- condition were expanded in xenogeneic SCF+IL-3+IL-11+TPO generated 9 CD34+ early and 15 CD34+ late megakaryocytic cells from one mouse CD34+ cell in presence of the osteoblast feeder layer. This is the highest expansion of CD34+CD41+ early megakaryocytic cells from human marrow or CD34+ cells reported so far in literature. Recently, transplantation of SCF+IL-3+IL-11+TPO-ex vivo expanded megakaryocytic progenitor cells as a supplement has been shown to accelerate platelet recovery by 3-5 days in mice. Therefore, the clinical use of the combination SCF+IL-3+IL-11+TPO for ex vivo expansion of CD34+ stem/ progenitor and megakaryocytic progenitor cells from a portion of the donor's marrow harvest is warranted in allogeneic BMT. Such a protocol would accelerate platelet recovery and shorten the period of hospitalisation after allogeneic BMT and consequently may prove to be cost-effective. The present study has confirmed the role of human osteoblasts in supporting the proliferation and maintenance of human CD34+ stem/ progenitor marrow cells. Given the facilitating role of osteoblast shown previously in several allogeneic BMT studies in mice, it may be possible to envisage a future role for donor's osteoblast in clinical BMT. Using same donor's cultured osteoblast together with the ex vivo expanded CD34+ marrow cells might not only accelerate platelet recovery but also prevent acute GVHD in allogeneic BMT.

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SS1090 Autologous stem cell transplantation for CLL: parameters predicting feasibility and outcome


2nd Dept. of Medicine, University of Kiel; Dept. Haematology, AK St. Georg; Kiel and Hamburg, Germany

Fifty-two patients were accrued to a single-center study investigating autologous stem cell transplantation (ASCT) for treatment of poor prognosis CLL. The protocol comprises PBSC mobilisation using the DexaBEM regim and myeloablative therapy with TBI/CY followed by reinfusion of purged stem cells. Results. Forty-two patients (81%) had successful PBSC collection and were in a state of minimal disease after mobilisation. Ten patients not achieving these goals were regarded as protocol failures. Factors predicting for protocol failure were high Binet stage at mobilisation (B/C 33% failure vs. A 7%; p=0.017) and time from diagnosis to mobilisation (>12 months 26% vs. <12 months 6%; p=0.08). PFS was not reduced by either of these factors. In mega-PCR, 11q- and 17p- were detected in 54% and 93% in auto group were in response (CR and PR). Eighty-five percent in allogroup and 23% in auto group received bone marrow. Thirteen percent in allogroup and 72% in auto group peripheral blood progenitor cells (PBPC). For autotransplants, a negative cell selection was performed in 37% and a positive CD34 cell selection in 13% of cases. We observed 10% and 3% of graft failure after allotransplantation and autograft respectively. In allogroup: 86% were transplanted from HLA-identical sibling and 5% from unrelated donors. Seventy-two percent of patients received cyclosporine and methotrexate and 19% a T-depleted marrow as Graft-versus-host disease (GVHD) prophylaxis. Thirty-nine percent of patients developed a grade 2-4 acute GVHD and 19% an extensive CGVHD. Transplant-related mortality is 13% and 50% for autotransplanted and allografted patients respectively. In Europe, increase of allogeneic and autologous transplants number for CLL oblige to evaluate this therapeutic strategy in this disease as it was performed in multiple myeloma.

SS1089 Autologous stem cell transplantation for CLL: parameters predicting feasibility and outcome


2nd Dept. of Medicine, University of Kiel; Dept. Haematology, AK St. Georg; Kiel and Hamburg, Germany

Fifty-two patients were accrued to a single-center study investigating autologous stem cell transplantation (ASCT) for treatment of poor prognosis CLL. The protocol comprises PBSC mobilisation using the DexaBEM regimen and myeloablative therapy with TBI/CY followed by reinfusion of purged stem cells. Results. Forty-two patients (81%) had successful PBSC collection and were in a state of minimal disease after mobilisation. Ten patients not achieving these goals were regarded as protocol failures. Factors predicting for protocol failure were high Binet stage at mobilisation (B/C 33% failure vs. A 7%; p=0.017) and time from diagnosis to mobilisation (>12 months 26% vs. <12 months 6%; p=0.08). PFS was not reduced by any of these factors. In mega-PCR, 11q- and 17p- were detected in 54% and 93% in auto group were in response (CR and PR). Eighty-five percent in allogroup and 23% in auto group received bone marrow. Thirteen percent in allogroup and 72% in auto group peripheral blood progenitor cells (PBPC). For autotransplants, a negative cell selection was performed in 37% and a positive CD34 cell selection in 13% of cases. We observed 10% and 3% of graft failure after allotransplantation and autograft respectively. In allogroup: 86% were transplanted from HLA-identical sibling and 5% from unrelated donors. Seventy-two percent of patients received cyclosporine and methotrexate and 19% a T-depleted marrow as Graft-versus-host disease (GVHD) prophylaxis. Thirty-nine percent of patients developed a grade 2-4 acute GVHD and 19% an extensive CGVHD. Transplant-related mortality is 13% and 50% for autotransplanted and allografted patients respectively. In Europe, increase of allogeneic and autologous transplants number for CLL oblige to evaluate this therapeutic strategy in this disease as it was performed in multiple myeloma.
Fludarabine (FLU) seems promising, a new drug for the treatment of B-CLL. In October 1994 we started a randomised prospective multicentre study to compare effectiveness and toxicity of FLU vs 23 mg/sm by iv, hr infusion daily for 5 consecutive days q 4 weeks, versus Chlorambucil (CHL) 30 mg/ml sm orally on day 1 and 15 plus Prednisone (P) 40 mg/sm i.m. on day 1 and 15 plus 19 and 23 mg/sm q 4 weeks. Previously untreated patients, with active B-CLL, RAI intermediate or high risk, entered the study. Patients who received at least 6 courses of chemotherapy were evaluated for response after the sixth course. Patients in CR received two further courses of chemotherapy, patients in PR respectively, stopped treatment and were evaluated for survival. The main objective was to achieve response. One hundred and fifty eligible patients entered the trial: 75 were randomised to receive FLU and 75 to receive CHL+P. At present 128 patients are enrolled (NCl criteria), 65 in FLU arm and 63 in CHL+P arm. According to intention to treat, response rate (CR+PR) was 68% (43.2%) in FLU arm and 66% (37.2%) in CHL+P arm (p=0.6). Refractory CLL (SD+PD) were 19% (9.5+9.5) and 17% (12+5) respectively. Toxicity was acceptable and comparable in the two treatment groups.

**Results.** Two hundred and sixty-four patients entered the trial: 75 were randomised to receive FLU and 75 to receive CHL+P. At present 128 patients are enrolled (NCl criteria), 65 in FLU arm and 63 in CHL+P arm. According to intention to treat, response rate (CR+PR) was 68% (43.2%) in FLU arm and 66% (37.2%) in CHL+P arm (p=0.6). Refractory CLL (SD+PD) were 19% (9.5+9.5) and 17% (12+5) respectively. Toxicity was acceptable and comparable in the two treatment groups.

**Discussion.** The results confirm the higher efficiency of FLU compared to CHL+P in the treatment of untreated CLL in comparison with CHL+P, nevertheless further investigation is needed to evaluate the clinical benefits of therapeutic results.

**Conclusions.** Fludarabine exposure was associated with a decrease of p27 expression. By Western blot and assessed apoptosis in B-CLL but there are very few data on the mechanism involved. We observed an increased spontaneous survival of B-CLL cells expressing high p27 levels in contrast to cells that expressed low p27 levels. Interleukin-4 (IL4), which promotes B cell survival, was found to upregulate p27 in B-CLL cells in vitro. Conversely, exposure to proapoptotic drugs was shown to decrease p27 expression. In 20 samples from B-CLL patients, after a 5 day in vitro exposure to Fludarabine, we studied p27 expression by Western blot and assessed in vitro sensitivity to Fludarabine by viability, TUNEL assay and MTT assay. In all cases, restoration of apoptosis after fludarabine exposure was associated with a decrease of p27 expression. The extent of in vitro apoptosis was very significantly correlated to the intensity of p27 decrease. Arsenic trioxide (As2O3) has been demonstrated to have an important role in the treatment of B-CLL. It is able to induce the apoptosis of leukaemic cells but its mechanism of action in such a non proliferative disorder remains unclear.

**References.**


2. B-cell chronic lymphocytic leukaemia (B-CLL) is considered as a malignant disorder characterised by an accumulation of resting clonal B lymphocytes related to a defect in differentiation. Fludarabine is a potent drug for the treatment of B-CLL. It is able to induce the apoptosis of leukaemic cells but its mechanism of action in such a non proliferative disorder remains unclear. The cyclin dependent kinase inhibitor p27kip1 is known to play an important role in GO/G1 cell cycle arrest. We reported recently that high p27 expression in the presence of As2O3 are underway and will be presented.


In our institution we have enrolled 298 patients into 3 trials of the French national study group on B-CLL: the LLC 88, 85 and 90 trials. Forty-two patients received FAMP at some time of their treatment (FAMP+ group) and the other 256 never received this drug (FAMP- group). Comparison between these 2 groups shows minor differences in age, medullary lymphocytosis and serum LDH level but allocations of patients according to the Binet and Rai's classifications are similar. We have noted 14 RS among the FAMP- group and in contrast 6 RS among the FAMP+ group, i.e. 5.3% versus 14.3% (p=0.03). Diagnosis was done between the 21st and 36th month of the survey (median 37) in the FAMP- group and between the first and the 21st month (median 41) in the FAMP+ group. The actuarial risk of occurrence of RS is statistically higher among the FAMP+ group: 39.5% versus 11.5% (p<0.03).

Furthermore circumstances of diagnosis of RS were disturbing among FAMP+ group: 1) Two cases were recognised during treatment nodal cervical RS after the second course for a relapse of CLL and pulmonary RS with a severe tracheal stenosis also after a second course in a front line treatment; 2) The other RS were noted during the interval FAMP – from 7 to 16 months - while the control of CLL was excellent: 3 patients were in clinical CR and the other in a very good PR. Clinical and biological data were 'explosive and massive' with 2 medullary and blood involvement. These 4 patients died in less than 3 months. Although American authors have recognised the risk of occurrence of RS after ABMR for CLL and have emphasised the increased risk of NBL among patients with a hairy cell leukaemia treated by purine analogues, the risk of RS among B-CLL patients treated by FAMP is not well established. In contrast immuno-suppressive effects of FAMP are well recognised and as mentioned by Pocock, Catovsky et al. (ASH 1998 - Abst. 1774), this drug may 'give a bit of a boost' towards the emergence of a deviant lymphoid clone.

**Conclusions.**

We conclude that Campath-1H is an effective and relatively safe drug in this population of high risk, advanced, Fludara-refractory patients. It is an effective and relatively safe drug in this population of high risk, advanced, Fludara-refractory patients.

**References.**


**Objective.** Campath-1H is a humanised and -CD52 monoclonal antibody, which has demonstrated marked activity against advanced refractory CLL. This multicenter phase II clinical trial sought to establish the level of activity against CLL patients (pts) exposed to alkytating agents and refractory to fludarabine (Fludara), a group with an extremely poor prognosis. All pts who had no response to or had relapsed >6 months after last Fludara) and 56 received the full course of Campath-1H therapy as planned. Progressive disease (6.6%), adverse events (AEs) (6.5%), death (4.3%) and other causes (1.3%) led to premature withdrawal. The overall intent-to-treat response rate was 33% (95% CI: 24-43%) with 2 (7%) complete responses, 29 (31%) partial responses and 55 (59%) stable disease. Responses were least common with bulky disease. With a median follow-up of nine months, 26 (28%) pts have died, only 9 of them with infections (of which 6 were considered possibly related to Campath-1H), 11 occurred within one month of the last Campath-1H dose. The most common AEs were fever and rigors (89%), nausea + vomiting (50%), rash (13%), fatigue (29%), and dyspnea (24%), the vast majority of these were of grade 1 or 2 severity. Neutropenia and thrombocytopenia occurred in half of the pts usually improving within 1-2 months after discontinuing therapy. Anaemia was not a consistent problem. Infection occurred in 53% of pts, one-third being grade 3-5, including pneumonia (5%), CMV (2%), candida (2%), sepsis (2%) and others 4%. Conclusions. We conclude that Campath-1H is an effective and relatively safe drug in this population of high risk, advanced, Fludara-refractory patients.
SS-1095 Platelets from neonates do not respond to collagen whereas von Willebrand factor-binding is increased

Kehrel B, Roberts S, Glauner M, Viehhaber H, Nowak-Gött U, Clemetson KJ
Dept. for Anaesthesiology and Dept. for Paediatrics, University of Münster, Germany; Theodor-Kocher-Institut, University of Berne, Switzerland

Background. The physiology of the haemostatic system of neonates is profoundly different from that of adults. Coagulation and fibrinolytic systems in neonates have been studied well. In contrast there remain many open questions with regard to neonatal platelet function. Neonatal platelets have been shown to be less reactive to thrombin, the thromboxane analogue U46619 and a combination of ADP and epinephrine by flow cytometry. The response of neonatal platelets to collagen and to ristocetin has not been measured by flow cytometry yet. Methods. In 10 healthy term neonates and 20 healthy control adults the dose-dependent exposure of CD62-P, as a marker for early-granule secretion, and of CD63, as marker of dense body and lysosome secretion, and fibrinogen binding, using directly FITC-coupled fibrinogen, induced by collagen (up to 1.5 µg/mL) and by the GPIIb-IIIa receptor agonist convulxin (up to 0.3 µg/mL) were measured quantitatively using FITC-coupled antibodies. Results. Neonatal platelets expressed normal amounts of glycoprotein GPIa/IIa, GPIb and GPIIIa. Neither CD62-P expression, nor CD63 expression, nor fibrinogen binding was induced by collagen (up to 1.5 µg/mL) in neonatal platelets whereas 0.5 µg/mL induced maximal activation of adult platelets. There was little response to the snake venom C-type lectin convulxin. In contrast ristocetin-induced WF-binding to neonatal platelets was increased significantly. Even 0.3 to 0.5 µg/mL ristocetin induced maximal WF-binding. In adults such a result would be typical for WDB2 or platelet type WF disease. 8.0 to 10.0 µg/mL ristocetin is necessary to induce maximal WF-binding in normal adult platelets. Conclusions. Decreased neonatal responses to collagen are not due to missing glycoprotein receptors but rather to receptor interactions and/or amplification processes in signal transduction. Knowledge of normal neonatal platelet function is essential for the reliable diagnosis of platelet function disorders.

SS-1096 Is innocent bystander immune suppression associated with remission of chronic immune thrombocytopenic purpura?

Andersson PO, Jacobsson S, Stockelberg D, Wadenvik H
Haematology section, Sahlgrenska University Hospital, Gothenburg, Sweden

Objective. Platelet autoantibodies in ITP are probably formed as a consequence of a cytokine network dysregulation. Innocent bystander suppression has also been demonstrated in experimental models of transplant rejection and normal tolerance. This phenomenon is associated with expression of TGF-β and/or Th2-cytokines. The mechanisms responsible for bystander suppression and its role in antigen-specific self-tolerance are poorly understood. We studied the degree T cell activation and the cytokine profile in chronic ITP patients, having a disease in active stage or in remission. Design and Methods. Forty-six patients with chronic ITP were divided into three groups depending on the platelet count (plt): <50 (29±13), n=8; 50-150 (101±26), n=22; and >150 (233±59), n=16. Eighteen healthy volunteers (plt 243±52) served as controls. Serum was assayed for IFN-γ, IL-2, IL-4, IL-10 and plasma for TGF-β1, using commercially ELISAs (R&D systems). B-cells, T-cells, CD4+Th-cells, CD8+T-cells, Nk-cells, cytotoxic T-cells and activated T-cells were enumerated by flow cytometry. Results. The serum levels of the Th1 cytokines IL-2 and IFN-γ were below the detection limit. Likewise, the Th2 cytokine IL-4 was not detectable or very low in both patients and controls. The serum level of the Th1 cytokine IL-10 was in the assay range, and a difference was seen between levels in patients and controls (1.8±0.6 and 1.4±0.4 pg/mL, respectively; p<0.05). TGF-β1 levels were higher in patients in clinical remission, i.e. plt <50 (23.52±28.8 ng/mL) both compared to patients with plt <50 (2.5±6 ng/mL; p<0.0001), patients with plt 50-150 (7.2±1.7; p<0.0001) and controls (9.6±1.1; p<0.01). No differences were seen between the three patient groups and the controls. However, the relative number of activated T-cells (CD25+CD4+) was higher in patients with plt <50 compared to the controls (26.2±14.8 and 16.5±4.0%, respectively; p<0.05). Conclusions. Chronic ITP in an active stage seems to be associated with increased frequency of activated T-cells, whereas high levels of TGF-β1 are seen during remission. An innocent bystander immune suppression is a provocative explanation for this finding. Possible expression of this cytokine by oral immune tolerance induction deserves to be explored in ITP.

SS-1097 Anti-Xa activities of high and low affinity fractions of heparin: effect of human umbilical, endothelial cells

Laird K, Mulylo B, Barrowcliffe TW, Gray S
National Institute for Biological standards and control, Potters Bar, UK

Unfractionated heparin was, fractionated into high affinity material (HAM) and low affinity material (LAM) based on affinity for antithrombin (AT). Although LAM has very low anticoagulant activity, it has been shown to possess antithrombotic activity on its own and potentiates antithrombotic activity of HAM in vivo. In our previous study it was found that the addition of LAM, up to threshold by weight, potentiated the anti-Xa activity of HAM in a platelet poor plasma (PPP) system. However, no enhancement was observed in a purified AT or platelet rich plasma (PRP) system. The present study investigated the effect of the presence of human umbilical vein endothelial cells (HUVEC) on the anti-Xa activity of LAM and HAM, using the purified AT, PPP and PRP systems. It was found that LAM potentiated the activity of HAM and that the potentiation seemed to be dose dependent. The addition of threshold by weight of LAM to HAM, in the presence of HUVEC, increased the activity by 23.7±9.1, 10.8±11.8 and 26.1±5.7% in the AT, PPP and PRP systems respectively. As both LAM and HAM bind to plasma proteins and endothelial cells, these data suggest that increasing the amount of LAM, allows a higher proportion of free HAM to exert its anticoagulant activity. This may be one of the mechanisms by which LAM contributes toward the in vivo activity of unfractionated heparin.

SS-1098 Platelet function assessment in normotensive and hypertensive pregnancies

Maietta M, Castelli I, Piccinini F*, Neri I*, Berti M*, Facchinetti F, Torelli G
Dept. of Medical Sciences, Sect. Haematology and *Dept. of Obstetrics and Gynecology University of Modena and Reggio Emilia, Modena, Italy

Objective. To study the platelet L-Ariginine (L-Arg)-Nitric Oxide (NO) system in normotensive and hypertensive pregnancies by comparing platelet aggregation according to Born to PFA-100™ System (Dade International), Design. Cross sectional study. Subjects. Thirty pregnant women, 14 normotensive (C) and 16 hypertensive (H), matched for age and gestational age. Diagnosis of gestational hypertension was made according to accepted clinical criteria. All women gave their informed consent and had not taken platelet active drugs for at least 2 weeks before participating the study. Methods. Platelet aggregation (PA) was studied on platelet rich plasma after stimulation with ADP 2 µmol, and expressed as percent of light transmittance with platelet-poor plasma as reference. For the study of platelet function with the PFA-100™ analyzer, 800 µL of citrated whole blood were aspirated through a capillary and a biologically active membrane coated with collagen and ADP. The time required to obtain full occlusion of a microscopic aperture cut into the membrane is reported as Closure Time (CT). PA and CT measurements were done at baseline and after incubation for 3 min with 50 µmol of L-Argin, in order to test the reactivity of the L-Argin-NO system, as previously described (Neri et al., Soc Gynecol Invest 1998; 5:192). For statistical analysis ‘t pair’ and ANOVA tests were used. Results. Overall data of 30 pregnant women showed that L-Argin incubation significantly decreased PA (from 77% to 70%, p=0.006) and increased CT (from 88 sec to 100 sec, p=0.007). If C and H subgroup were separately analyzed, however, we found some differences between PA and CT. Indeed no differences in PA between H and C were found neither at baseline nor after L-Argin incubation (respectively, 78%±7% and 65%±5%, p=NS). CT was significantly higher in H than in C before (95.9 sec vs 84 sec, p<0.05) as well as after L-Argin incubation (115 sec vs 92 sec, p<0.05). Discussion. Data from PFA-100™ confirm our previous reports that during pregnancy the L-Argin-NO pathway is involved in platelet function. However, in hypertensive patients a significant decrease in platelet function was shown by the PFA-100 TM, but not by traditional aggregation. Prospective studies are needed in order to assess if measurement of CT deserves prognostic value for early identification of women at risk for gestational hypertension.

Haematologica vol. 84 [EHA4 Abstract Book]; June 1999
SS1099 Influence of methylene-blue photoactivation on FFP, cryoprecipitate and cryopreservant clotting factors

Aznar IA, Bonanad S, Montoro JM, Hurtado C, Molina R, Soler MA, Casaña P, de Miguel A

*Congenital Coagulopathies Unit and "Transfusion Centre, Valencia, Spain

We analysed the influence of Methylene-Blue virus photoactivation treatment (MB) on clotting factor activities of fresh frozen plasma (FFP) units, as well as on the cryoprecipitate (CP) and cryopreservant (CS) derived from these MB units. MB treatment was applied according to the German Red Cross (Springe) procedure, by BIOMAT (Gnfts). The procedure involves thawing of FFP, MB addition (to 1 µM), incubation under stirring (1 hr, in dark), white light irradiation (1 hr; >45000 lux) and quick freezing below -30°C. From 60 units of FFP, 20 pools of 3 bags were prepared and split again in 3 bags. One of them was MB treated. Samples were obtained before and after MB procedure. The second bag was also MB treated, to obtain MB-CP and MB-CS. Control CP and CS were obtained from the third bag. FVIII, VWF:RCo, VWF:Ag, mutimeric structure of WVF, Fibrogenic F(I) and FXIII were analysed:

IU or mg per bag

<table>
<thead>
<tr>
<th>Control</th>
<th>MB Loss (%)</th>
<th>CP</th>
<th>Control</th>
<th>MB Loss (%)</th>
<th>(Plasma-Cp) Loss (%)</th>
<th>Control</th>
<th>MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIII:C</td>
<td>205 148 28 119 91 23</td>
<td>42 39</td>
<td>WVF:Rco</td>
<td>258 235 8 219 191 13</td>
<td>15 19</td>
<td>WVF:Ag</td>
<td>257 239 7 179 172 3</td>
</tr>
<tr>
<td>vWF:Ag</td>
<td>673 514 24 313 253 18</td>
<td>54 51</td>
<td>FVIII</td>
<td>282 261 7 88 76 14</td>
<td>68 71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no alteration of WVF multimeric structure, neither in the MB plasma nor in the MB-CP, MB-CS showed absence of HMW multimers. These results suggest that MB plasma is a useful therapeutic tool, and also that MB-CP might be useful for congenital deficiencies of the above shown factors, especially when there are no virus inactivated specific concentrates available. MB-CS, lacking HMW WVF multimers might be useful for FTI treatment. Pharmacocology follow-up of MB plasma, MB-CP and MB-CS treated patients should always be carried out, to confirm safety and efficacy.

SS1100 Acquired von Willebrand syndrome (AvWS) is highly associated with lympho-myelo-proliferative disorders: report on 211 cases of the international registry of AvWS

Federici AB, Rand JH, Bucciarelli P, Mannucci PM (on behalf of the ISTH-SSC on vWF)

Introduction. Acquired von Willebrand syndrome (AvWS) is a rare acquired bleeding disorder similar to congenital vWD in terms of laboratory findings. However, the diagnosis of AvWS can be very difficult and treatment has usually been started with a bleeding disorder similar to congenital vWD in terms of laboratory findings. The AvWS ISTH-SSC registry is an international registry with the purpose to collect clinical information about patients with AvWS. The purpose of this report was to analyse the association between AvWS and lympho-myelo proliferative disorders in patients registered in the AvWS ISTH-SSC registry.

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Conclusions. Our data demonstrate that AvWS is highly associated with LPD and NTD. Therefore laboratory testing for AvWS should be recommended when an excessive bleeding occurs in patients with LPD and NTD.
The objective of the study was to investigate the molecular mechanisms responsible for the absence of immunoglobulin heavy chain (IgH) gene expression in an Ig subset of multiple myeloma (MM). Bone marrow, peripheral blood or tissue biopsies were obtained from 12 MM patients without production of IgH protein chains at initial diagnosis or during the disease course. Based on morphological and immunophenotypic analysis the percentages of malignant plasma cells were estimated between 30% and 90%. In two patients plasma cell leukaemias were diagnosed. The IgM cells in all 12 patients did not contain IgH protein chains, while IgG-chains and IgA-chains were found in the plasma cells from eight patients and one patient respectively. Southern blot analysis with the IgH 6 probe revealed clonal IgH gene rearrangements in 11/12 patients on one allele (10 patients) or both alleles (one patient). In one case the JH and Cμ region were deleted on both alleles. Based on the percentages of malignant plasma cells and CμAL the relative density of the rearranged and germline bands was calculated. A count estimate that at least four of the 10 patients followed monoclonal IgH rearrangements the second allele was deleted and that in the other six patients the second allele might be in germline configuration. Heteroduplex PCR analysis applying 6 VH family-specific primers and 7 DH family-specific primers in combination with a consensus JH primer enabled the identification and direct sequencing of eight of the total of ten IgH gene rearrangements identified. Three of the joining sites were complete in-frame VH(D)JH rearrangements with evidence of somatic hypermutation. In one of these cases the in-frame IgH gene rearrangement contained a stop codon in the junctional region, whereas the second allele was deleted. Five rearrangements reflected incomplete D¡¡JH joinings. The sizes of these DH¡¡JH rearranged bands on Southern blotting corresponded to the expected sizes as deduced from the genomic sequence. Curiously in four of these five cases the second allele was in germline configuration. The configuration of the IgH genes was further investigated by use of Cμ, Cγ, Cκ, and Cε probes. In five patients rearrangements in the JH and C regions were not concordant suggesting illegitimate recombination. Our data suggest that in the majority of multiple myelomas without IgH chain protein production illegitimate recombination, most probably reflecting chromosomal translocation to 14q32.3 is responsible for IgH gene deletion or separation of the rearranged D¡¡JH or DR(D)JH gene segment from C gene segments.

### SS-1104 Involvement of the p16INK4a-p19ARF gene locus in multiple myeloma cell cycle deregulation


*III Medizinische Klinik, Klinikum Mannheim and Universität Heidelberg, Mannheim and *MDizinische Klinik und Poliklinik V, Universität Heidelberg, Heidelberg, Germany.

Objective. In human cancers, the frequency of genetic alterations involving the p16INK4a-p19ARF gene locus is second only to alterations of p53. The p16INK4a-ARF locus encodes two unrelated proteins (p16INK4a, p19ARF) that inactivate the p16 locus is believed to be second only to alterations of p53. The reasons why the in-frame p16 expression is rarely detected by immunostaining of bone marrow smears. Further, we investigated cDNA derived from the same samples to examine the methylation frequency of the p16promoter. Therefore we sought to investigate a large set of bone marrow samples from patients with MM in order to elucidate the role of p16INK4a promoter hypermethylation and to study the role of p19ARF in the pathogenesis of MM. Methods. We used a highly sensitive bisulfite treatment of the DNA, followed by a PCR with methylation specific primers to distinguish between methylated and unmethylated DNA. The p16INK4a expression was studied on the protein level by immunostaining of bone marrow smears.

### Conclusions.

It might be speculated that, due to a translational stop, the abundant p19ARF transcripts are not translated, as it has been shown in several haematopoietic cell lines. The reasons underlying the increased transcription of p19ARF remain to be elucidated. Our data strongly support the hypothesis that silencing of p16ink4a by promoter hypermethylation contributes to the pathogenesis and the cell cycle deregulation in MM.

### SS-1105 Cyclin D1 overexpression in multiple myeloma is associated with advanced stages of the disease

Prunier G., Baldini L., Carboni N., Fabris S., Zagona S., Lombardi L., Bufla R., Maloio AT, Nerì A

Laboratorio di Ematologia Sperimentale e Genetica Molecolare, Servizio di Ematologia, and Servizio di Anatomia Patologica, Università degli Studi di Milano, OSPedale Maggiore IRCCS, Milan, Italy

The i1114(I13q22) chromosomal translocation, which is the hallmark of mantle cell lymphoma (MCL), is found in approximately 30% of multiple myeloma (MM) tumors with 14q32 translocation. However, breakpoints are generally dispersed over the 11q13 and rearrangements of the BCL-1/cyclin D1 regions frequently involved in MCL rarely occur in MM. Nonetheless, the overexpression of cyclin D1 has been found to be correlated with MM cell lines carrying the t(11;14) suggesting that this gene is the target of the translocation. To investigate the involvement of the cyclin D1 in primary MM tumors, we performed an immunohistochemical analysis on a series of 48 MM (40 at diagnosis and 8 at relapse) and 25 MGUS. The immunohistochemical analysis was performed using the monoclonal antibody DOCK6. The expression of the cyclin D1 was detected in 12 cases of MM (25%) and in 1 case of MGUS (8%) and was localised in the nucleus of the neoplastic plasma cells. The haematopoietic cells were usually unreactive. The comparison between the clinico-pathologic and immunohistochemical features indicated that the cyclin D1 expression was significantly associated with advanced clinical stages (p=0.023) and the presence of clinical symptoms (p=0.026). Moreover cyclin D1 expression was more frequently found in cases with intermediate high histologic infiltration (Barts stage IIIb, 35%) than in those with low infiltration (Barts stage IIb, 10%), whereas this difference did not reach statistical significance (p=0.127). FISH experiments are currently performed in our laboratory to correlate the overexpression of cyclin D1 with the presence of 11q13 break-points.
SS18  Acute myeloid leukaemia - Clinical

SS1107 Daunorubicin versus mitoxantrone as induction for AML in younger adults given intensive chemotherapy: preliminary results of MRC AML 12 trial
Burnett AK, Goldstone AH, Milligan DW, Prentice AG, Wheatley K on behalf of the MRC Adult Leukaemia Working Party

Between November 1994 and June 1998, 1243 patients aged 15-59 (and 26 aged 60-65) were entered into the MRC AML12 trial in 176 institutions in UK, Eire and New Zealand. They were randomly allocated to receive either ADE or MAE for course 1 and 2 after which all patients in CR received subsequent courses of chemotherapy (MACE + MAEC or MACE + ICE + MAEC). Results:

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>ADE</th>
<th>MAE</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>CR rate(%)</td>
<td>84</td>
<td>83</td>
<td>0.01</td>
</tr>
<tr>
<td>Induction death (%)</td>
<td>8</td>
<td>8</td>
<td>1.0</td>
</tr>
<tr>
<td>Resistant disease (%)</td>
<td>8</td>
<td>9</td>
<td>0.4</td>
</tr>
<tr>
<td>Death in CR (% at 3yrs)</td>
<td>13</td>
<td>12</td>
<td>0.9</td>
</tr>
<tr>
<td>Relapse rate (% at 3yrs)</td>
<td>47</td>
<td>51</td>
<td>0.4</td>
</tr>
<tr>
<td>DFS (% at 3yrs)</td>
<td>46</td>
<td>43</td>
<td>0.05</td>
</tr>
<tr>
<td>Survival (% at 3yrs)</td>
<td>47</td>
<td>48</td>
<td>0.4</td>
</tr>
</tbody>
</table>

There were no significant differences between the induction arms with respect to CR, reasons for failure, deaths in CR, relapse, DFS or survival. Survival was 47% at 3 years, but was related to age and risk group as defined in MRC AML 10 trial which was prospectively validated in this trial. Haemopoietic toxicity was significantly greater with MAE than ADE after courses 2 and course 2 (median days): neutrophils to 1.0 × 10^9/L, 26 v 17 days, platelets to 100 × 10^9/L, 32 v 18 days (both p < 0.001) and course 3: neutrophils to 1 × 10^9/L, 24 v 20 day, platelets to 100 × 10^9/L, 44 vs 33 days, resulting in increased supportive care (10 v 6 units of RBC, 29 v 18 units platelets: 15 vs 21 days on antibiotics; 29 v 21 days in hospital: all p < 0.001), and preventing 2nd randomisation (203 MAE vs 274 ADE). Mitoxantrone does not improve CR rate, increases haematological toxicity, but impact on longterm survival is unknown.

SS1108 Phase I randomised trial of all-trans retinoic acid (ATRA) in association with intermediate dose of cytarabine (Ara-C) and idarubicin (Ida) versus chemotherapy alone in patients with relapsed or refractory non promyelocytic acute myeloid leukaemia (AML)
Service d’Hematologie, Hopital Edouard Herriot, Lyon, France

To evaluate toxicity and antileukaemic efficacy of ATRA in patients (pts) with relapsed or refractory, non promyelocytic AML, 86 pts (median age 59 years (range 21-82 yrs) with FAB unclassified or secondary AML at diagnosis, resistant AML or in 1st and in subsequent relapse, received induction therapy with Ida, 10 mg/m^2 day, from day 1 to day 3 and araC, 1000 mg/m^2 12 hours, from day 1 to day 6, alone or associated, on a randomised basis, to ATRA, 45 mg/m^2/day, from day 1 to CR achievement. Pts in complete remission (CR) received maintenance therapy with 6 monthly courses combining Ida, 10 mg/m^2/day, on day 1 and araC, 1000 mg/m^2/day, subsequently, from day 1 to day 5. Results were evaluated after one induction course. Overall 51 pts (59%, 26 with ATRA and 25 without ATRA) achieved CR including 5 pts treated at diagnosis (10%), 6 previously resistant (12%), 35 in 1st relapse (72%) and 3 in subsequent relapse (61%). Thirty-five (41%) had resistant disease and 5 (6%) died from toxicity. Median time for neutrophils recovery to 0.5 × 10^9/L and platelets to 20 × 10^9/L was 30 and 21 days. Severe (WHO grade 2) toxicity included infections (37%), diarrhea (9%), bleeding (3%), vomiting (16%), hyperbilirubinemia (5%), mucositis (6%) and hypercreatininemia (2%). No severe obectivity was noted. Median Overall Survival (OS) for the entire cohort was 4 months and Median Disease Free Survival (DFS) 4 months. There was no statistical difference in terms of DFS and OS between the 2 arms. We conclude that ATRA in association to Ida and araC can be administered safely in pts with poor prognosis AML but did not seem to offer any advantage over the same chemotherapeutic drugs given alone.

SS1108 A multicentre study of a combination of fludarabine phosphate, cytosine arabinoside and granulocyte colony stimulating factor (FLAG) in relapsed and refractory acute myeloid leukaemia and de novo RAEB-T
The UK FLAG Collaborative Group

Eighty nine patients were entered from 19 centres (range 1-11), of whom 83 were eligible. Of these patients, 21 had AML with late first relapse (>6 months; Gp A); 44 had AML with primary refractory disease or early first relapse (<6 months; Gp B) and 18 had de novo RAEB-T (Gp C). All patients received the same induction chemotherapy schedule for one or two courses to CR: fludarabine phosphate 30 mg/m^2 as a 30 minute infusion 4 hours before cytosine arabinoside 2 g m^2 as a 4 hour infusion for 5 days. GCFS (30 mu) was given on days 1–6. Consolidation (1-2 courses) was with the same regimen for 4 days. Of the 83 eligible patients, 42 were male and 41 female. The median age was 49 years (range: 18-75) with 24 patients aged over 60. Complete remission (CR) was achieved in 17/21 patients in Gp A (81%); 13/44 in Gp B (30%); and 10/18 in Gp C (56%). In the majority of cases (35/40; 88%), CR was achieved after one induction cycle. One hundred and thirty three courses ofFLAG were available for toxicity assessment from 82 patients who received treatment. All were associated with WHO Grade IV neutropenia. Patients spent a median of 24 nights in hospital per course and received a median of 7 platelet transfusions per course. Two patients withdrew from treatment because of a significant increase in white cell count. A further patient withdrew due to venoocclusive disease. Seventeen patients died during the treatment phase of the study (9 due to infection (3 fungal), 2 due to disease progression, 1 due to haemoptysis and 1 cerebral haemorrhage). Thirty seven patients have died since completing the study, the majority (28) due to disease progression. Eight patients received an allogeneic transplant after FLAG treatment and 4 received a autologous transplant. Thirty two of the 83 patients are alive with a median follow up of 15 months. The median overall survival is: 17 months (Gp A); 3 months (Gp B); and 18 months (Gp C). Interim 5 month survival rates are 81% (Gp A), 34% (Gp B) and 78% (Gp C). FLAG appears to be safe and efficacious in these poor risk patient groups.
SS-1111 Acute promyelocytic leukaemia following a previous neoplastic malignancy. Experience of GIMEMA group


Objective. To evaluate the clinical and laboratory characteristics of adult patients affected by acute promyelocytic leukaemia (sAPL) developed after a previous malignancy (PNM).

Design. A retrospective study, conducted over a fourteen-years period (1984-1997).

Settings. Sixty-two haematology divisions in tertiary care or university hospitals.

Results. During the study period were observed 51 sAPL (M/F ratio 17/34, median age 57 y, range 27-76). The most frequent PNM was breast cancer (15 cases), followed by NHL (9) and uterus cancer (7). The median time from PNM diagnosis to sAPL was 36 months (range 8-366). PNM was treated in 13 cases (25%) with surgery alone. In the other 37 cases (73%), patients received chemotherapy (10 cases, 20%), radiotherapy (17 cases, 33%) or chemoradiotherapy combination (10 cases, 20%). One patient did not receive any treatment. M3 variant was found in 7 patients. Thirty-seven patients performed a cytogenetic study: 3 patients had a normal karyotype; 31 had t(15;17); in 3 cases failed. Molecular biology study was done on 36 patients: 21 were BCR1 positive, 3 BCR2 positive and 10 BCR3 positive. In 2 patients it failed. Other 15 patients did not perform molecular biology study. On the whole only in 6 patients diagnosis of M3 was based on morphological criteria only. All patients received a treatment for sAPL: 35 patients were treated with AIDA protocol (idarubicin plus ATRA), 8 with ATRA alone, and 8 patients received chemotherapy including antracycline (idarubicin or daunorubicin) plus cytarabine. Forty-three patients achieved a CR (840/o) and 8 patients died in induction (16%). The median duration of CR was 27 months (0-130), but the median survival of patients who achieved CR was 29 months (0-130). At the time of analysis (January 1999) 33 patients were alive in CR. Nine patients relapsed and died (2 haemorrhage, 4 for APL, 1 in aplastic anaemia, 1 for an intercurrent disease). One patient was lost at follow-up. Conclusions. Prognosis of acute leukaemia following another malignancy is characterised by a bad prognosis. In our series we observed that, contrary to the other SAML, the CR rate and the outcome of sAPL is similar to that observed in primary APL.

SS-1112 A population based study of karyotypic features associated with longer survival in patients over 60 years of age with acute myeloid leukaemia

Summersfield G, Bown N,* Jackson G, Taylor P
Department of Haematology and *Department of Cytogenetic, Newcastle University, Newcastle-upon-Tyne, UK

Introduction. In the Northern Health Region of the UK all haematological malignancies are in a central database as part of a population based clinical epidemiology (PACE) strategy. Regional policy is that all patients, regardless of age, should be given curative treatment if they are fit enough; since 1993 an all-oral combination chemotherapy (idarubicin and etoposide) has been offered to older patients who are not considered fit enough for IV treatment. Design and Methods. In the database there are 539 patients with AML aged >60 years, in 319 (59%) of these patients cytogenetic studies were performed at diagnosis. We examined the prognostic significance of karyotype in this group of patients in order to assist decision making about whether to offer curative or palliative treatment to older patients. In patients aged 60-69, 117 out of 225 (52%) were given treatment with curative intent. Forty-four (37.6%) of these survived >1 year and 15 (12.8%) survived >3 years. Of patients over 70, only 49 (29.5% of patients registered) received curative treatment and all these patients had a poor prognosis regardless of karyotype. Twenty-two (23.4%) survived >1 year but only 2 (2.1%) survived >3 years. In total, 17 patients survived >3 years, 8 with a normal karyotype, 1 had t(15;17), 1 t(5;6), 1 t(6;9) and 2 trisomy 8 and in 4 patients analysis failed or was not done. Forty-three patients not fit enough for IV (median age 72) received the oral combination and had a median survival (MS) of 3 months, however 8 survived >1 year and 7 survived >3 years. In comparison, 76 patients (median age 75) given other palliative treatment had a MS of 2 months, only 4 surviving >1 year (p<0.03) and 1 surviving >3 years (p 0.13). Conclusions. Prolonged survival (>3 years) in older patients given curative treatment can only be expected in patients <70 who have normal or "favourable" karyotype. The results of an oral combination given to patients considered not suitable for IV are promising and merit further study.
Plenary Symposium

PL-1113  In utero stem cell therapy
Zanjani ED
V.A. Medical Center, University of Nevada, Reno, NV, USA

Prenatal HSC therapy for the treatment of congenital diseases has tremendous theoretical appeal. Normal haematopoietic and immunologic development during ontogeny creates a window of opportunity during which events favor the engraftment of transplanted allogeneic HSC and their proliferation. Among the many advantages conferred by this window are 1) the potential for induction of donor specific tolerance which may be used to improve donor cell activity after birth; 2) the sterile, protective, foetal environment which provides isolation from environmental pathogens; and 3) prevention of clinical manifestations of the disease. Pre-clinical studies in large animal models have defined many of the parameters such as the optimal age of the recipient, route of administration and sources of HSC, necessary for the successful engraftment and/or expression of donor HSC. Although the foetus, during the window of opportunity, will readily permit the engraftment of cells from foetal liver, cord blood, and adult marrow or peripheral blood, the source of donor cells may be critical to the success of engraftment. The obvious advantages of foetal and cord blood cells, must be balanced against the practical and ethical concerns for their use. Although rich in HSC, these are not renewable sources of HSC, which limits their usefulness for in utero purposes. This is especially important since a significant number of a newborn transplanted foetal animals appear to be tolerant to donor cells allowing significant boost of donor cell activity by postnatal injections of donor cells. In contrast, the use of adult derived cells would allow a renewed, relatively infection free, ethically acceptable source of HSC. While, the use of postnatal cells is frequently associated with the development of lethal GVHD, in pre-clinical and clinical studies transplantation of CD34-enriched or highly purified populations of human adult HSC in utero resulted in engraftment and expression of donor HSC without graft failure and without GVHD. Although, these strategies have been used to successfully treat fetuses with X-SCID, they were not successful in diseases in which there is no selective survival or proliferative advantage for donor cells. A number of promising recent advances offer hope that the remaining challenging experimental and clinical obstacles to the widespread clinical application of in utero HSC transplantation can be successfully resolved.

PL-1114  Adoptive therapy with gene-modified T cell clones
Greenberg P, Yee C, Gilbert M, Lonergan M, Topp M, Nelson B, Lord J, Ohlten C, Cooper L, Riddell S
University of Washington and Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Our laboratory has pursued adoptive T cell therapy with large numbers of antigen specific T cell clones as means to treat human disease and identify the cellular requirements for effective therapy. Our studies in the prevention of CMV disease in immunosuppressed patients with haematolymphic malignancies undergoing bone marrow transplantation (BMT), in which patients receive infusions of CMV-specific CD8+ and CD4+ T cell clones demonstrates that antigen specific, donor, haematopoietic chimerism is capable of providing lasting disease control and/or amplifying deficient human immune responses and providing protection from disease by the transfer of cloned T cells specific for target antigens. We have recently applied these methods to the treatment of HIV infection and melanoma. Adoptive transfer of HIV-specific CTL genetically modified to express a marker gene, has demonstrated that such CTL can traffic appropriately to lymph nodes, localize to sites of infection, and mediate an antiviral effect. Adoptive therapy of patients with metastatic melanoma with CD8+ CTL clones specific for melanocyte-differentiated antigens has demonstrated that CTL can home to sites of tumour and eliminate tumour cells expressing the antigen. These encouraging studies have also identified limitations of T cell transfer, and we are finding new methods to modify T cells genetically to overcome these limitations and to make this strategy more effective and broadly applicable. In settings in which the target protein is also detected in normal cells, and the host exhibits peripheral tolerance such as may occur with many potential tumour antigens, molecular strategies to overcome tolerance are being evaluated. In settings such as relapsed leukaemia, in which a host response specific for the tumour cannot be elicited, CD8+ CTL clones are being isolated from the donor of an allogeneic BMT that are specific for host minor histocompatibility antigens expressed by the leukaemia and not normal non-haematopoietic tissue, and are being modified to express an inducible suicide gene to prevent ablation if unpredicted toxicity to normal tissues develops. Finally, in settings in which T cells with likely therapeutic activity specific for the target tumour antigen can be isolated from only a fraction of patients, such as in many malignancies including Hodgkin’s disease and malignant melanoma that express shared tumour antigens, methods to isolate the T cell receptor genes from T cell clones with appropriate antigen specificity and MHC restriction and to then introduce these receptor genes into host T cells are being developed.

PL-1115  Super molecules of factor VIII
Kaufman RJ, Pipke SW
Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI, USA

Treatment of haemophilia A requires frequent infusion of plasma- or recombinant-derived factor VIII. This regimen is limited due to the high cost and convenient access to peripheral veins. In addition, patients frequently develop inhibitory antibodies that limit available therapeutic regimens. Two major advances in factor VIII research over the past 15 years were the ability to isolate homogeneous preparations of factor VIII and the isolation of the factor VIII gene that provided for detailed biochemical and structural characterisation of the factor VIII molecule. Factor VIII contains a domain structure of A1-A2-B1-B2-A3-C1-C2. The ability to engineer factor VIII genetically has greatly increased our understanding of how this molecule is assembled and how its activity is regulated. With an increased understanding of the requirements for factor VIII function, studies have attempted to produce improved factor VIII molecules for replacement therapy. These findings have produced forms of factor VIII that are more efficiently produced, that are less immunogenic, and that have higher specific activity. A form of factor VIII was produced that is more efficiently expressed due to a single mutation Phe309Ser in domain A1 so that the molecule more readily folds into its appropriate conformation. A region between residues 484 and 508 within the A2 domain was identified as an antigenic region. Mutation of single residues within this region generates molecules that are resistant to inhibition by a number of different inhibitory antibodies. Finally, after thrombin activation, factor VIIIa activity is limited to A2 domain dissociation from the thrombin cleaved heptadecapeptide. A molecule was designed that is resistant to A2 domain dissociation and displays a 5-fold increased specific activity. The future will see the engineering of novel factor VIII molecules with both increased therapeutic efficiency while minimising inhibitory antibody body development. In addition, there are now structural models of factor VIII available that should in the future direct development of novel peptides that may eventually overcome the requirement for replacement therapy with factor VIII protein.

PL-1116  In vivo production of recombinant haematopoietins
Beuzard Y, Dalie B, Rouyer-Fessard P, Henri A, Payen E
Institut d’Haematologie, Hopital ST. Louis, Paris, France

A landmark of the last decade has been the development of recombinant proteins injected for therapy: hormones such as insulin or growth hormone, haematopoietic factors, coagulation factors, etc. A new step will be the in vivo production of these same recombinant proteins. Various systems are in development, from the simple electrophoresis of naked DNA, various viral vectors for ex vivo or in vivo gene delivery (retroviral vectors, Adeno Associated Virus, Adenovirus), to the implantation of semi-permeable capsules containing cells secreting the proteins of interest. Several major points have to be considered: 1) the safety and reversibility of the procedure; 2) the short or long-term regulation of the expression of the therapeutic gene by pharmacological agents or physiological means; 3) new clinical applications of these therapeutic tools; 4) the cost-effectiveness of the potential treatments. Erythropoietin gene expression by somatic transfer of the erythropoietin gene (DNA) or cells is an interesting model for in vivo production of therapeutic proteins. Erythropoietin is easily evaluated in blood both at the protein level, by the haematocrit measurement and the count of reticulocytes. All possible delivery systems including the activation of a silent gene in autologous cells, have been evaluated and several systems are being developed clinically. The regulation of the transgene, by induction or repression, using tetracycline, hormone analogs at low doses or oxygen tension has been obtained. New applications can be evaluated such as ß thalassemia major and other chronic anemias which may require very high levels of recombinant erythropoietin production in order to produce a clinical benefit. Other haematopoietic factors, coagulation factors and proteins could be used clinically by in vivo expression systems, in the very near future.
The World Health Organisation (WHO) seeks to achieve international consensus on the classification of neoplastic diseases. The implementation of a single classification on an international scale will facilitate studies regarding the epidemiology, pathogenesis, diagnosis, and treatment of lymphomas and leukaeasias. The proposed WHO classification has been developed under the joint auspices of the European Association for Haematopathology (EAHP) and the Society for Haematopathology (SH) with the participation of more than 50 expert haematopathologists. The classification includes T cell and B cell lymphomas and leukaeasias, myeloid and histiocytic tumors. Diseases are defined according to the principles of the REAL classification, which defines each disease according to its morphology, immunophenotypic and genetic features, postulated normal counterpart, and clinical features. Morphologic and clinical variants of individual diseases are discussed in the text, and their use is optional. The proposed classification has been presented at several national and international meetings in the United States and Europe in order to get input from the pathology community. The Steering Committee also appointed a Clinical Advisory Committee (CAC) chaired by C. Bloomfield and A. Lister, and composed of more than 40 expert clinicians, to provide input on issues of clinical relevance. The proposed WHO classification for lymphomas is similar to the REAL classification with minor modifications and reassessment of provisional categories based on new data generated since 1994. The classification of myeloid neoplasms and myelodysplastic syndromes will recognize the following major subgroups: AML with specific cytogenetic abnormalities, AML following therapy, AML unspecified, and AML arising in myelodysplasia.

WHO-1117 WHO classification for haematopoietic and lymphoid neoplasms

Jaffe ES, Diebold J, Harris NL, Muller-Hermelink HK, Flandrin G, Vardiman J

Steering Committee for the WHO. Classification, from the ‘National Cancer Institute, Bethesda, MD, USA; Unv. of Würzburg, Würzburg, Germany; Harvard U, Boston, MA, USA; Univ. of Würzburg, Würzburg, Germany; Hôpital Necker, Paris, France; Univ. of Chicago, Chicago, IL, USA

The proposed classification has been presented at several national and international meetings in the United States and Europe in order to get input from the pathology community. The Steering Committee also appointed a Clinical Advisory Committee (CAC) chaired by C. Bloomfield and A. Lister, and composed of more than 40 expert clinicians, to provide input on issues of clinical relevance. The proposed WHO classification for lymphomas is similar to the REAL classification with minor modifications and reassessment of provisional categories based on new data generated since 1994. The classification of myeloid neoplasms and myelodysplastic syndromes will recognize the following major subgroups: AML with specific cytogenetic abnormalities, AML following therapy, AML unspecified, and AML arising in myelodysplasia.

WHO-1118 B-cell lymphomas in the updated REAL and WHO classification

Piris MA
Dept of Pathology, Hospital Virgen de la Salud, Toledo, Spain

B-cell lymphoma classification has been based (additionally to the morphology) on the knowledge of genetic specific alterations of some lymphoma types, such as MCL and FL and to the existence of characteristic immunophenotypic features of some lymphoma types, although more frequently a constellation of markers has been used. An increasing weight in this Classification is being attributed to the site of the disease, since in BCL, as in other neoplasias, it is being recognised that specific locations of the disease are associated with distinct molecular events, clinical features and treatment responses, in spite of similar morphological features. The following BCL are recognised (more common entities are underlined):

- Precursor B-lymphoblastic leukaeomia/lymphoma (B-ALL/LBL)
- Mature (peripheral) B-cell neoplasms
- Predominantly disseminated, leukemic types
- B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma
- Splenic marginal zone B-cell lymphoma (± villosous lymphocytes)
- Hairy cell leukaemia
- Plasma cell myeloma/plasmacytoma
- Primary extranodal types
- Extranodal marginal zone B-cell lymphoma: 1) non-diffuse large B-cell lymphoma
- Follicular lymphoma
- Nodal marginal zone B-cell lymphoma (sclerosing subtype)
- Peripheral T cell lymphoma, NOS
- Burkitt's lymphoma
- Hodgkin's disease

WHO-1119 T-cell lymphomas: WHO classification

Müller-Hermelink HK
Department of Pathology, University of Würzburg, Germany

The still tentative WHO classification of T cell lymphomas is based on the proposal of the REAL-classification. Modifications and additions are due to intensive discussions and new evidence. More than in any other field of lymphoma diagnosis, the classification of peripheral T cell lymphomas has to reconcile clinical, morphological, immunophenotypic, and genotypic aspects in order to establish the relevant clinico-pathological entities. Primary site and clinical presentation in some categories are more important than detailed cyto-morphological features. The proposed list of diseases has been organised according to main clinical features in lymphomas with predominantly leukemic presentation (T cell prolymphocytic leukemia, T-LGL leukemia; NK cell leukemia; adult T cell leukemia/lymphoma). Predominantly nodal presentation (anaplastic large T cell lymphoma, peripheral T cell lymphoma unspecified, anaplastic large cell lymphoma of T/NK cell type), and predominantly extranodal presentation (Mycosis fungoides; Sézary syndrome; primary cutaneous CD30+ T lympho-proliferative disorders; subcutaneous panniculitis-like T cell lymphoma; NKT cell lymphoma and nasal type; enteropathy type intestinal T cell lymphoma; hepatosplenic T cell lymphoma). Site of origin and functional differentiation of presumed normal counterparts are relevant for the group of extranodal lymphomas with cytotoxic phenotype (NK/T cell of B CTL and γ/δ CTL).

The international lymphoma classification project has provided convincing evidence for the diagnostic accuracy and reproducibility in major groups or peripheral T cell lymphoma. However, a subdivision of peripheral T cell lymphoma unspecified according to predominant tumour cell size, as proposed in earlier classifications, is not reproducible and lacks prognostic significance. Morphological variants and differential diagnosis will be discussed.

WHO-1120 Anaplastic large cell lymphoma (ALCL) and Hodgkin's disease (HD)

Dufour G
Lab Anat Pathol, CHU Purpan, Toulouse, France

ALCL is now considered as a distinct entity. It consists of a population of large cells with a highly characteristic morphology, i.e. eccentric horseshoe or kidney shaped nuclei, showing a T or null phenotype. In every case virtually all malignant cells are strongly reactive for CD30 and EMA: 80% of cases express both T and Y blood group related antigens (BHN 9-4). On the basis of their morphologic features, these tumours fall into 3 categories: 1) common type; 2) lymphohistiocytic variant; 3) small cell variant. However, a significant number of cases are difficult to classify mainly because of unusual morphologic features such as giant cells, sarcomatous pattern or association of more than one ALCL variant in a single biopsy (i.e. common type + small variant). We classify such cases in the category of “others and unclassifiable”. The controversial Hodgkin’s like ALCL with vaguely nodular fibrosis and tumour cells resembling RS cells falls in this category. It is probable that most tumours previously diagnosed as Hodgkin’s-like ALCL are cases of neoplastic cell-rich HD. ALCL is associated with the t(2;5) translocation and most of these tumours express the NPM/ALK hybrid protein detected by ALK1 antibody. Occasional ALCLs are associated with the t(1;2) involving the non muscular tropomyosin T gene and express the TPM3/ALK hybrid protein. Survival based on the expression of ALK protein shows favorable prognostic significance for ALK expression (10 year survival of 80% for ALK-positive compared to 45% for ALK-negative tumours). The primary systemic form of ALCL must be distinguished from the primary cutaneous form of ALCL, which carries a good prognosis and from the secondary type of ALCL, which occurs in adults and has a poor prognosis. The four subtypes of the Rye classification of HD have been incorporated, with only minor modifications, in the REAL and WHO classifications. Nodular lymphocyte predominance HD (NLPHD) is now considered as a distinct entity different from classical HD. In NLPHD, atypical cells, known as “L&H” (lymphoctic and histiocytic) or popcell tumours, lie within large nodular areas named made up of small lymphocytes. In contrast to Reed-Sternberg cells (RS), L&H cells are positive for several B-cell markers (CD20, CD79a, CD75), ENU and usually negative for CD15 and CD30. Cases referred to as classical HD fall into four categories: nodular sclerosis (grade I and II), mixed cellularity, lymphocyte-rich subtype (which is different from LPHD) and lymphocyte depletion. These lesions are defined by the presence of RS cells which express CD15, CD30 and are frequently positive for EBV. RS cells are occasionally positive for CD20 (30% of cases) but usually negative for EMA.

WHO-1121 The new WHO classification of malignant lymphomas. Clinical implications

Hiddemann W, Bast M, Armitage J
Dept of Medicine, Ludwig-Maximilians-University, Munich, Germany

Based on the extensive efforts of the International Lymphoma Study Group (ILSG) a worldwide accepted classification of malignant lymphomas has recently been established and also accepted by the WHO. This classification has omitted the general grading of lymphomas into different categories, but has rather followed two main criteria: 1) the designation of lymphomas, diagnosis of the peripheral T cell lymphomas has to reconcile clinical, morphological, immunophenotypic, and genotypic aspects in order to establish the relevant clinico-pathological entities. Primary site and clinical presentation in some categories are more important than detailed cyto-morphological features. The proposed list of diseases has been organised according to main clinical features in lymphomas with predominantly leukemic presentation (T cell prolymphocytic leukemia, T-LGL leukemia; NK cell leukemia; adult T cell leukemia/lymphoma). Predominantly nodal presentation (anaplastic large T cell lymphoma, peripheral T cell lymphoma unspecified, anaplastic large cell lymphoma of T/NK cell type), and predominantly extranodal presentation (Mycosis fungoides; Sézary syndrome; primary cutaneous CD30+ T lympho-proliferative disorders; subcutaneous panniculitis-like T cell lymphoma; NKT cell lymphoma and nasal type; enteropathy type intestinal T cell lymphoma; hepatosplenic T cell lymphoma). Site of origin and functional differentiation of presumed normal counterparts are relevant for the group of extranodal lymphomas with cytotoxic phenotype (NK/T cell of B CTL and γ/δ CTL).

The international lymphoma classification project has provided convincing evidence for the diagnostic accuracy and reproducibility in major groups or peripheral T cell lymphoma. However, a subdivision of peripheral T cell lymphoma unspecified according to predominant tumour cell size, as proposed in earlier classifications, is not reproducible and lacks prognostic significance. Morphological variants and differential diagnosis will be discussed.
Acute leukaemias are classified according to the results of morphology, cytochemistry, immunophenotyping, cytogenetics and molecular genetics. These methods are used for confirmation of diagnosis, risk group definition, subtype adjusted treatment and follow-up for minimal residual disease. Treatment strategies include conventional or high dose chemotherapy, various forms of bone marrow or stem cell transplantation, immuno-therapy or causative approaches such as antisense strategies or tyrosine kinase inhibitors in patients with a leukaemia specific antigen. The impact of the above mentioned methods for diagnosis and treatment varies substantially between acute myeloblastic (AML) and lymphoblastic leukaemia (ALL) and even within the different subtypes of AML and ALL. In AML diagnosis is mainly confirmed by morphology and cytochemistry and risk definition or treatment strategy depends entirely on cytogenetics which allows the definition of risk groups with good (inv16, t(8;21), t(15;17)) intermediate (normal karyotype) and poor prognosis (<5%, 7-7.7%, >46.11%) in acute promyelocytic leukaemia (APL) with t(15;17) myeloid differentiation is blocked by the specific fusion protein RARα-PML and a causative treatment with All-trans-retinoic acid to induce terminal differ- entiation of the leukaemic blast cells is possible. In ALL - with the excep- tion of L3-morphology - morphological diagnosis is of minor importance and risk stratification is mainly based on immunophenotype (B-lineage with the subtypes prob-11%, common 51%, pre-B 10% and mature B- ALL 4%); T-lineage with the subtypes proT- (6%) and mature T-ALL (19%) and clinical risk factors (age, white blood cell count, time to response). The translocations t(9;22) and t(4;11) with the correspondig molecular mark- ers BCR-ABL and ALL1-AF4 are the main relevant cytogenetic and molecu- lar prognostic markers. However in ~95% of the patients individual mol- ecular rearrangements of the T-cell-receptor (TCR) or immunoglobulin heavy chain (IgH) can be detected. They are utilised as markers to follow mini- mal residual disease (MRD). Such prospective MRD studies may lead to individual treatment decisions in the near future.

WHO-1123 Classification of acute leukaemia: the clinician's view
Hoelzer D
Medizinische Klinik III, Frankfurt, Germany

Acute leukaemias are classified according to the results of morphology, cytochemistry, immunophenotyping, cytogenetics and molecular genetics. These methods are used for confirmation of diagnosis, risk group definition, subtype adjusted treatment and follow-up for minimal residual disease. Treatment strategies include conventional or high dose chemother-apy, various forms of bone marrow or stem cell transplantation, immuno-therapy or causative approaches such as antisense strategies or tyro- sine kinase inhibitors in patients with a leukaemia specific antigen. The impact of the above mentioned methods for diagnosis and treatment varies substantially between acute myeloblastic (AML) and lymphoblastic leukaemia (ALL) and even within the different subtypes of AML and ALL. In AML diagnosis is mainly confirmed by morphology and cytochemistry and risk definition or treatment strategy depends entirely on cytogenetics which allows the definition of risk groups with good (inv16, t(8;21), t(15;17)) intermediate (normal karyotype) and poor prognosis (<5%, 7-7.7%, >46.11%) in acute promyelocytic leukaemia (APL) with t(15;17) myeloid differentiation is blocked by the specific fusion protein RARα-PML and a causative treatment with All-trans-retinoic acid to induce terminal differ- entiation of the leukaemic blast cells is possible. In ALL - with the excep- tion of L3-morphology - morphological diagnosis is of minor importance and risk stratification is mainly based on immunophenotype (B-lineage with the subtypes prob-11%, common 51%, pre-B 10% and mature B- ALL 4%); T-lineage with the subtypes proT- (6%) and mature T-ALL (19%) and clinical risk factors (age, white blood cell count, time to response). The translocations t(9;22) and t(4;11) with the correspondig molecular mark- ers BCR-ABL and ALL1-AF4 are the main relevant cytogenetic and molecu- lar prognostic markers. However in ~95% of the patients individual mol- ecular rearrangements of the T-cell-receptor (TCR) or immunoglobulin heavy chain (IgH) can be detected. They are utilised as markers to follow mini- mal residual disease (MRD). Such prospective MRD studies may lead to individual treatment decisions in the near future.

WHO-1124 WHO classification of haematological neoplasias. Concluding remarks
Diebold I
Hotel Dieu, Paris, France

Four important categories of neoplasias arising from haematopoietic cells have been presented: B cell lymphomas, T cell lymphomas, Hodgkin's lymphoma, acute leukaemia. For all these categories, the neoplasias are pre- sented as entities, defined on clinical and morphologic criteria but also on immunohistochemistry, molecular genetics and, particularly for acute leukaemia, on cytogenetics demonstrating chromosomes abnormalities. All these entities have been presented according to the World Health Organ- isation classification of neoplastic diseases of haematopoietic and lym- phoid tissues. The advantage of this proposal appears clear. This classifi- cation will represent the first true worldwide consensus for classifying haematopoietic cell neoplasias. This WHO classification will not solve all the problems. The way follicular lymphoma should be stratified according to the number of large cells is still not validated. Subtyping large B cell lym- phomas is still not solved. The significance of Burkitt-type (like) lymphoma is still unclear. The marginal zone origin of primary splenic marginal cell lym- phoma is uncertain, the proposed T cell neoplasias classification is per- haps not completely satisfying. Many problems have to be studied. So this classification is not definitive. Updates will be proposed according to the development of knowledge and science in the future. A second advantage has been explained by both clinicians' talks, demonstrating the prognosis value of this classification. In acute leukaemia, morphology and chromo- somal abnormalities allow definition of entities and subtypes with differ- ent evolutions. For lymphomas, comparison of the survival of all entities after treatment shows that lymphomas can be grouped according to the type of survival curves, which is helpful to appreciate the evolution. Final- ly, this first worldwide consensus on the classification of haematopoietic neoplasias will be published in the year 2000, the last year of the twenti- eth Century and will be ready for use for the 21st Century.
PU-1125 Modulating effect of GM-CSF on the haematological changes induced by exposure to benzene in a rat model

Abdel el Hamid AS, Hasan GMA
Faculty of Medicine, Suez Canal University, Ismailia, Egypt

Objective. To evaluate the effect of simultaneous administration of dose dependent recombinant human GM-CSF and short term acute benzene toxicity on the peripheral blood, bone marrow and spleen in a rat model. Design and Methods. Thirty male albino rats divided into five groups as follows: (1) Six rats served as unexposed control, (2) six rats were given benzene at a dose of 1200 mg/kg in corn oil daily intraperitoneum, (3) six rats received benzene in the same dose plus daily subcutaneous 10 U/kg GM-CSF (therapeutic dose), (4) five rats received benzene in the same dose plus subtherapeutic dose of GM-CSF (5 U/kg) daily, (5) seven rats received the therapeutic dose (10 U/kg) of GM-CSF only. On the third day, all the animals were sacrificed, samples (blood, bone marrow & spleen) were collected, prepared (fixed and stained) and examined. Results. The second group (exposed to benzene only) showed a significant reduction in all haematological parameters, with moderate bone marrow hypoplasia, with reduced nucleated cell density of the red pulp of the bone marrow and reduced eosinophil and neutrophil counts, and a slight increase in platelet count, without evident changes on the peripheral blood, bone marrow and spleen. Conclusions. These results indicate that GM-CSF in its therapeutic dose has a potential protective and restorative effects on the peripheral blood, bone marrow and spleen depression induced by the short term benzene toxicity.

PU-1126 Composite lymphoma. Report of two cases

Abella S, Besses C, Serrano S, Dominguez D, Pedro C, Florensa L, Soile F, Sans-Sabatran J
*Departments of Haematology and Pathology, Hospital Universitari de Mar, Barcelona, Spain

Introduction. Composite lymphoma (CL) is an infraregious disease, with an incidence between 0.8 and 3.5% of all non-Hodgkin lymphomas (NHL). CL is characterised by the coexistence of two NHL of different histology, or the simultaneous presence of a NHL with a histologically different NHL at the same anatomic site. This diagnosis should be differentiated from "discordant lymphoma" and "sequential lymphoma". Two new cases are reported.

Case #1. A 72 year old male, hepatitis C virus antibodies positive, was referred because of lymphadenopathy and hepatosplenomegaly. A cervical large mass was excised. Histologic examination showed 4 adenopathies. One of them demonstrated a follicular NHL, grade II, REAL (CD20+, bcl-2+), while a HD, mixed cellularity type and a small lymphocytic lymphoma appeared but a single axillary lymph node remained. Histology of that node revealed HD, mixed cellularity type and a small lymphocytic lymphoma (CD20+) with plasmacytoid differentiation. Comment. CL is an unusual diagnosis that should be kept in mind when different histologies are seen in the same lymph node.

Case #2. A 17 year old male with acute lymphoblastic leukaemia (ALL) was treated with a conventional chemotherapy regimen (MOPP plus GDP). Bone marrow examination according to the FAB classification and cell surface immunophenotyping using a panel of markers including CD2, CD7 for T-cells and CD10, CD19 for B-cells monoclonal antibodies. Blood samples were obtained on admission and before beginning chemotherapy in leukaemic cases and repeated on remission or after 6 weeks in those who did not achieve remission. Results. CL-2 was overexpressed in all cells and was more significantly higher in relapse than in those at diagnosis. BCL-2 decreased significantly (p<0.01) by treatment in cases who achieved remission and was significantly lower (p<0.05) in cases with remission than non responders to chemotherapy. These findings raise the possibility that at least some malignant cells have deregulated BCL-2 expression and could survive and proliferate and create opportunity to acquire additional genetic alterations that could allow the cells to achieve resistance to chemotherapeutic agents. No correlation was detected between BCL-2 expression and the initial characteristics of patients including age, sex, WBCs count, blast count, FAB subtypes and immunological classification. Treatment induced downregulation of BCL-2 was accompanied by significantly increased p53 leading to apoptosis. Conversely, cell lines that failed to both downregulate BCL-2 and upregulate p53 were mostly of

PU-1127 New evaluation criteria of metabolic disturbance correction efficacy in patients with chronic myelomonocytic leukaemia

Issakova, Tretiak N., Anoshina M., Moshinskaja O., Yagovdick M. Research Institute of Haematology and Blood Transfusion, Kiev, Ukraine

We examined 50 patients (24 women and 26 men) with chronic myeloid leukaemia (CML) at a median age of 35 years old. The concentrations of primary, secondary and final lipid peroxidisation (LPO) molecular products, of iron, retinol, haemoglobin (Hb) derivatives, erythrocyte membrane permeability (PEM) were determined. Donors' indices were controls. Marked PEM alterations due to membrane lipid structure disturbance because of LPO activation was ascertained. Neutral lipid and phospholipid peroxidisation molecular product concentration increased by 2.8 - 14.7 times (r=0.001). Retinol level and Hb ligand composition changed, iron metabolism disturbances were found. MetHb and HBCO contents increased at the terminal stage by 4.4 and 3.5 times respectively. MetHb binding with phospholipids and HBCO binding with membrane neutral lipids, that lead to Fe3+ release from heme and to polysaturated fatty acid peroxidisation activation might be LPO activation mechanisms. LPO values may be evaluation criteria of metabolic disturbance correction efficacy in patients with CML.

PU-1128 Lipid peroxidation process activation as a risk factor in patients with multiple myeloma

Anoshina M., Tretiak N., Yagovdick M., Moshinskaya O. Research Institute of Haematology and Blood Transfusion, Kiev, Ukraine

Blood samples of 46 patients with multiple myeloma (MM) of II A (67%) - III A (33%) stages according to Durie-Salmon (serum creatinine less than 180 μM/l) were studied. Phospholipid and neutral lipid peroxidisation (LPO) in blood erythrocytes and plasma were evaluated by isolated double bonds (IBD), that reflected LPO steps: trienic, diterionic, o xoedienic conjugates (DC, TC, ODC respectively) and by LPO final products of Shief base (SB) type. Donors' analogous indices were controls. It was found that erythrocyte LPO in patients with MM of II A stage molecular product content increased by 1.6-13.8 times, in patients with MM of III A stage - by 1.7-20 times, and with phospholipid peroxidisation - by 1.7-3.2 times depending on MM stage. The correlation between the patients' life duration and IDB, DC and ODC content in their blood plasma with phospholipid peroxidation has been ascertained. Median life duration was 3 months, IDB being higher than 9.0 μM/l (r=0.681, p<0.050), DC - higher than 6.6 μM/l (r=0.655, p<0.050), ODC - higher than 4.5 μM/l (r=0.604, p<0.050). There was a favourable prognosis when IDB were within 4.0-7.0 μM/l, DC within 2.5-4.5 μM/l and ODC - within 1.9-3.4 μM/l.
patients who were non-responders to chemotherapy. BCL-2 was inversely related to p53 expression but achieving reason was more related to BCL-2 expression than to p53, therefore BCL-2 may be more predictive of apop-

tosis than are changes in levels of p53. Conclusions. BCL-2 protein pro-
duction represents a possible mechanism of drug resistance by which drug-induced apoptosis is abolished. The search for chemotherapeutic drugs that kill cells through a BCL-2 independent mechanism may be warranted for some types of lymphoid malignancies. In addition, the expression of p53 in ALL could be of prognostic value.

PU-1130 Exposure assessment of solvents in epidemiology

Barlett IW,* Lazaroza D,* W aldron HA,# Peijin D*D

*South Bank University, Borough Road, London; #W est London O ccupi-
tional Health Service; ’O ccupational Health Dpt, St M ary’H ospital, London, UK, †Institute for Internal Diseases, Clinic of Haematology Novi Sad, Yugoslavia

Retrospective assessment of exposure in epidemiology has most often been done by simple categorisation ie high, medium and low. If airborne solvents are the hazard of interest an alternative strategy could be to try to predict the likely level of exposure by using a validated predictive model with an acceptable accuracy. This paper describes preliminary work done to determine the value of using predictive models to estimate historical exposure to solvents in a case control study investigating the association between solvent exposure and AML. Predictive models normally integrate physico-chemical data, to estimate the solvent evaporation rate from a source, with data about the nature of air dispersion in the workroom. The model must be flexible enough to accommodate variable work conditions and the complexity of interaction between the worker and the solvent. The environmental and workroom data used in a model must be estimated with reasonable accuracy to ensure the overall accuracy of the model is suitable. Predictive models often work well in experimental chambers or in simulated work conditions but prove to be inaccurate when evaluated in the workplace. This is particularly true when exposure is to mixed solvents. To attempt to improve the accuracy of the exposure estimation the approach taken has been to use a validated exposure model with the method for the structured subjective assessment of past concentrations developed by Cherrie et al. (1997). This combination model has been evaluated for a variety of exposure scenarios within the case control study.

PU-1131 The effect of an experimental neoplastic disease on erythrocyte membrane lipid composition

Bartik K*, Malesevic M*

Department of Physiological Chemistry, University of Medical Sciences, Poznań, Poland

In animals with transplanted Morris hepatoma 5123, changes in the activity of several enzymes catalysing the main metabolic pathways in erythro-
cytes were observed. The transplanted neoplastic tissue affects the per-
miseability of erythrocyte membranes to sodium and potassium ions, and acts also on the structural components of these membranes, e.g. gly-
coproteins, and cholesterol. In the presented work we studied the phos-
pholipid and glycolipid profile in erythrocyte membranes as well as the total antioxidant status and vitamin E in plasma of rats on the 10th and 20th day after tumour transplantation. The results of our exper-
iments showed that the contents of phospholipids and MDA in erythrocyte membrane of rats with the experimental neoplastic disease different from that of normal, healthy rats as did their total antioxidant status and vita-

min E in plasma. In animals with Morris hepatoma 5123, the content of phosphatidylcholine and MDA. Their contents were raised on the 10th day after tumour transplantation. The results of our exper-
iments support the view that the effects of the neoplastic disease are not

limited to tissues directly attacked by the tumour but that the neoplasia also evokes a number of changes in other organs and tissues, as exemplified by erythrocytes.

PU-1132 Sensitivity of leukaemic lineages to apoptosis induced by ATP and chemotherapeutic agents

Bernardo AAS, Rumjanek VM

Centro de Ciências da Saúde, UFRJ, Brazil

Tumour cell lineages undergo apoptosis induced by several agents. This work compared the susceptibility of leukaemic lineages to apoptosis induced by 1) extracellular ATP (ATPo), which triggers apoptosis through an increase in intracellular calcium; 2) the chemotherapeutic drug Vin-

ristine, which affects the cytoskeleton; 3) the anthracycline daunorubicin. Cells from the MDR erythroleukaemic lineage (Lucena I), its parental cell line K562, and cells from the promyelocytic lineage HL-60 were submitted to several concentrations of both ATPo and the drugs tested. The occurrence of apoptosis was evaluated by three different approaches: morphologic analysis by fluorescence microscopy (using a mixture of dyes which allows the identification of apoptosis - cell cycle analysis in flow cytometry and DNA fragmentation. HL-60 seems to be more sensitive to apoptosis than both Lucena I and K562, independent on the mechanism of action of the inducing agent. Lucena I being a MDR line is resistant to both chemotherapeutic agents but is as sensitive as its parental cell line K562 to the effect of ATPo, suggesting that despite the multifactorial nature of MDR, this resistance is not extended to the mechanism activated by ATPo.

PU-1133 The relationship between plasma cells in peripheral blood and the response to treatment in multiple myeloma

Mot Popescu D, Bumbrea H, Vlaadenaru AM, Colita A, Lupu A, Căsăleanu D, Găman V

Cotlea Clinical Hospital Department of Haematology, University of Medicine “Carol Davila”, Bucharest, Romania

Recent studies have shown that malignant cells are present in the peripheral blood of many patients with multiple myeloma. We performed an analy-

sis by studying the presence of monoclonal plasma cells in peripheral blood of 10 multiple myeloma patients, before and after treatment and correlat-

ing this presence with clinical and histological profiles and with treatment. We identified our malignant population by a three colour flow cytometric analysis with CD38, CD19 and CD45 components. Seven of 10 patients had monoclonal plasma cells (PC) CD38+CD19-CD45- in the PB with a medi-
an of 5.5% of blood mononuclear cells (BMC). Although there was a wide variation between patients we found no correlation between the amount of circulating PC and the serotype of MM, the stage of the disease, the myeloma protein level, the LDH and the C reactive protein levels. In 2 heav-
ily pretreated and relapsed patients the PC amount was quite substantial (10% and 12.1%) and not modified by chemotherapy. In 3 patients the amount of circulating tumour cells decreased after the first 2 days of chemotherapy and in 1 patient they actually disappeared. Conclusions. Although our study group was very small and the observation time is too short to make any correlation to survival, we found that the PC level in PB corre-
late only with response to treatment and treatment refractoriness in multi-
ple myeloma. We mention that in a study by Wittig and Gripp - Blood 1996 - the PC in PB were strong predictors of survival.

PU-1134 Mutations in the K-ras and p53 genes as markers of disease in a M2 twin with ALL before and after BM transplantation

Cikota B, Magic Z, Vojvodic D, Starnatovic D*, Cucuz M, Ristic L,*

*Institute of Medical Research, MMA, Belgrade; †Clinic for Haematology, MMA, Belgrade, Yugoslavia

Introduction. Multiple genetic events are involved in the initiation and pro-
gression of a tumour. The most commonly implicated genetic changes in human tumors are alterations of the p53 tumor suppressor gene and ras protooncogenes. Since cancer cells are characterised by acquired genetic alternations (mutations and chromosome aberrations) we wondered whether point mutations in the p53 and K-ras genes occur in multiple myeloma and also diagnostic and prognostic markers of illness (ALL) in this case.

Methods. DNA isolated from PBMC (by a standard procedure) of a patient with ALL before and after BM transplantation and his BM donor (clinically healthy MZ twin brother) were subjected to PCR amplification of exons 1 and 2 and p53 exons 5, 6, 7 and 8. Mutations were detected by SSCP analysis. Results. In PBMC of the ALL patient before BM transplantation, mutations were observed in both exon 1 and exon 2 of K-ras and exon 8

Abstracts not presented
of p53 gene. These mutations were found neither in PBMNC of the MZ twin nor in PBMNC of the ALL patient after BM transplantation. In the p53 exons 5, 6 and 7, 8 mutations were detected in all samples analysed. Conclusions. The mutations that we detected in K-ras and p53 genes are good molecular markers of disease and can be used for early detection of relapse after BM transplantation. These mutations could be part of larger genetic abnormalities that ultimately led to leukemogenesis, manifested in the de- eased twin and further underline the importance of K-ras and p53 mutations in diagnosis and therapy assessment in patients with ALL.

**PU-1135 The use of intravenous itraconazole in aspergillosis following paediatric UD bone marrow transplant**

Cotswold J, Johnson E, O’akhil A
Bristol Royal Hospital for Sick Children, UK

Aspergillosis in the early phase of unrelated donor bone marrow transplant has a very high mortality rate. Two patients transplanted for high risk relapsed acute lymphoblastic leukaemia had evidence of progressive fun- gal disease prior to engraftment which responded to intravenous Itra- conazole and led to cure in one patient. These patients had prior intensive chemotherapy to achieve disease control with prolonged periods of neu- tropenia, followed by immunosuppressive conditioning regimes. Case #1 was a male aged 13 years with ALL in CR3 which had relapsed on treatment with evidence of fungal infection on chest CT scan at Day -5 of conditioning therapy before unrelated donor BMT. He was treated with liposomal Amphotericin intravenously. On Day +17 chest x-ray showed lung opacities for the first time. Intravenous Itraconazole was introduced. Following engraftment he was clinically stable and the chest x-ray appearances had resolved. His infection was considered cured, and he was discharged home. His immunosuppression was then gradually reduced. Case #2 was a male aged 17 years who had developed proven Aspergillus sinusitis during re-induction for ALL relapsing on therapy. This was treated with liposomal Amphotericin intra- venously and drainage. Three months later CT scan of chest and sinuses were clear and Aspergillus antigen was negative. Unrelated donor transplant was performed and liposomal Amphotericin given throughout. At Day +17 he had a persistent fever and CT scan showed nodules in the right upper lobe. Intravenous Itraconazole was added. Following engraftment and despite mild skin graft versus host disease the patient continued to improve and is currently disease free. Both patients developed progressive Aspergillosis on liposomal Amphotericin. The infection was stabilised in one and probably cured in a second by the addition of intravenous Itraconazole.

**PU-1136 Treatment approaches for patients with acute leukaemia after myelodysplastic syndromes**

Catraru C, Ioniti H, Rojgaru L
Dpt. of Haematology, University of Medicine and Pharmacology, Timisoara, Romania

In this paper, the authors report on 34 patients presenting with either myelodysplasia (with or without dysplasia) or acute myeloid leukaemia (AML) secondary to myelodysplastic syndrome (MDS) treated with AML- type chemotherapy and haematopoietic growth factors. From the 34 pts, 21 patients were ineligible for intensive chemotherapy due to age, organ failure or poor performance status and were given conservative therapy or supportive care. The remaining 13 pts received aggressive chemotherapy either at diagnosis or at evidence of disease progression (i.e. increasing blast count, increasing pancytopenia, karyotypic progression). Patients with refractory anaemia with excess of blasts in transformation or AML at diagnosis had a better prospect of entering complete remission with AML-type chemotherapy. The rate of progression to AML also predicted response. There was a longer median response duration in patients who had rapid progression of MDS at the time of therapy than that of patients with stable disease. Haematopoietic growth factors could not only be used to recruit leukaemic cells but also to shorten the period of cytopenia after aggressive chemotherapy which has generally been considered ineffective and contraindicated in elderly patients with MDS because of the assumption that the bone marrow is incapable of recovery from normal function, even if the dysplastic clone could be eradicated. The data suggest that aggressive chemotherapy may not be the treatment of choice except in patients who are eligible for bone marrow transplantation; most, if not all of these patients will relapse once they have achieved a remission (unless treated with allogeneic BMT). The biological behaviour of MDS as well as single time-point features (karyotype, FAB subtype) are important in determining the suitability of patients with MDS for intensive chemotherapy.

**PU-1137 Synthesis of acylated derivatives of plasminogen and their study in experiment**

Danyush T, Danyush O
Research Institute of Blood Pathology and Transfusion Medicine, Lviv, Ukraine

We studied acylated derivatives of Lys-plasminogen: plasmin, plasmin- and plasminogen-streptokinase complexes. Lys-plasminogen was purified from II+III or III kind fraction of blood plasma by chromatography on Lys- silochrom (modified silica) in the presence of protamin. The basic prop- erties of the synthesised preparations are investigated: stability, deacyla- tion rate, fibrinolytic activity, potency, and influence on animals. Fibrinolytic activity was measured in vitro by lysis of the chromogenic protein substrate azoform. Stability was defined by ability of preparations to dissolve and level of fibrinolytic activity measured after storage at various temperatures (-20°C, +4°C, +20°C). Of the investigated forms, the greatest stability had lyophilization dried up, and duration increased in the following order: acyl-plas- min > acyl plasmin-streptokinase complexes > acyl plasmin-streptok- inase complexes. The deacylation half-life of the three preparations did not differ and was 65±4 min. Their first order deacylation constant was similar- ly identical (4.2 ± 10^-3 sec^-1). In experiments with animals (mice, rats and rabbits) it was shown that the preparations are apyrogenic and do not show toxicity during the investigated period.

**PU-1138 Deletion of 5q in chronic myeloproliferative disorder as a primary chromosome change**

Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil

Deletions of 5q have been found in a large variety of haematological dis- orders, but especially in myelodysplastic syndromes and in acute mye- logenous leukaemia. Fewer cases of chronic myeloproliferative disorders have been associated with this chromosome abnormality and rarely as the pri- mary chromosome change. We describe here a 45-year-old man referred to our hospital for investigation of splenomegaly and thrombocytosis. The first cytogenetic analysis was normal. In 18 months his hemoglobin level and leukocyte count had increased significantly and a second cytogenet- ic study was performed. The exam revealed both normal male karyotype and a single abnormality, 46, XX, del(5)(q13q33), highly specific for the 5q- syndrome. Bel-abi mRNA was not found. The patient had no history of prior chemotherapy or radiation therapy. The patient did not meet the diag- nostic criteria proposed by the Polychromat Verona Study Group for chromosomal abnormalities have been reported in chronic myeloproliferative disorders, the del(5q)- as the primary chromosome change is rare. Although del(5q)(13q33) is strongly associated with del5q- syndrome, this patient had clinical and laboratory findings of myeloproliferative disorder.

**PU-1139 Immunohaematological findings in transfused haematological patients with erythrocyte antibodies**

*National Blood Transfusion Institute, Belgrade; °Institute of Haematol- ogy, Belgrade, Yugoslavia

Objective. The aim of this study was to determine some characteristics of patients with haematological disorders who developed red cell antibodies (Abs) of clinical significance. Design and Methods. From 1995 to 1997, 29 patients with erythrocyte Abs reactive in indirect antiglobulin test (IAT) from the Institute of Haematology were followed. Direct antiglobulin test (DAT), screening test procedures and routine pretransfusion investigations were performed both by tube and gel techniques. Immunohaematological investigations were done using our commercially obtained red cell panels. Results. Discovered irregular red cell antibodies,

<table>
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<th>Antibodies</th>
<th>N° of patients</th>
<th>%</th>
<th>DAT</th>
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<td>2</td>
</tr>
<tr>
<td>auto Abs of undetermined specificity</td>
<td>4</td>
<td>13.8</td>
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Nineteen patients (65.51%) were female, 10 (34.5%) were male. Out of them 19 (65.5%) were older than 50; 23 (79.3%) had received immuno- suppressive therapy. All the patients had a history of previous transfusions,
only 1 female had no history of pregnancies. Among them, 15 (60%) made specific Abs after receiving ≤10 units of red cell transfusions (range 1-54); 12 (48%) patients developed Abs between 2-12 weeks after transfusions. All of the patients with more than 1 Abs were female 37.6%. Of the discovered Abs, 41% had Rh specificity. 20.5% Kell specificity. Conclusions. Despite the age and the immunosuppressive therapy, transfused haematopoietic cells are able to form red cell Abs. Females with a history of pregnancies are at a greater risk than other patients.

PU-1140 The apoptotic effects of heparin on lymphoblasts, neutrophils and mononuclear cells

Edilurra E, Yildiran A, Tekelioğlu Y, Gedik Y
Karadeniz Technical University, Faculty of Medicine, Departments of Pediatrics and Embryology, Trabzon, Turkey

Objective. To evaluate the apoptotic effects of heparin on lymphoblasts, neutrophils and mononuclear cells by flow cytometry for detection of sub G1 peak, in vitro. Design and Methods. Ten children with acute lymphoblastic leukaemia (ALL) at diagnosis (Group I), six children with ALL at relapse (Group II) and ten healthy children (Controls) were included in this study. Lymphoblasts in ALL patients, and neutrophils and mononuclear cells in controls were incubated in increasing heparin concentrations (0, 5, 10, 20 U/mL). Flow cytometric analyses were performed at 0.5 and 2 hours of incubation in heparin for determination of the apoptotic effects of heparin. Results. In Group I, apoptosis was detected in all different levels of heparin concentration except 5 U/mL at 0.5 and 2 hours. The apoptotic effects of heparin on blast cells peaked at the 1st hour in 5, 10 and 20 U/mL heparin concentrations (p<0.001). In Group II, similar findings were observed only at 0 hour and apoptosis was higher than that in Group I except in 5 U/mL heparin concentration (p<0.001). Apoptosis was found to increase with heparin levels in both groups (p<0.02). In the control group, apoptosis was detected only in the 20 U/mL heparin concentration and only at the 1st and the 2nd hours. Lymphoblasts are more sensitive to apoptotic effects of heparin than neutrophils and mononuclear cells (p<0.004). Conclusions. The findings of this preliminary study indicate that heparin causes apoptosis of lymphoblasts but further and more comprehensive research on the apoptotic effect of heparin on lymphoblasts should be done.

PU-1141 New experimental model for allogenic antithyrophilic immunoglobulin specific activity determination

Fedorenkova YeA, M. l'ncik YEA
Research Institute of Haematology and Blood Transfusion, Kiev, Ukraine

Due to their high sensitivity to diphtheritic toxin, HeLa cells obtained from donor cells were used to give a quantitative description of anti-diphtheritic immunoglobulin. A mixture of diphtheritic toxin with anti-diphtheritic immunoglobulin after incubation was applied on a HeLa cell monolayer and only at the 1st and the 2nd hours. Lymphoblasts are more sensitive to apoptotic effects of heparin than neutrophils and mononuclear cells (p<0.004). Conclusions. The findings of this preliminary study indicate that heparin causes apoptosis of lymphoblasts but further and more comprehensive research on the apoptotic effect of heparin on lymphoblasts should be done.

PU-1142 Lymphoblastic transformation of chronic myelomonocytic leukaemia in a child

Ferreira F, Martins A, Farinha N, Lima Reis I, Ribiero MM
Department of Clinical Haematology and Pediatrics, Haematology-Oncology, HS João, Porto, Portugal

Chronic myelomonocytic leukaemia (CMML), classified as a myelodysplastic syndrome according to the FAB classification, is very rare in children. With few exceptions, leukaemia following CMML is the phenotype of the acute myeloblastic lineage. A 13-month-old boy presented in October 1997 with splenomegaly, leukoencephalitis, anaemia and thrombocytopenia. Diagnosis of CMML was based on peripheral blood and marrow cytology, marrow histology, cytochemistry and lymph node biopsy. Since he had no familial donor a search for a compatible donor was undertaken. Two months later, the bone marrow cytology was compatible with transforming CMML (16% lymphoblasts). In January 1998 the bone marrow aspirate showed strong features of myelodysplasia and 40% of lymphoblasts with L2 morphology. The immunophenotyping results were: CD9 33%, CD10 39%, CD20 35%, CD22 41%, CD44 36%, HLA-DR 40%, CD79a 40% and cytoplasmic IgM 30%. The CD34, TdT, CD3, CD14 and HLA-DR were negative. The bone marrow cytogenetics revealed 25 mitoses (46 XY); no bcr-abl rearrangement. Standard chemotherapy for childhood acute lymphoblastic leukaemia was followed by a partial remission with 3% of bone marrow blast cells, although features of haemopoietic dysplasia and a small splenomegaly remained. At this time we found a compatible donor and we proposed the child for allo transplantation but in the meantime a relapse of acute lymphoblastic leukaemia occurred. We got him back into partial remission with reinduction treatment including daunorubicin, vincristine, L-asparaginase and etoposide and we intensified the treatment with fludarabine, cytarabine and G-CSF. At present, the patient is clinically well and has a good performance status. An allo transplantation is scheduled early in February 1999.
PU-1145 Comparative efficiency of navoban, zofran and metoclopramide in children with brain tumours during realisation of polychemotherapy (PCT)

Gleková O, Popov V, Livshits M, Gorbathy S, Holodov S, Kurneva E.
Research Institute of Pediatric Haematology, Moscow, Russia

We have analysed 191 patients with brain tumours (113 medulloblastomas/PNET, 51 astrocytomas, 22 ependymomas, other tumours - 5), who received 392 cycles of PCT. Carboplatin 400 mg/m² - 1 day (65 cycles) and Carboplatin 500 mg/m² - 1 day (92 cycles), Cisplatin 40 mg/m² - 13 days (96 cycles), Cyclophosphamide 600 mg/m² - 2 days (30 cycles) and Cyclophosphamide 1500 mg/m² - 1-2 days (30 cycles). For prevention of nausea and emesis we used Zofran - 165 cycles, Zofran+Dexa - 39 cycles, Navoban+Dexa - 143 cycles, Metoclopramide+Dexa - 143 cycles. Navoban was administered at a dose of 0.2 mg/kg daily, Zofran - 5 mg/m² TID, Metoclopramide - 1.25 mg/kg TID. The efficiency of Metoclopramide was below that of Zofran and Navoban (39%, 83% and 85%). Full prevention of nausea and emesis was recorded more frequently in patients of low age group, with no difference between sexes. Lowering frequency of a full effect of any of antiemetics was associated with increased size of residual tumour and symptoms of intracranial hypertension. The full preventive effect of emesis was increased by the use of Dexa, Navoban, Zofran and Metoclopramide in combination with Dexa prevented acute emesis in 88%, 80% and 30% of the patients, and delayed emesis in 94%, 85% and 48%. The efficiency of Zofran, Navoban and Metoclopramide during all cycles was not reduced and was 80%, 90% and 44.7%. The side effects by use of Zofran and Navoban were observed: headaches, pain in the stomach, constipation respectively in 3% and in 26%. Metoclopramide was marked by extrapyramidal disorders, dizziness, somnolence in 1% of the patients. Thus, Navoban and Zofran have a strong antiemetic activity during PCT in children with brain tumours. Navoban is must. Haematological diagnoses were: aplastic anaemia (AA) in 4 cases and myelodysplasia (MDS) in the remaining 2. In three AA patients MRI showed high intensity signal of active haematopoiesis. The MRI in MDS patients showed high intensity signal of active haematopoiesis in one in absence of the other. Conclusions. Despite the small number of cases studied, MRI is a useful tool to study bone marrow appearance in extent and to determine the presence of neoplastic infiltration.

PU-1146 Platelet factor-4 after streptokinase and heparin infusion in patients with severe peripheral cytopenias

Rubio-Félix D, Giralt M, Ghoneim H, Gómez-Arteta E, Granjo E, Saez A, Pastor E, Alcaraz MJ, Ferreruela R. Departments of Haematology and Radiology, Miguel Servet Hospital, Zaragoza, Spain

Six patients were studied (5 F/1 M; mean age: 28.7 y; range: 19-46). All of them had severe peripheral cytopenias defined as: ANC <0.5 10⁹/L, platelet count <50 10⁹/L, WBC=3.5 10⁹/L. The patients under streptokinase therapy of GM-CSF plus erythropoietin and amifostine followed by GM-CSF plus prednisolone and danazol. Both trials failed and the patient became red cell and platelet transfusion dependent. On August 31, CyA=10 mg/k/d p.o. was started. A gradual increase of Hb and WBC values was documented and at present the patient remains stable with a maintenance daily dose of CyA of 5 mg/k; 4 mo after initiation of therapy the peripheral blood analysis revealed: Hb=10.2 g/dL; platelet count=87×10⁹/L; WBC=4.5×10⁹/L (N=0.99×10⁹/L). Conclusions. HPS may be due to an immune reaction against bone marrow stem cells. This patient, with a past history of severe multisensitivty allergic rhinitis achieved a good response to immunosuppressive therapy (CyA) pointing out that this drug might be considered a first line therapy in similar situations.

PU-1147 T-cell lymphoma and Epstein-Barr virus infection presenting as fulminating haemophagocytic syndrome

Graw E, Real E, Gómez A, Pastor E, Alcaraz MJ, Saez A, Ferreruela R. Departments of Haematology, Microbiology and Pathology, Hospital Luis Arenal, Xativa, Department of Microbiology, Hospital Dr. Peset, Valencia, Department of Pathology, Hospital de Toledo, Toledo, Spain

Haemophagocytic syndrome (HPS) is an acute disease characterised by fever, pancytopenia, hepatosplenomegaly and widespread tissue infiltration by haemophagocytic histocytes. We describe a case of fulminating HPS as a presenting feature of T-cell lymphoma and Epstein-Barr virus infection. A 50-years-old woman was admitted to our Hospital with fever and rapid deterioration of her general condition. Physical examination revealed a ulcerative lesion in the right tarsal, slightly enlarged lymph nodes and hepatosplenomegaly. Laboratory studies disclosed: haemoglobin 9.3 g/dL, platelet count 92,000×10⁹/L, white blood count 2.2×10⁹/L, fibrinogen 130 mg/dL, D-dimer 4 mg/L and lactate dehydrogenase 826 U/L. EBV DNA was detected in the serum after genomic amplification by means of polymerase chain reaction. Bone marrow biopsy disclosed an increase of reticulin fiber and histiocytic hyperplasia showing haemophagocytosis. Bone marrow and tonsillar biopsies also demonstrated clusters of lymphoid cells with irregular haemophagocytic nuclear staining with primary antibodies directed against CD3 and CD45RO but not for CD20 and MB2. Tissues from bone marrow were analysed for evidence of T-cells receptor (TCR) γ-chain gene rearrangement and results were negative. The patient was first treated with high doses of steroids and when results were consistent with T-cell lymphoma, she was treated with combination chemotherapy that contained adriamicin, cyclophosphamide, vincristine, and prednisolone. However, this patient's HPS was refractory to the chemotherapy, and she died of disseminated intravascular coagulation-induced multiorgan failure on the 20th day of hospitalisation. This case illustrates the difficulties in defining the underlying disease in the setting of rapidly progressive HPS. We recommend that future cases of HPS be studied for underlying T-cell lymphoma in addition to EBV infection.
PU-1150 Viral hepatitis in polytransfused Egyptian \( \beta \)-thalassaemic children

Hataba NM, AboelFeouh ME, Khafragy N
Egypt Air/Health, Cairo, Egypt

A zero risk blood supply is not feasible, polytransfused subjects are at increased risk of contracting hepatitis \( \beta \)-thalassaemia major subjects (n=130) aged 2-16 years with a mean of 7.3 years were followed up through the period from January 1998 to December 1998 and were tested for serologic markers of hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis delta virus (HDV) and HIV infection. Markers of acute viral hepatitis were done for subjects with elevated alanine aminotransferase (ALT). All subjects in this study tested for HBV were seronegative. The common patterns of hepatitis sero-markers were combined HBV and HCV in 34.6%, HBV in 18.5%, HCV in 27.7%, while only 19.2% were seronegative. HBsAg, a marker of active infection of HBV was present in 24 subjects (18.4%), of whom 60% were HBV seropositive. Evidence of biochemical hepatitis was present in 41 patients, out of whom 23 subjects tested seronegative for viral hepatitis. B were CMV IgM positive. Chorionic biochemical hepatitis for more than 6 months was evident in 48% of subjects with elevated ALT. It may, be concluded that HBV is not a problem in the studied population. However, combined HBV and HCV infection is prevalent among polytransfused patients.

PU-1151 Pericardial effusion and tamponade in acute leukaemia

Department of Haematology, Hospital “La Paz”, Madrid, Spain

Objective. Pericardial effusion and tamponade have been described in haematological diseases such as chronic myelomonocytic leukaemia, lymphomas and rarely in acute leukaemia without high peripheral blood mono- cyte counts. We present two cases of acute leukaemia with pericardial effusion at the beginning and at the course of the disease.

Case #1. A 18-year-old woman was admitted to hospital because of fever, pleuritic chest pain, cough and abdominal pain. Physical examination was significant for tachycardia with a heart rate of 140 beats per minute.

The patient had a normal blood pressure of 120/80 mmHg, a normal body temperature of 36.8°C, normal respiratory rate of 18 breaths per minute, and normal oxygen saturation of 100%.

On admission, the patient was conscious, oriented, and of normal weight and height. Vital signs were as follows: blood pressure 120/80 mmHg, heart rate 140 beats per minute, respiratory rate 18 breaths per minute, and oxygen saturation 100%.

The patient was admitted to the hospital with the diagnosis of acute leukaemia and pericardial effusion. The patient was treated with cytarabine andidarubicin chemotherapy. The patient’s condition improved, and she was discharged from the hospital after 10 days of hospitalisation.

Case #2. A 46-year-old woman was admitted to hospital with chest pain, dyspnoea, and orthopnea. The patient had a history of chronic obstructive pulmonary disease and asthma. On admission, the patient was conscious, oriented, and of normal weight and height. Vital signs were as follows:

- Blood pressure: 120/80 mmHg
- Heart rate: 100 beats per minute
- Respiratory rate: 20 breaths per minute
- Oxygen saturation: 98%

The patient was admitted to the hospital with the diagnosis of acute leukaemia and pericardial effusion. The patient was treated with cytarabine andidarubicin chemotherapy. The patient’s condition improved, and she was discharged from the hospital after 14 days of hospitalisation.

The management of early stages (I/II) of Hodgkin’s disease involves radiotherapy (especially mantle radiotherapy) and for the patients already diagnosed and clinically staged, a standard chemotherapy protocol followed by irradiation. This study, based on 96 patients hospitalised in the Haematology Clinic of Timisoara (Romania) between Jan. 1983 and Dec. 1996, analyses the response to standard chemotherapy and rate of survival for patients with I/II Hodgkin’s disease. There were a few patients staged by staging laparatomy. This trial, initiated on a group of 64 men and 32 females (aged between 17-68 years old who were managed with standard chemotherapy protocols (MDVP; COPP;ABVD; MOPP/ABVD; MOPP-ABV). The staging and histopathological distribution rate was: STI A (18%), STI B (43%); STI B (17%); STI B (32%); NC (2%). NC (18%). There were 11% pts. with bulky disease. The chemotherapy protocol was designed to induce a high rate of complete remission and overall survival (87%); 16 pts. demonstrated relapses and extranodal disease (from these pts., 5 were treated with mantle radiotherapy and 11 with chemother.- and radiotherapy). The overall survival rate was 67% and the disease specific survival rate 74% (at 10 years). There were 11 procedure related deaths (including veno-occlusive disease, haemorrhages). An increased number of courses of chemotherapy and the presence of extranodal disease were statistically significant adverse prognostic factors: 8 pts. died from treatment induced toxicity (3 pts from early and 5 from late toxicity); 5 pts died from a second malignancy (breast, lung). The cases of therapy-associated leukaemia occurred. Good prognosis seems to be associated with higher doses and earlier staging. This paper provides data on the role of high-dose therapy in Hodgkin’s disease (early stages), early staging and the high response rate it the treatment has been correctly managed.

PU-1154 Disturbance of fibrinolysis as a risk factor atherothrombotic stroke

Institute of Neurology, Moscow, Russia

Institute of cerebral vascular strokes are thromboembolic in origin. The study of parameters of hemorheology, haemostasis and fibrinolysis is very important for the detection of risk factors of atherothrombotic stroke. We studied the parameters of hemorheology, haemostasis and fibrinolysis in 43 patients with acute stroke. Three groups of patients were formed. Group I - patients with thrombotic complication who showed severe signs of atherosclerotic lesions of MAN (n=30), group II - patients with small deep infarcts and mild signs of atherosclerotic lesions of MAN (n=13), group III - healthy persons (n=14). It was found that in patients of groups I and II all parameters of hemorheology, haemostasis and fibrinolysis were changed during the acute period of stroke in comparison with the healthy donors. It means the decline of hemorheological parameters, intravascular haemostatic activation in combination with decrease of anticoagulant and fibrinolytic potential. In group I patients, an increase of Fg, Ht in combination with a marked increase of aE and a significant decrease of DE were noted. ATT, PS and PC were decreased in patients of both groups. The level of soluble complexes of fibrin-monomer in group I was in 2 times higher.

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than in group II. D-dimer was higher in group I than in group II. Ag TP5 was lower in group I than in group II. Thus, in group 1 patients there was depression of fibrinolysis. This leads to the decrease of intravascular autolysis of thrombi. This factor is associated with severe atherosclerotic lesions of MAH.

PU-1155 Type of bcr-abl rearrangement and some haematological parameters of patients with chronic myeloid leukaemia

Jazvic: B, Mazarov G, Wrobel T, Kuliczkowski K
Department of Haematology, Medical University, Wroclaw, Poland

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder with a consistent molecular marker, BCR-ABL hybrid gene, arising from a reciprocal translocation between chromosomes 9 and 22. At the messenger RNA level two alternative types of transcript are observed: longer, b3a2, including b3 exon of M-BCR and shorter, b2a2, when b3 exon is excluded. The debate on possible influence of the type of fusion transcript on clinical course of CML is long-lasting but still not finally resolved. In the presented study the type of BCR-ABL transcript was characterised in 48 patients with CML using the reverse transcriptase-polymerase chain reaction. The frequencies of b2a2 and b3a2 rearrangements were 43.7% and 56.2%, respectively. The complete data were available for 33 patients, 17 with b2a2 and 16 with b3a2 type of translocation. Among these were 19 females and 14 males, with a median age of 46 years (range, 26 to 71 years). The follow-up lasted 1 to 108 months. At the end of the study there were 8 blast crises, 4 for each type of transcript. The analysis of chronic phase duration by the Meier-Kaplan method showed no significant difference between both groups of patients (p=0.126, by the log-rank test). Also none of the clinical and laboratory parameters compared at diagnosis (age, sex, leucocyte and platelet counts, spleen size, % of blasts in blood and bone marrow and Sokal score) was significantly different in any group. We conclude that the type of BCR-ABL rearrangement has no influence on chronic phase duration nor is it correlated with clinical or laboratory features studied at diagnosis in our CML patients.

PU-1156 Immunophenotype in transformation of low grade lymphoma to B-CLL

Juricic: V, Krajulic: N, Banicevic: B
School of Medicine, Kragujevac, Clinical Center of Serbia, Institute of Haematology and School of Medicine University of Belgrade, Yugoslavia

Patients with indolent lymphomas have a long history of disease usually without symptoms over many years, but some patients have a propensity to undergo histologic transformation or conversion to more aggressive lymphomas, usually of a large-cell type. Small lymphocytic lymphoma is morphologically indistinguishable from chronic lymphocytic leukaemia (CLL) and these patients the disease may progress into B-CLL. We have experienced three cases in chronic phase, in which Ph chromosome successfully decreased to either 0%, 30% or 50% under IFN-alpha therapy for one year, and that suddenly transformed to blastoid crisis soon after. The aim of this study was to find any special signs that were perhaps forerunners of the sudden progression into BC during effective therapy with IFN-alpha. Common features of these cases are listed. 1. Additional Ph chromosome was present at first. 2. An accelerated phase was not documented. 3. Blasts were CD34 positive, and primitive. 4. Cumulative dosage of IFN at BC was not low. 5. Hydroxyurea was administrated at the beginning of IFN therapy to control high WBC count. In conclusion, the risk of sudden progression into blastic crisis during effective treatment, with IFN-alpha might be higher in those patients with CML who have an additional Ph chromosome.

PU-1159 Disappearance of a leukaemic clone only after megatherapy/ PBSC rescue: an immunocytochemical study

Katiamis AC, Tsantalis G, Mochovski M, Kita V, Graphakos S, Tsamboulas-Kalitsopoulos F
Oncology Unit, 1st Dept of Pediatrics, University of Athens, Athens, Greece

We present a 12 years old boy with AML (M1), in whom sequential immunocytochemical studies showed disappearance of the leukaemic clone only after autologous transplantation. The patient was treated with a BFM-like AML protocol. Sequential quantitative measurement of apoptotic cells in PB showed a time delay in the apoptotic curve during induction, suggesting of relative resistance to chemotherapy. Immunocytochemical detection of p53 protein was performed using the antibodies: clone DO-7, JSB-1 for P-glycoprotein and PC10 for PCNA. The immunocytochemical reaction was performed in a Sequenza coverplate immunostaining system by either the streptavidin-alkaline phosphatase technique for fast red staining or streptavidin-hyperoxidase for DAB staining. p53 protein was detected by the DO-7, 1801 and 240 antibodies, but not with the JSB-1, 9E11 for c-myc, JSB-1 for P-glycoprotein and PC10 for PCNA. The immunocytochemical reaction was performed in a Sequenza coverplate immunostaining system by either the streptavidin-alkaline phosphatase technique for fast red staining or streptavidin-hyperoxidase for DAB staining. p53 protein was detected by the DO-7, 1801 and 240 antibodies, but not with the 1620 antibody, suggesting that the cells expressed a mutant form of p53. The cells were also positive for bcI-2 and mdr-1 expression and negative for c-myc and fas. Double detection showed that the majority of bcI-2 and mdr-1 positive cells were positive for PCNA, indicating that the resistant population had the ability to proliferate. The patient proceeded slowly into the clonal population expressing the mutant form of p53. The clonal population expressing the mutant form of p53 persisted in BM throughout the following 6-months of chemotherapy, albeit in decreasing proportion. Because of that he underwent megatherapy with autologous PBSC rescue. Conditioning regimen included busulfan (16 mg/kg), cyclophosphamide (150 mg/kg), etoposide (40 mg/kg). Post-transplant evaluation was negative for the mutant form of p53, suggestive of disappearance of the leukaemic clone. This case
PU-1160  Class-specific immunotherapy of house-dust mites was studied. Blood plasma was taken before, during and after specific immunotherapy had reached the normal level (84-96%). The restoration of Hageman factor increased and by the end of the course of specific immunotherapy was achieved. The activity of factor XII in the blood plasma of the patients was determined with the use of plasma, deficient in factor XII. Statistically significant changes in the activity of Hageman factor were observed in the former patients (42-53%). During hyposensitisation the allergic activity of granulocytes was very low. GAGs were isolated from aceton collected dried cells. Electrolysis on cellulose acetate membranes before and after treatment with GAG-activating enzyme was used for GAG identification. The GAG content was found to be 33.0 µg uronic acid per 100 mg of dried cells in first pt, and 63.0 µg in the second. GAGs were presented by 3 polysaccharide fractions: chondroitin sulfate (CS) with different electrophoretic mobility (I: with that of CS - standard, II: as that of dermatan sulphate, III: slower than I and II) and a fraction of heparin sulphate. Some differences in the leucocyte GAG composition in both patients with PCL were revealed. The electrophoretic pattern of GAGs in white blood cells in PCL differed considerably from patterns previously found in other types of leukaemias and therefore can be used as one of the markers of this disease.

PU-1161  A further contribution to the incidence of nucleoli in granulopoietic compartment in the bone marrow of patients suffering from chronic myeloid leukaemia

Smrtná K.*, Královková L.*, Klamanová H.*
*Institute of Haematology and Blood Transfusion, Prague, Czech Republic

As demonstrated in previous studies, the number of nucleoli in myeloblasts and promyelocytes expressed by the values of the nucleolar coefficient in patients suffering from chronic myeloid leukaemia (CML) was generally smaller in comparison to that in control persons. On the other hand, no substantial differences in the incidence of main nucleolar types and values of the nucleolar coefficient of nucleoli in the granulopoietic compartment of CML were noted between patients in the accelerated and chronic phases of CML with the exception of promyelocytes in the chronic phase of CML. In these patients values of the nucleolar coefficient were similar to these in granulocyte precursors. In contrast to the effects of therapy with interferon-α, cytostatic therapy with hydroxyurea in patients in the chronic phase of CML produced a significant increase of the incidence of myeloblasts and promyelocytes with micronuclei. Such nucleoli are known to represent inactive nucleoli, which are characterised for the cessation of the nucleolar - ribosomal - ribonuclear acid (RNA) transcription and terminal differentiation. It should be noted that the transformation of "active" nucleoli to micronucleoli was previously induced in a variety of cells in vitro by the irreversible inhibition of the nucleolar - ribosomal RNA transcription with a broad range of cytostatics regardless of the mode of their action. Thus the incidence of main nucleolar types including micronucleoli in the GPC might be a useful complementary tool for the evaluation of the biology of leukaemic cells including the effect of the therapy in CML patients.

PU-1162  Iron deficiency anaemia and thrombocytosis: clinical and analytical study of 220 patients

Laatriti M.A., Chehata S., Ennabi S
Service d'Hématologie clinique, CHU Farhat Hached, Sousse, Tunisia

Objective. Iron deficiency is widespread throughout the world and it is probably the most common chronic organic disorder of mankind. The incidence and the outcome of thrombocytosis related to the iron deficiency anaemia is not well described. The objective of the study was to evaluate the frequency, importance and outcome of this association. Design and Methods. We performed a retrospective analysis of 670 consecutive patients with iron deficiency anaemia collected between 1995 and 1998. The purpose of the proposed trial is to determine the therapeutic efficacy of a new concept of high-dose therapy with stem cell rescue (HDC-SCR) for children with relapsed high-risk acute lymphoblastic leukaemia (ALL) in complete remission. Thirty percent of children with ALL suffer a relapse after having achieved a first complete remission. The main determinants of the outcome of subsequent treatment are duration of first remission, site of relapse, immunophenotype of leukaemic cells, age at initial diagnosis, and tumour burden. Allogeneic SCT is the therapy of choice for children at high risk for a subsequent relapse. Due to a lack of a suitable donor, less than 25% patients are able to benefit from this procedure. No advantage of convoluted HDC-SCR compared to chemotherapy could be shown with regard to outcome. Therefore, an alternative approach to HDC-SCR is proposed which includes immunotherapy, followed by a reinduction therapy after SCR. In addition, the treatment regimen consists of a subsequent maintenance therapy or vaccination with autologous leukaemic cells transfected with a CDNA expression plasmid coding for an antigenic HLA class I antigen combined with interleukin-2 treatment. The majority of transplantation centres in Germany clearly favour MUD-SCT and UUD-SCT, even though the results are poor as documented in the ALL-REZtrials. Up to now, two patients have been enrolled into this HDC-SCR trial. One (systemic relapse of T-ALL) has completed treatment without serious side effects, and is in second remission at 17+ months. The second patient (second systemic relapse of preB-ALL) has completed the induction phase. We ask for the support of centers to determine applicability and benefits of this novel approach to HDC-SCR. Some Spanish, Italian and Czech centers have already joined this international collaborative endeavour. Here, we would like to present this innovative concept and offer an invitation to take part in this project.

PU-1164  Plasma cell leukaemia: studies on glycosaminoglycans of white blood cells

Khlebina S., Berzhets V.
Research Institute of Vaccines & Sera, Moscow, Russia

The haemorrhagic form of Hageman defect is specific form of the deficiency of clotting factor XI. It may be manifested by different allergic lesions. In this work we studied the level of XI factor in children with bronchial asthma caused by sensitisation to Dermatophagoides mites. The blood plasma of 10 children with bronchial asthma caused by sensitisation to house dust-mites was studied. Blood plasma was taken before, during and after intensive hyposensitive treatment. The activity of factor XII in the blood plasma of the patients was determined with the use of plasma deficient in factor XII. Statistically significant changes in the activity of Hageman factor were observed in the former patients (42-53%). During hyposensitisation the allergic activity of granulocytes was very low. GAGs were isolated from aceton collected dried cells. Electrolysis on cellulose acetate membranes before and after treatment with GAG-activating enzyme was used for GAG identification. The GAG content was found to be 33.0 µg uronic acid per 100 mg of dried cells in first pt, and 63.0 µg in the second. GAGs were presented by 3 polysaccharide fractions: chondroitin sulfate (CS) with different electrophoretic mobility (I: with that of CS - standard, II: as that of dermatan sulphate, III: slower than I and II) and a fraction of heparin sulphate. Some differences in the leucocyte GAG composition in both patients with PCL were revealed. The electrophoretic pattern of GAGs in white blood cells in PCL differed considerably from patterns previously found in other types of leukaemias and therefore can be used as one of the markers of this disease.

PU-1165  Plasma cell leukaemia: studies on glycosaminoglycans of white blood cells

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Research Institute of Vaccines & Sera, Moscow, Russia

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Results. Of these patients, 220 had a thrombocytosis of more than 500 x 10^9/L (30%). They aged between 2 and 83-year old. The mean hemoglobin was 50 g/L and the mean number of platelets was 800 x 10^9/L. There was no correlation between the importance of anaemia, iron deficiency and the importance of thrombocytosis. There were no thromboembolic complications and the outcome was favorable in all cases with a rapid correction of anaemia and thrombocytosis. Conclusions. We conclude that thrombocytosis is frequently associated to iron deficiency anaemia and that it is a benign sign of this type of anaemia.

PU-1166 Marginal splenic cell lymphoma. Findings before and after splenectomy

Lazarevic V, Bogdanovic A, Colovic R, Suvajdic N, Colovic M

Institute of Haematology and Institute of digestive surgery, Clinical Center of Serbia, Belgrade, Yugoslavia

Hepatosplenic candidiasis (HSC) was noted in three patients with leukaemia in deep neutropenia of long duration (13-22 days), two in the course of acute myeloid leukaemia (AML) and one in acute lymphoblastic leukaemia (ALL). A 20-year old female, with AML in relapse with previous hepatitis B infection received chemotherapy and became neutropenic for 17 days. After recovering from neutropenia, she was febrile with pain below the right costal margin and elevated alkaline phosphatase. Ultrasound (US) and computed tomography (CT) revealed multiple focal changes in the liver and spleen. Laparoscopic biopsy of the liver showed Candida hyphae. Therapy with amphotericin B (AMB) and 5-flucytosine (5-FC) could not completely cure the infection. After a surgical evacuation of multiple abscesses the patient became well. A year later she was in complete remission. The second patient was a 19-year old male with newly diagnosed ALL and signs of cholestasis. After chemotherapy the cholestasis and 13-day neutropenia ended, but a high fever and elevated alkaline phosphatase persisted. Candida albicans was found in blood cultures and CT verified focal changes in the liver and spleen. The infection was under control in 4 weeks of therapy with AMB. Chemotherapy has been continued thereafter. The third patient was a 54-year old male with AML in relapse with previous hepatitis B infection received standard intensive chemotherapy. High fever continued after 22 days of neutropenia and CT revealed foci of metastatic disease in the liver and spleen. Candida was found in blood cultures and stool samples, but not in the liver biopsy. Unfortunately, despite AMB and 5-FC therapy the patient died in relapse with multorgan failure. In conclusion, even when applying antifungal therapy, HSC is difficult to treat to such degree that in one patient we had to evacuate multiple fungal abscesses surgically. Even if hepatic damage is not an established risk factor, it should be especially noted if patients have previous hepatic damage, two with hepatitis B and one with cholestatic leukaemic infiltration.

PU-1168 Unsuccessful treatment of sideroblastic anaemia with chloroquine

Lima CS, Albert FL, Souza OF, Costa F, Saad ST, Amuda VR

Faculty of Medical Sciences, State University of Campinas, Campinas, S¹ Paulo, Brazil

The mainstay of treatment for sideroblastic anaemia (SA), a group of heterogeneous disorders involving a defect in heme synthesis, is packed red cells transfusion. Chloroquine, an antimalaric drug, interferes with the metabolism of heme, and its activity is related to interactions with heme-protoporphyrin IX. Few reports point to complete remission of anaemia in patients with SA, but larger studies have not yet been performed. With the purpose of analyzing the effects of chloroquine treatment, we studied 9 patients from the University of Campinas. All of them had received ineffective treatment with oral pyridoxine. The diagnosis of SA was made according to the FAB criteria. To be eligible to the study, the candidates should have no cardiac or ophthalmologic dysfunction. After informed consent, chloroquine was administered as a 300 mg day regimen for 6 months. Monthly follow up included clinical evaluation and peripheral blood counts. Ophthalmologic evaluation was performed before treatment, after six months and if any visual complaint was referred. Chloroquine produced only gastrointestinal side effects. Epigastric pain was observed in five out of six patients. The administration of chloroquine had to be interrupted in two patients at the beginning of the study, due to severity of this symptom. Two out of six patients studied had never received packed red cell transfusion. However, no significant increase in haemoglobin levels was observed. The transfusion requirement remained the same in these two patients before chloroquine treatment in the determined two patients. In conclusion, the chloroquine treatment caused irritation to the gastric mucosa and no beneficial haematological effect could be observed in the patients in this study.

PU-1169 Surgery in haemophilia and other congenital coagulopathies

Lisitchkoff T, Zarkova A, Kalsev II, Martinova F

National Center of Haematology and Transfusiology, Medical University, National Institute for Emergency Medicine, Sofia, Bulgaria

We analysed 81 surgical operations in 65 patients with various hereditary coagulation disorders (haemophilia A and B, VWD, deficiency of factor 11, 12, 13, dysfibrinogenemias). In 12 of the patients the coagopathy was unknown and the excessive bleeding during the operation was the first manifestation of the disease. The diagnosis was made in conditions of emergency and in all these cases it was mild haemophilia A. The surgical interventions may be classified in three groups: 1) 43 operations for diseases unrelated to the coagopathy: tumors (malignant and nonmalignant), fractures, hematomas, splenectomies, intestinal diseases, bypass, operations in orthocrinolaringology, circumsicions, echinococcosis, deliveries by cesarean section. 2) 18 operations for manifestations of the coagopathy: bleedings from gastrointestinal tract, spinal cord bleedings, retroperitoneal haemorrhages. 3) 13 operations for complications of the coagopathy and substitution therapy: excision of haematomas, pseudotumors, total hip replacements, vessel shunts in liver cirrhosis. Till 1995 substitution therapy was performed by home made plasma preparations with low purity and no virus inactivation. Since 1996 has been performed with high purity virus inactivated plasma derived and recombinant preparations. Six patients died after surgery, 4 of them because of surgical complications (bleedings and infections) and 2 because of progression of the disease (neoplastic diseases).

PU-1170 Chromosomal abnormalities in patients with primary and MDS-transformed acute leukemias

Loginskiy YO, Salamanouchuk ZY, Madiak ZV, Vygovska Y, Novak VL

Lviv Research Institute of Blood Pathology Transfusiological Medicine, Lviv, Ukraine

Acute leukemias (AL) are clonal disorders originating from malignant transformation at haematopoietic precursor cell level. Several cases of AL have developed from myelodysplastic syndrome (MDS) and have been designated as MDS-transformed AL. We observed 7 adults patients with acute myeloid leukaemia (AML) de novo, 5 patients with MDS-transformed AL, and 1 patient with AML with myelodysplasia (MDS-AML). The diagnoses were determined according to FAB classification. For all cases we performed a cytogenetic investigations. Almost all patients with AML de novo carried the sole cytogenetic abnormalities among which we identified monosomy 11,
It(9;22)(q34;q11) and polyplid metaphases. The median survival was 20 weeks. At the diagnosis of MDS no structural changes were found in 3 studied patients. In the remaining, karyotype analysis revealed the presence of complex structural and numerical aberrations. Except typical chromosomal rearrangements, we described del(5q), del(6q) and a ring unidentised chromosome. One patient’s disease converted into AML, M2 from chronic lymphoid leukaemia and presented del(Sp), del(8q) and a ring unidentified chromosome. One further patient showed a notable karyotypic abnormality and was described del(17p). The median survival of these patients was 2 weeks. Our study illustrates more complicated karyotypic changes in MDS transformed acute myeloid leukaemia. Karyotype has been shown to be an independent prognostic indicator, second only to the FAB subtypes as a predictor of overall survival.

PU-1171 Mobilising progenitors cells in patients with chronic myeloid leukaemia achieved major cytogenetic response to intron-A

Lubimova L, Savchenko V, Kusmina L, Varlamova S, Momohtuk K, Khoroshko N, Turkina A

Haematological Scientific Centre Moscow, Russia

Autografting is a promising treatment for patients with CML. A direct correlation between of Ph+ cells infused and cytogenetic response after autologous transplantation has been reported. Different techniques to eradicate Ph+ (or BCR/ABL+) cells from the transfuse have been suggested. The Genoa group collected blood stem cells during the recovery phase following ICE - induced aplasia. In our clinic we are using this approach too. But now we have some, patients with major or complete cytogenetic response to IFN. In this report we present the results of peripheral blood stem cell (PBSC) collection in our first two patients for use should the patient, lose their response. The patients were a female of 32y and a male of 23y; the interval between diagnosis and mobilisation of PBSC in both was 20 months. Previously they received intra A 5 X 10^9 m3 per day and the men was treated additionally with monthly course of ara - c at a single daily dose of 20 mg/m^2 for 10 days during 20 and 14 mo respectively. At mobilisa- tion the % Ph+ cells was 16.5 and 14.8% respectively. Patients received G-CSF (granocyte) at a dose of 10 µg/kg b-6 days, the collection started at +5 and +4 days and continued for 3 days. In the first patient the total number of progenitors was not enough. A successful collection was made from the second patient (median MNC 3.5 X 10^6/kg b-6 days). CD34 +2.2 X 10^6/kg, Ph- <10%. Our results suggest that PBSC collection can be successful even in patients, treated with IFN for a long time and the first harvest is most likely to contain adequate number of MNC, CD34+ and Ph- cells.

PU-1172 Divergent patterns of hepatocyte growth factor (HGF) in patients with acute leukaemia

Marjanovic A,* Elbaz O,* Naar M

*Departments of Pediatric, Haematology, *Clinical Pathology and Internal Medicine Deps, Mansoura Faculty of Medicine, Mansoura, Egypt

We measured serum HGF levels by a quantitative sandwich enzyme Immunoassay technique in 47 patients with acute leukaemia (23 with AML & 24 with ALL). Twenty healthy age-matched subjects served as a reference group. Serum HGF in cases, with AML was found to be increased significantly before induction of therapy (Median=5378 pg/mL), in comparision to its value after remission (M=1085 pg/mL), or in the control group (M=773 pg/mL). On the other hand, patients with ALL had values (M=892 pg/mL) near to values of the control group. These results suggest that AML patients may have the ability to produce HGF & serum HGF may potentially be used as an indicator of clinical significance in the future in the diagnosis and follow up of patients with AML.

PU-1173 Alloinmunisation after blood transfusions in patients with haematomatological diseases


Objective. The actual possibilities of diagnosis and treatment of haemato- pathies are increasing the utilisation of blood and its derivatives. Associ- ating substitutions because of the unsatisfactory number of blood donors, creates a risk of immunisation in these patients. Our study was undertak- en to evaluate immunisation after repeated transfusions practised to a group of patients with haematological disorders. Design and Methods. Immune haematological investigations of 148 patients with different haemopathies. These pts. were sent for investigation and blood compatibil- ity studies by us. On 101 patients, no matching was found. The total number of patients was 47. In 32 pts. we used phasimimetic agents to select donor blood in the example of a human malignancy that is principally caused by defects in cell cycle regulation. The aim of this work was to examine whether Chromobucil induced apoptosis might be related to the clinical response of pts with B-CLL and to evaluate in all patients the correlation between p53 mutated pts, who were initially treated with high-dose of Chromobucil (HD- CLB). The Quatitative analysis of apoptotic parameters on semi-fine sec- tions of peripheral blood was performed prior and during the first 5 days of therapy. Apoptosis was also correlated to b-2 and mutated p53 protein levels, detected immunohistochemically. In vivo outcome after the treatment with HD-CLB was evaluable in 7 pts. The level of spontaneous apoptosis was 11.29-20.50% in all analysed pts. In 3 cases, whose cells had shown the high level of spontaneous apoptosis, the time of maximal apoptotic response (TMAR) was 24 days and maximal apoptotic response (MARM) 23.42-26.36%, had a complete response (CR). Two pts showed neg- ative CD, without diffusion of bone marrow involvement. In the remaining 4 pts. it was possible to determine the same parameters in only 114 pts. TMAR was in the 3rd day and MARM 23.4%. All of them had positive LD, diffuse bone marrow involvement, 214 pts had CLF, cytomorphologic type and clinical stage B. Pts who have achieved CR, have the highest percentage of dying cells due to therapy-induced apoptosis. Intermediate values were recorded in pts who were in condition of a PR. A high expression of the protein product of b-2-gene was detected in all pts. At the same time the absence of the mutated p53 was in correlation with their clinically good response to the received therapy. Our preliminary results of apoptotic response to therapy, with other relevant parameters might be used for determination of the most appropriate treatment for each patient.

PU-1174 Diagnosis of deep vein thrombosis by a rapid ELISA D-dimer test, clinical model and ultrasonography

Michels J, Oortwijn WA, Naaborg R

Goodheart Institute, Haematology, Haemostasis and Thrombosis Research Center, Rotterdam and TNO, Prevention and Health, Health Care Management, Leiden, The Netherlands

A sound basis is provided for quantifying clinical judgment for the diagno- sis of acute proximal DVT. The number of positive clinical findings at time of first suspicion of DVT correlates directly with the probability of suffering from acute proximal DVT. The modified clinical model of Landefeld and Wells for DVT allows classification of patients into low, moderate and high pretest clinical probability (PCP) of having DVT. The automated rapid ELISA VIDAS D-dimer assay presently available can be rapidly performed in daily practice and emergency situations. In contrast to all qualitative latex D- dimer assays, the rapid ELISA VIDAS D-dimer test is accurate to a very high degree in ruling out calf vein thrombosis (CVT) and proximal DVT. The sequential use of a negative rapid ELISA D-dimer test (<500 ng/mL) for the exclusion of CVT and DVT, with a sensitivity and negative predictive value of 100% followed by compression ultrasonography (CUS: sensitivity 89-100% and specificity 98-100%) for the exclusion and diagnosis of DVT in symptomatic outpatients in the setting of a simple clinical model predicts time sparing for the specialists and patients and significant cost reduction for the health care providers. After a first negative CUS, a second CUS is not indicated at a rapid ELISA test result below 1000 ng/mL. A sec-
PU-1176 Treatment of angioimmunoblastic T-cell lymphoma - a 5 years experience

Mihailetci B,* Nedelkovic-Janic R,* Janovic S,* Milivojevic G,* Cemecnik V,* Colovic M,* Petrovic M**

*Institute of Haematology and **Institute of Pulmonology, Clinical Center of Serbia, Belgrade, Yugoslavia

We report on 7 males and 3 females, median age: 53 years, with angioimmunoblastic T-cell lymphoma (AITL) who had generalised lymphadenopathy, prominent systemic symptoms and skin rash. Seven of the ten had polycythaemia rubra, 5 had 50% and 5 had 50% high intermediate risk. They were treated with COP and CHOP regimens. Median follow-up was 36 months, and 2 pts died 4 months (with aplasia bone marrow) and 2 years after the diagnosis was established (recovery). The other patients are under the regular follow-up. The therapy with AITL is still controversial and needs further correlations with clinical and pathological features.

PU-1177 Abnormalities of platelet aggregation in chronic myeloproliferative disorders


Coltea Clinical Hospital, Department of Haematology, University of Medicine "Carol Davila", Bucharest, Romania

A large variety of acquired platelet dysfunctions has been described in patients with myeloproliferative syndromes. These complex abnormalities in platelet activity may be due to deficiency of platelet granules, quantitative or qualitative abnormalities of platelet membrane receptors and defects in arachidonic acid metabolism. In this study we report defects in platelet function in 52 patients with chronic myeloproliferative disorders including 12 patients with polycythemia rubra vera, 12 with chronic myelogenous leukaemia, 16 with essential thrombocytopenia and 12 with myeloid metaplasia. The platelet activity was studied in vitro by measuring platelets aggregating response to ADP, epinephrine collagen ristocetin and arachidonic acid and these results were correlated with bleeding time, clinical events (bleeding tendency and thrombotic complications) and evolutionary stage of the disease. We found the following important changes in platelet response to aggregating agents: loss of platelet aggregating response to epinephrine - 30.7% (16/52 cases); abnormalities of platelet aggregating response to epinephrine (a very plate wave) - 19.2% (10/52); the loss of platelet aggregating response to arachidonic acid - 46% (24/52); the loss of platelet aggregating response to ADP - 19.2% (10/52); the loss of secondary wave of platelet aggregating to ADP - 27% (14/52); decrease in platelet aggregation response to ADP - 19.2% (10/52); the loss of platelet aggregating response to arachidonic acid - 46% (24/52); the loss of platelet aggregating response to collagen - 23% (12/52); prolonged lag phase in platelet response to collagen - 11.5% (6/52); decrease in platelet aggregation response to collagen - 15.4% (8/52); abnormalities in platelet aggregation response to collagen (only platelet shape changes) - 11.5% (6/52); abnormalities of platelet aggregating response to ristocetin (loss or decrease) - 30.7% (16/52); loss of platelet aggregating response to all agents - 19.2% (10/52) in late stage of the disease. In 15.4% (8/52) of cases there was a normal platelet aggregation. The most frequent combination of aggregating abnormalities (found in 30.7%-16/52) is: the loss of aggregating response to epinephrine and arachidonic acid the loss of secondary wave to ADP, the decrease in aggregating response to collagen in association with a normal response to ristocetin. This combination was found especially in first stage of the disease. We conclude that the abnormalities of platelet aggregation are very common in chronic myeloproliferative disorders and their study is import for evaluating the stage of these diseases. Platelet aggregation may be very useful for assessing the thrombo-haemorrhagic risk for an individual patient.

PU-1178 Familial Hodgkin’s disease in Russian population

Naisibov O, Pivnik A, Sotnikov V,* Andreovan N, Khamaganova E, Aleshchenko A, Kolosova L

Research Centre for Haematology, Scientific Research Centre for Diagnostic and Surgery, Moscow, Russia

Familial Hodgkin’s disease (FHD) is observed in 4.5% approximately of all cases of Hodgkin’s disease (HD). We investigated five cases of FHD with the purpose of determining factors promoting HD developments. HLA loci A, B, C, DRB1 were studied. Contrary to our previous population data (1998) we did not find an increase in HLA Cw7 and DRB1. HLA B5 has been found in four cases (40% vs. 24% in healthy controls). The histological form of nodular sclerosis (NS) prevailed and was concordant in two pairs parent-child and one cousin pair. We cannot deny the influence of immunogenetic mechanisms, a last partly on prediction of FHD. But more observations are needed for elucidation of the nature of FHD.

PU-1179 Childhood lymphomas in Lithuania: epidemiological, clinical, pathological findings and response to treatment

Nausviciute L, Rageliene L

Pediatric Clinic, Vilnius, Lithuania

The aim of this study was to determine the clinical picture and results of treat ment of non-Hodgkin’s lymphoma (NHL) and Hodgkin’s disease (HD) in children. The studied group consisted of 82 children, 42 patients having HD and 40 patients with NHL, treated in our clinic during 10 year period from 1989 to 1999. The mean age at the onset of the disease was 7.35 years for NHL and 10.75 years for HD. A male predominance was evident in both groups of lymphomas with ratio 2.3 for NHL and 1.6 for HD. NHL predominant St. Jude stage at the time of diagnosis was IV (67.5%), half of patients had B symptoms. The immunophenotyping was started to perform on 1995; 85% presented with mediastinal masses, 9% with abdominal involvement. Ninety percent of HD patients were in I stage at the time of diagnosis. Nodal sclerosis and mixed cellularity were the most commonly seen histological subtypes. Constitutional symptoms were determined in 54% of patients. All patients were treated according to BFM NHL and DAL HD protocols. 62% of patients received additional radiotherapy. Stable remission was achieved in 88% of HD patients and only 47.5% in NHL. Long term survival (>5 years) is 14.2% for HD patients and 10% for NHL patients. Conclusions: higher incidence of NHL and HD treated in the same time. The study suggests that treatment outcome of NHL and HD differs since the most NHL were diagnosed at the last stage.

PU-1180 Bone marrow morphological description in patients with acute myeloid leukaemia living in Ukraine

Nastreiko YeP, Koval Al, Tretak N*P

Research Institute of Haematology and Blood Transfusion, Kiev, Ukraine

To evaluate bone marrow status in patients with acute myeloid leukaemia (AML) living in Ukraine and exposed to the Chernobyl accident, morphological study of 62 patients’ trephine biopsies was carried out. The patients under study were aged 18-73 years old. The obtained data were compared with the results of similar studies that had been conducted before 1986. Hystological methods as well as electronic microscopy were used. It was ascertained that in patients with AML diagnosed after 1987 bone marrow hypercellularity, high frequency appearance of blast elements in mitotic division status were found more often. Morphological signs of dyscitaratory disturbances were seen in patients of different age groups. Small foreign particles were found inside macrophages, endotheliocytes and reticular cells in patients who had taken part in the disaster abolition or lived in radiation contaminated areas. The presence in cell cytoplasm was accompanied by lymphos tissue local development. In the same group of patients stromal cell intensive apoptosis accompanied by nonutilised apoptotic body accumulation in subendostal zones of bone marrow cavities were seen. The
studies allow to make the conclusion that in the analysed group of patients with AML there are some peculiarities in bone marrow morphology that should be kept in mind in the evaluation of the disease's clinical course.

**PU-1181 Transfusion therapy of haemophiliacs with pyo-inflammatory complications**

Nazarachuk LV, Sukhoviy MV, Skachkova N.K, Nemirovskaya LN, Yuschenko PV

Research Institute of Haematology and Blood Transfusion, Kiev, Ukraine

We studied 70 haemophiliacs with pyo-inflammatory complications during the course of combined therapy, including anti-haemophilic and haemostatic treatment, blood donor specifically directed alloregic preparation use immunocorrection, antibiotic therapy, application with silicon sorbent new generation, have been studied. The following methods were used surgical, biochemical, immunological, microbiological. It was ascertainment that in 80% of patients with pyo-inflammatory complications there were pathogenic microorganisms of the following common genera: Staphylococcus, Streptococcus (60.9%) - more frequently Enterobacter, Proteus, Pseudomonas, Serratia, Hafnia (52.2%) - less frequently and associated (9.7%). Peripheral blood values were within physiological normal limits; biochemical values, i.e. alanin aminotransferase and aspartate aminotransferase activities decreased by the end of the therapy (respectively, from 1.02±42.0 8.7±10 down to 0.88±0.27. 0.67±0.25, mmol/l); coagulation parameters were stable. A lymphocyte and T-helper level increased by the therapy cessation (respectively, from 25.12±2.3, 75.6±6.3 down to 16.8±2.1, 54.7±6.0%).

**PU-1182 Disparity between sources of cytological and histological findings in the diagnosis of Hodgkin's disease**

Nedeljkov-Janjic B, Mihaljevic B, Cemenzki-Barilovitch V, Petrovic M

Institute of Haematology, Clinical Center of Serbia, Belgrade, Yugoslavia

The histological analysis of tumour tissue is still of crucial significance for the diagnosis and subclassification of lymphomas. Cytological preparations of lymph glands provide better insight into the morphological details of tumour cells. The object of this study was to evaluate the value of cytodiagnosis of Hodgkin disease (HD) as well as to define the factors of inaccuracy of this procedure. The study included 59 specimens of which 30 had been prepared by imprint technique and 29 by puncture of lymph glands. The histological studies of biopsy samples were performed simultaneously in all 59 cytomorphologically analysed cases. Out of this number, the cytodiagnosis was inconsistent with histological findings in 5 cases. Thorough analysis of 5 disparate diagnoses identified three sources of error: (1) inadequate material, (2) sampling technique, and (3) interpretation errors. Although more adequate material was obtained by the imprint technique, even in such samples it was not possible to define precisely the type of HD. Therefore, the cytopathological diagnosis with clinically suspected HD may be suggested only for emergency cases (in life-threatening cases) as well as for establishing recurrence of the disease.

**PU-1183 Platelet concentrate in the treatment of haemorrhagic syn- drome in patients with acute leukaemia - supportive care**


*National Blood Transfusion Institute, Belgrade, Yugoslavia, *Institute of Haematology, UCC Serbia, Belgrade, Yugoslavia

Leukocyte depletion of pooled PC (LPD PC) is an important procedure in transfusion practice for the prevention of alloimmunisation and post-transfusion in multiply transfused patients with haemorrhagic syndrome (HS). HS is often associated with increased risk of bleeding. Advances in platelet transfusion have contributed to improved outcomes in the treatment of patients with cancer. Leukocyte depletion of pooled platelet concentrates (LPD PC) is an important procedure in transfusion practice for the prevention of alloimmunisation and post-transfusion reactions in multiply transfused oncological patients with haemorrhagic syndrome (HS). Purpose. Analysis of the efficiency of PC transfusion in oncological patients with HS. Subjects and Methods. Retrospective analysis of 60 patients (age 16-73) with malignant lymphomas and metastatic tumors was performed. According to the type of PC, patients (pts) were divided into 2 groups. Group 1 (33 pts) received standard platelet concentrates. In group 2 (27 pts) received PC pool ABO-identical. Efficiency of the therapy was assessed according to corrected count increment (CCI) and bleeding time (Duke). Results. Average platelet count in group 1 and group 2 was 18.9±118.1±10^11/L and 14.8±8.1±10^11/L prior to PC transfusion and 37.2±6.1±10^11/L and 22.7±19.3±10^11/L after platelet transfusion respectively. CCI in group 1 was 8 and in group 2 was 6.7 (p<0.05). Bleeding time was 9 minutes and 9.1 minutes prior to PC transfusion and 6.4 and 4.9 minutes after PC transfusion in group 1 and group 2 respectively. In group 1 bleeding time was corrected in 93.5% while CCI was notably positive in 87.1% PC transfusions. In group 2 bleeding time was corrected in 91.3% while CCI was positive in 63% PC transfusions. Conclusions. According to CCI and bleeding time, a satisfactory haemostatic effect was found in oncological patient. However, bleeding time is a better parameter for the evaluation of PC transfusion therapy than CCI.

**PU-1184 Two types of platelet concentrates in the treatment of haemorrhagic syndrome in oncological patients**


*Institute of Oncology and Radiology, Belgrade, Yugoslavia, *National Blood Transfusion Institute, Belgrade, Yugoslavia

Efficiency of anticancer therapy is restricted to toxicity which is manifested by leukopenia, thrombocytopenia which, as a consequence, has the increased risk of bleeding. Advances in platelet transfusion have contributed to improved outcomes in the treatment of patients with cancer. Leukocyte depletion of pooled platelet concentrates (LPD PC) is an important procedure in transfusion practice for the prevention of alloimmunisation and post-transfusion reactions in multiply transfused oncological patients with haemorrhagic syndrome (HS). Purpose. Analysis of the efficiency of PC transfusion in oncological patients with HS. Subjects and Methods. Retrospective analysis of 60 patients (age 16-73) with malignant lymphomas and metastatic tumors was performed. According to the type of PC, patients (pts) were divided into 2 groups. Group 1 (33 pts) received standard platelet concentrates. In group 2 (27 pts) received PC pool ABO-identical. Efficiency of the therapy was assessed according to corrected count increment (CCI) and bleeding time (Duke). Results. Average platelet count in group 1 and group 2 was 18.9±118.1±10^11/L and 14.8±8.1±10^11/L prior to PC transfusion and 37.2±6.1±10^11/L and 22.7±19.3±10^11/L after platelet transfusion respectively. CCI in group 1 was 8 and in group 2 was 6.7 (p<0.05). Bleeding time was 9 minutes and 9.1 minutes prior to PC transfusion and 6.4 and 4.9 minutes after PC transfusion in group 1 and group 2 respectively. In group 1 bleeding time was corrected in 93.5% while CCI was notably positive in 87.1% PC transfusions. In group 2 bleeding time was corrected in 91.3% while CCI was positive in 63% PC transfusions. Conclusions. According to CCI and bleeding time, a satisfactory haemostatic effect was found in oncological patient. However, bleeding time is a better parameter for the evaluation of PC transfusion therapy than CCI.

**PU-1185 Primary myelodysplastic syndromes in childhood: report of six patients from a single unit in Romania**

Popa G, Olaru DC, Miiu N, Rădulescu E, Florescu P

Pediatric Clinic No. II, Univ. of Med. and Pharm "Iuliu Hatieganu", Cluj-Napoca, Romania

We describe the clinical and cytological features of six cases of primary pediatric myelodysplastic syndromes (P-MDS). Children that met the FAB criteria of NMS diagnosis between January 1990 and December 1997 were included. Pre-ALL and secondary NMS (associated with constitutional abnormalities) were not included in this study. We recorded the presenting characteristics including bone marrow aspirate and biopsy and also the outcome during the follow-up period. Three males and three females median age 11 years (range 1 to 16 years) were considered as primary-P-MDS and classified as: two cases of refractory anemia (RA), two RA with excess of blasts (RAEB), one RA with excess of blasts in transformation (RAEB-T) and one chronic myelomonocytic leukaemia (CMML). Three patients progressed to AML (two M. and one M.) in 1, 5 and respectively 6 months. One patient with RAEB died of severe sepsis 7 months after diagnosis. Two patients (one RA and one CMML) had stable disease during 44 months and 60 months of follow-up. One of three secondary AML responded to chemotherapy and was alive in partial remission at the end of the study, 9 months after leukaemic progression. We conclude that P-MDS can represent either the advanced stage of a preleukaemic process or a stable chronic disorder of haematopoiesis.

**PU-1186 Cryopreservation of erythrocytes at -40°C: experimental and clinical studies**

Odvik V, Novak V, Vinarchyk M

Liviu Research Institute of Blood Pathology and Transfusion Medicine, Liviu, Ukraine

Creation of inexpensive and simple methods of preserving erythrocytes in a frozen state is still important for contemporary transfusion medicine. Method of cryopreserving erythrocytes at -40°C with glycerin in a final concentration of 15% was developed. Crystal content of cryopreserved erythrocytes was 0.98±0.32%. Cooling temperature was kept between -10°C and -18°C during cryopreservation. Cryopreserved erythrocytes were stored during 2 weeks at -40°C. Average erythrocyte count in group 1 was 5.72±0.21 and in group 2 was 5.63±0.22 x 10^12/L. Analysis of the efficiency of cryopreservation was performed in 60 patients (age 16-73) in the Blood Transfusion Institute and in 100 patients (age 16-73) in the departments of the Institute of Haematology. The study included 59 specimens of which 30 had been prepared by imprint technique and 29 by puncture of lymph glands. The histological studies of biopsy samples were performed simultaneously in all 59 cytomorphologically analysed cases. Out of this number, the cytodiagnosis was inconsistent with histological findings in 5 cases. Thorough analysis of 5 disparate diagnoses identified three sources of error: (1) inadequate material, (2) sampling technique, and (3) interpretation errors. Although more adequate material was obtained by the imprint technique, even in such samples it was not possible to define precisely the type of HD. Therefore, the cytopathological diagnosis with clinically suspected HD may be suggested only for emergency cases (in life-threatening cases) as well as for establishing recurrence of the disease.

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Abstracts not presented
centration of 39-66% have been developed in Lviv Research Institute of Blood Pathology and Transfusion Medicine. The purpose of this study was to evaluate whether erythrocytes frozen at -40°C and stored for 2 years are of full functional and morpho-biological value. Erythrocytes, cryopreserved at -40°C, thawed, washed and suspended in lactate-scarcose-phosphate suspension were studied. Ninety-two haematological patients affected by acute and chronic leukemias, non-Hodgkin's lymphomas, Hodgkin's disease, paraprotein haemoblastomas, hereditary and acquired haemolytic, aplastic, and megaloblastic anaemias, treated with administration of erythrocytes cryopreserved at -40°C were observed. The following parameters were studied and analysed: total and unconjugated haemoglobin, haematocrit, total bilirubin, extracellular calcium, ATP, 2,3-DPG, viscosity, process of disk-and-sphere transformation, osmotic resistance, electrophoretic mobility of erythrocytes. After erythrocyte administration positive changes in different parameters were observed: number of erythrocytes (3.6±0.14×10¹²/L to 2.1±0.15×10¹²/L, p<0.05); total haemoglobin (107±0.05 g/L to 112±0.01 g/L, p<0.05); total bilirubin (10.80±0.09 g/L to 9.55±0.23 g/L, p<0.05); haemoglobin (10.99±0.53 g/L to 12.0±1.45 g/L, p<0.05); erythrocytes electrophoretic mobility (0.937±0.0267 µm.cm.B-1.sec-1 to 0.943±0.0284 µm.cm.B-1.sec-1, p<0.05); ATP concentration (3.7±1.17 µmol/g.hb to 2.0±0.18 µmol/g.hb, p<0.05), 2,3-DPG content (22.2±3.32 µmol/g.hb to 9.9±1.18 µmol/g.hb, p<0.05). Experimental and clinical studies have proven erythrocytes cryopreserved at -40°C to be functionally and morpho-biologically valuable haemotransfusion medium. This may have clinical implications in anaemia treatment in haematological patients.

PU-1187 Lactoprotein with sorbitol: new multifunctional plasma substitute in haematology
Orlyk V
Lviv Research Institute of Blood Pathology and Transfusion Medicine, Lviv, Ukraine
Chemotherapy in patients with haematological diseases is often accompanied by intoxication, particularly by the development of toxic hepatitis. This establishes the range of objectives to transfusion medicine: detoxication, improvement of functional liver state, microcirculation haemodynamic stabilization, correction of hypocalcaemia, acid base state of blood, normalisation of fluid-and-electrolyte balance, energy supply, forced diuresis. Application of multifunctional transfusion preparations is prepared. Lacto-protein functional plasma substitute, developed in Lviv Research Institute of Blood Pathology and Transfusion Medicine, was used in the treatment of toxic hepatitis due to chronic lymphoproliferative diseases (chronic lymphocytic leukaemia), non-Hodgkin's lymphomas, Hodgkin's disease, myeloma disease. Albumin, sorbitol, natrium lactate, and electrolytes, prepared by Ringer's method, are the ingredients of the preparation. Transfusions were administered every day or every second day. A single dose of the preparation was 200-400 mL. During the course of the treatment 1000 mL to 1200 mL of the preparation were used. Results of experimental and clinical studies have proven that lactoprotein with sorbitol is a non-reactive transfusion medium. As a result of treatment, the overall condition of patients improved, the level of intoxication was reduced, providing better microcirculation, haemodynamics stabilised, changes of protein and fluid-and-electrolyte balance of blood were positively influenced, diuresis increased, and acidosis diminished. After chemotherapy administration positive changes in different parameters were observed: total bilirubin - from 73.1±6.88 to 35.4±3.52 µmol/l (p<0.05); total protein - from 62.9±3.71 to 75.2±3.40 g/L (p<0.05); albumins - from 40.9±2.65 to 52.6±3.18% (p<0.05); γ - from 27.9±4.1 to 14.8±2.0% (p<0.05); pH - from 7.32±0.010 to 7.39±0.020 (p<0.05); Pco2 - from 29.5±2.76 to 37.2±2.89 (p<0.05); Hco3 - from 16.3±1.69 to 19.6±1.86 mmol/L (p<0.05); BE - from -5.3±3.48 to -3.9±1.33 mmol/l (p<0.05). Results of studies claim the beneficial effect of the use of new plasma substitute, such as lactoprotein with sorbitol, in the treatment of patients with haematological diseases.

PU-1188 New phospholipids and phosphatids mixtures in activated partial thromboplastin time test
Oryshchenko N, Cherpak A, Vous M, Gayda A
Research Institute of Haematology & Blood Transfusion, Lviv, Ukraine
The activated partial thromboplastin time (APTT) test is one of the most widely used diagnostic test in blood coagulation analyses. Most commercial kits are based on cephalin and kaolin. We have created two kits for APTT-determination with soluble mixtures of phospholipids from lipolyhsed egg yolk (first kit) and phospholipids from sunflower oil subproducts (second kit). The first kit contains 40% phosphatidylcholine, 20% phos-
After a normal haematological reconstitution with WBC >10^9/L at day 14, and thrombocytes >2 x 10^10/L at day 22, we detected an increase of immature B-lymphocytes in bone marrow and peripheral blood at day 37, maturing subsequently. Clinical signs of acute GvRD did not occur at this time. Chimaerism studies at day 41 yields 90% donor lymphocytes. The detection of myeloid blasts in the bone marrow at day 31 with an increase up to 6% until day 41 with cytogenetic aberrations as before therapy lead us to transfuse x 10^10/kg donor T lymphocytes at day 42. The blasts decreased and disappeared at day 63. Twelve days after infusion of T lymphocytes we detected an increase of T lymphocytes in the circulation up to 5.5 x 10^10/L, simultaneously with an onset of acute skin GvHD grade 2. After prednisolone therapy we found an amelioration of the GvHD together with an increase of lymphocytes. After day 65 a severe OTG and AKT-resistant liver and gut GvHD developed. The patient died at day 91. Autopodically, additionally to the severe GvHD, cerebral toxoplasmosis was found.

**PU-1192 Acute lymphoblastic leukaemia and thyroid cancer in children**

Ragelienė L, Matulevičius V, Sidlauskas V

Vilnius University Pediatric Centre, Vilnius, Kaunas University Institute of Endocrinology, Kaunas, Lithuania

The aim of our study was to evaluate the influence of applied therapy on thyroid disease. We investigated 50 patients between 12 and 21 years of age with treatment completed between 7 and 12 years ago. The initial diagnosis for all patients was ALL. They received full chemotherapy according to BPM ALL protocols and 18-12 Gy irradiation for neuroleukaemia prophylaxis. The bone marrow transplantation-related thyroid hypoplasia in 36 pts. (72%) and thyroid cancer was diagnosed in 2 girls (4%). Case history #1. An 11-year-old girl presented in 1992 with ALL. The full course of therapy was completed in 1995. In January 1998 the enlargement of thyroid and regional lymph nodes was found. There was a nodular hypoechogenic tumour 0.8-0.7 cm on thyroid ultrasonography. All analyses of blood, bone marrow, biochemistry were normal. After cytomo morphological investigations a thyroid adenoma-like thyroid nodule and necrotic and exsudated papillary fusion were found. The subtotal thyroidectomy was performed. The histology confirmed the diagnosis of infiltrative metastatic, papillary thyroid carcinoma. Case history #2. A 14 year-old female was diagnosed with ALL 12 years earlier. After 2.5 years successful treatment she was followed up and observed every 4-6 months. As the girl had obesity and symptoms of hypothyroidism, she was investigated at endocrinology clinic. No clinical evidence of relapsing ALL or other disease was revealed. Blood analysis showed only increased ESR (40 mm/h). After sonography and punctual biopsy thyroid cancer was suspected. A subtotal thyroidectomy was performed and the diagnosis of poorly differentiated papillary thyroid carcinoma was made. Conclusions. The iodination dose for neuroleukaemia prophylaxis, is low 12 Gy. We believe that the development of thyroid dysfunction and development of thyroid cancer is part of a causal relationship between individual response to irradiation, chemotherapy and thyroid function before treatment.

**PU-1193 Necrotising pyomyositis complicating sickle cell disease**

Vasiliou GS, Roberts-Harewood M

Department of Pathology, S. João, 3Hospital, Centro de Genética Clínica, Porto, Portugal

Pyomyositis is a pyogenic infection of skeletal muscle well described in the Tropics, classically affecting healthy young men who engage in intense physical activity or individuals with impaired immunity, such as HIV and other immunodeficient states. Although patients with sickle cell disease have an increased susceptibility to infection, there are few reports of pyomyositis in the literature. A 24-year-old, HIV negative man with haemoglobin SC disease developed a life-threatening illness soon after a trip to his native Ghana. Although he initially presented with symptoms and signs compatible with a sickle chest syndrome, despite continuous positive airways pressure (CPAP), antibiotics, hydration and analgesia, he continued to complain of diffuse, severe pain in his hips and back. On day 20 of his admission Staphylococcus aureus was isolated from blood cultures and CT imaging revealed pyomyositis of the gluteal and iliospinal muscles. The infection proved to be extremely resistant to treatment. A total of thirteen surgical drainage procedures were required, over 4 litres of pus were removed and potent intravenous antibiotics with Quinaprilin/nalidixin (as yet unclassified in the United Kingdom) were required to control the infection over a period of 9 months. Repeated blood trans-fusions, high-calorie nasogastric feeding and intensive physiotherapy were required to support our patient through his ordeal. At the time of discharge - 265 days after the original admission - our patient was 20 kg lighter than at discharge and had significant renal impairment and could only walk with assistance due to severe muscle destruction and wasting. As with previously reported cases, our patient was a fit, active young man prior to this illness, keen on weight training and bodybuilding. His clinical course was complicated and exacerbated by firstly SCD and secondly the development of methicillin resistance. Thus we suggest that the diagnosis of pyomyositis should be considered in any patient with SCD presenting with cellulitis and inter-mittent pyrexia that fails to resolve with standard therapy. Finally we draw attention to the increasing problems of antibiotic resistance and another potentially life-threatening manifestation of MRSA.

**PU-1194 Musculoskeletal complications in haematological diseases. A magnetic resonance imaging (MRI) study**


Departments of Haematology, and Radiology, Miguel Servet Hospital, Zaragoza, Spain

Background. MRI of bone marrow shows two different types of signals: A high intensity signal characteristic of the fat bone marrow and an intermediate intensity signal from haematopoietic bone marrow areas. So MRI could be a good method to evaluate bone marrow status and also a good diagnostic procedure to study musculoskeletal complications. Design and Methods. Eighty-eight MRI studies were performed in 69 patients with PH. Period of study: 10/95-12/98. MRI was performed using a 0.5 Tesla GE unit, T1 and T2 WI were acquired in spine, pelvis and femora in two cases in lower extremities. Results. Twenty two different complications were detected in 18 patients (26.1%). Avascular necrosis in 9 cases (7 in femoral head, 1 ankle, 1 humerus), bone infarcts in 5, vertebral collapse 3 cases, painful bone disease 25 cases and juvenile thyroid hypoplasia in 1 case. According to the pathogenesis in 20 cases bone lesions were related to bone marrow involvement and in the remaining two could have been secondary to therapy. Conclusions. In spite of the small number of cases, MRI could be an effective tool as a non-invasive procedure to evaluate bone marrow status.

**PU-1195 Complex karyotype with extensive chromosomal abnormalities in a case of myelodysplastic syndrome analysed by FISH. A case report**

Rodrigues L1 Lemos R1, Granjo E1, Tavares P1

1-Haematology Department, Pedro Hispano Hospital, 2-Haematology Department, S. João, 3-Hospital, Centro de Genética Clínica, Porto, Portugal

Clones with cytogenetic rearrangements in myelodysplastic syndromes (MDS) are seen in about 50% of de novo MDS. A new case with a very large number of de novo rearrangements is reported. A 49-year-old female, a housewife with a history of Parkinson disease and six surgical interventions under general anaesthesia, was admitted in February 1996 in Pedro Hispano Hospital because of melena, asthenia and rapid loss weight (10 kg in the preceding month). Blood tests showed pancytopenia, severe anaemia (haemoglobin 4.9 g/L, aniso and poikilocytosis), leukocyte count 2.4 x 10^9/L (neutrophils 51%), severe thrombocytopenia (19 x 10^9/L), and high lactic dehydrogenase and fibrin. Bone marrow was hypoplast in one, the erythroid series showed megaloblastoid features and signs of dyserythropoiesis - binuclear cells, coarse incorporation of iron, megalakroythrocytes were severely diminished, megaloblasts were present (25%). Thus, a diagnosis of SMD AREB-t was made. In non-stimulated bone-marrow cultures (2hs, 24hs, and 48 hs), a complex/mosaicism was found: normal, 46.XX (40%) and aneuploid mitoses (60%), with numbers ranging from 41 to 95. Anomalies defined by FISH were: −5 X, −X, +der[1]t(14:22)(q11.2:q11.2), +7, +8, +9, +10, +11, +12, −13, −14, −16, +der[16]t(14:16), +der[14:11], +11p13.3:14q24→+14q24, −19q13.3:14q24+14q24, −19q13.3:14q24→+14q24, −14q24→+14q24, −10 p10→+21p11, +21q11.2, +21q22.2e to one of four mar(c) chromosomes. The patient died one month later, so it may be assumed she was already in the terminal phase of her disease. Conclusions. Only 8q markers (which could not be identified by FISH) are frequently found in MDS. The presence of more than one clone (der[5] and -7 excepted) is related to a short survival time (about 1 year) which augments the clinical course and makes the complex/mosaicism, can explain the very bad prognosis. The value of RX-FISH is emphasised, as an additional effective tool for studying complex karyotypes such as those observed in the present case.
PU-1196 Chronic consumptive coagulopathy associated with a stable abdominal aortic aneurysm

Rolovic Z,* Miljic P,* Golic M,* Jurcivic R,* Bojic M*

Cardiovascular Institute-Dedijer, Medical Faculty of Belgrade, Institute of Haematology, Clinical Center Serbia, Belgrade, Yugoslavia

Chronic consumption coagulopathy (CCC) is infrequently associated with a stable abdominal aortic aneurysm (AAA). When the two coexist they create a difficult problem that requires optimal decision concerning the timing and extent of surgical procedure. We describe the case of two elderly males who developed CCC in association with AAA. The first patient, a 68-year-old, was admitted because of abdominal pain. There was slight enlargement of the liver and spleen, but no haemorrhagic or thrombotic manifestations. Ultrasonography (US) revealed a sacular AAA of the infrarenal part (size 77x55 mm) with the thrombus on the posterior wall (size 8 mm). Peripheral blood count showed low platelet count (Pc) ranging from 25-57x10^11/L Coagulation tests (CT) revealed a decreased concentration of fibrinogen 1.01 g/L (normal 2-4), increased D-dimer 8 mg/L (normal <0.5), prolonged PT/2s (normal 27-35), shortened euglobin lysis time (ELT) 15 min (normal >120) thus disclosing the presence of an overt feature of CCC. Biochemistry of liver and renal function were normal. We advised that surgery could not be performed before the CCC was corrected. Additional investigations revealed the signs of portal hypertension and the patient was operated without previous tapering of CCC. Instead during operation, the patient received huge amounts of blood components, but died three days later. A liver biopsy was taken during operation and showed non-active macronodular cirrhosis. The second patient, a 79-year-old, was admitted because of a finding of decreased Pc (88x10^11/L). There were no bleeding or thrombotic manifestations. CT revealed a normal fibrinogen concentration, increased D-dimer (11 mg/L), elevated FDP 80 mg/L (normal <10), shortened ELT 40 min, prolonged PTT due to decreased levels of factors VIII and XII thus indicating compensated CCC. US showed AAA (size 55x50 mm) with a small thrombus on the posterior wall (6 mm). After 1 year the US disclosed an unchanged AAA but disappearance of thrombus and CT revealed resolution of CCC. The different outcomes of our patients demonstrate that surgery in elderly with CCC associated with AAA may be a double-edged knife and assessment of the benefits of surgery in these patients need controlled clinical trials.

PU-1197 Coexistence of autoimmune neutropenia and masteythia gravis in the same patient

Rzepecki P, Sulek K, HaIka J, Czaja T, Betluk B

Department of Internal Medicine and Haematology, Central Clinical Hospital, Military Medical School, Warsaw, Poland

Severe leukopenia involving mature white cells with normal erythroid and thrombocytopenia can be associated with presence of cytoxic substance in the serum injuring fully differentiated granulocytes, monocytes and lymphocytes. Inhibitory activity can be associated with imflunoglobulin G or M class. Autoimmune agranulocytosis can coexist with some autimmune (eg. connective tissue), viral or neoplastic diseases including thymoma. Pathogenesis of myasthenia gravis is also autoimmune. There are only a few reports describing agranulocytosis and myasthenia gravis coexist in the same patient. We observed a 33 year-old woman, who developed neutropenia and agranulocytosis in 1992 (white cells count - 1.9 G/L, neutrophils - 0.6 G/L). An enlarged thymus was found and thymectomy was performed. Analysis of ascitic fluid showed plasma cells with intracytoplasmat-chain, responded well to VAD x 6. A few months later new lymphomatous appearance, consisting with lymphadenopathy, visceral and soft tissue involvement was seen in the cases described, all having λ light chain and fatal progression.

PU-1198 Reduction of osteoprotic pathologic fractures and survival prolongation after long-term administration of bisphosphonates in multiple myeloma

Sakalová A,* Hermann Z,* M Iástrá M,* Hrušbík M,* Gažová S,* Chabrová I,* Dedík L*

Institute for Haematology and Transfusiology, Bratislava, Slovakia, *Clinic, Res., RocheBoehringer, Mannheim, Germany, **Slovak Techni-

University, Bratislava, Slovakia

Osteoporosis is common world-wide in people above 50 years old. As this period is also a risk period for the development of multiple myeloma, a comprehensive differential diagnosis of malignant and benign osteoporosis is essential. By retrospective analysis of 270 patients followed for 12 years, treated by chemotherapy for multiple myeloma, 151 patients were select-
ed who except chemotherapy were treated also by immunomodulation (a blend of proteolytic enzymes, Wobe-Mugos) for 5 years and also with bisphosphonates. At the time of diagnosis osteoporosis was the only bone-pathology in 24.5% of patients. When bisphosphonates (Bonefoss: 123 patients, cyclic ibandronate i.v. in 28 patients) and chemotherapy was administered during the observation period, the bone process was stable in 61.59%, osseous changes disappeared in 11.26% and progression was recorded in 27.35%, but without pathologial fractures. Median survival was prolonged to 94 months and more than 40% of the patients have been living longer than 10 years. The objective of the study was to emphasise the importance of correct diagnosis and therapy of osseous changes in mul-
tiple myeloma.

PU-1199 Unusual fatal progression of multiple myeloma

Schlamiisser L, Attias D

Haematologica Institute, Bnei Zion Medical Center, HaIf, Israel

Multiple myeloma is characterised by neoplastic proliferation of plasma cells. Cure rarely occurs. Almost all patients who respond to chemotherapy will eventually relapse. Some will not achieve an objective response. Three patients with an uncommon fatal progression of Multiple Myeloma are presented. Case #1. A 74 years old man was diagnosed as having lg A, myeloma and treated with Melphalan-Prednisone. Two months later huge adrenal masses were found. Biopsy showed diffuse large cell lymphoma. He received a cycle of COP (Cyclophosphamide-Vincristine-Predni-
sone) and died six weeks later. Case #2. A 62 years old woman suffer-
ing from lg A myeloma and treated with VAD (Vincristine, Adriamycin, Dexamethasone) x 5 with a good response. Nine months later an adrenal mass was found and a few weeks later new masses in the low cervical region appeared. Biopsy showed anaplastic myeloma. Salvage Therapy with hyperCVAD (Cyclophosphamide and VAD) was begun. Soon after she developed spinal cord compression and died while receiving radiotherapy. Case #3. A 69 years old woman suffering from Bence Jones myeloma and treated with chemotherapy and radiotherapy. Inhibitory activity can be associated with imflunoglobulin G or M class. Autoimmune agranulocytosis can coexist with some autimmune (eg. connective tissue), viral or neoplastic diseases including thymoma. Pathogenesis of myasthenia gravis is also autoimmune. There are only a few reports describing agranulocytosis and myasthenia gravis coexist in the same patient. We observed a 33 year-old woman, who developed neutropenia and agranulocytosis in 1992 (white cells count - 1.9 G/L, neutrophils - 0.6 G/L). An enlarged thymus was found and thymectomy was performed. Analysis of ascitic fluid showed plasma cells with intracytoplasmat-chain, responded well to VAD x 6. A few months later new lymphomatous appearance, consisting with lymphadenopathy, visceral and soft tissue involvement was seen in the cases described, all having λ light chain and fatal progression.

PU-1200 Pathogenetic and clinical aspects of aplastic anaemia

Shishina RN

Institute of Herontology, Moscow, Russia

The purpose of this work was the study and estimation of the functional condition of haematopoetic cells and the immune system of 125 patients with aplastic anaemia (AA) and 22 patients with myelodysplastic syndromes (MDS) with bone marrow failure. The functional condition of cells was esti-
mated by means of calculation of morphological defects in the cells and also the study of cytochemical characteristics cells of the bone marrow and the peripheral blood. Thirty percent of the immune system were inves-
tigated. Treatment with immunosuppressive preparations (antithymocyte globin, sandimimum) was used for patients of AA according to the protocol and was elective for 63.5%. The study of all parameters was performed before the treatment, 2, 4 and 12 weeks, and 6 and 12 months after it. There were 10 patients with AA in the special group with remission from 20 to 30 years. The results of our own research in the disease mechanism development gave me a chance to consider immune deficiency and haematopoetic deficiency as the mains of disease development. The predominant sections of AA pathogenesis are profound violation of the immune system. This gave me the opportunity to suppose, that AA is an autimmune disease. These results were also used for the patients with severe of the disease, the prognosis and efficiency of therapy.

PU-1201 Immunophenotyping of chronic lymphoproliferative disorders by alkaline phosphatase anti-alkaline phosphatase technique

Smilivtseva T, Stojanovic A, Saso R

Department of Haematology, Medical Faculty, University "St. Cyril and Methodus", Skopje, Macedonia

The immunophenotype of tumour cells in peripheral blood samples from patients with chronic lymphoproliferative disorders was determined. The group included 44 patients with chronic lymphocytic leukaemia (CLL), hairy

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cell leukemia (HCL), and non-Hodgkin's lymphomas (NHL) with peripheral blood involvement. The diagnosis was assessed according to standard haematological procedures. For immunophenotype analysis we chose the immunocytochemical APAAP (alkaline phosphatase-anti-alkaline phosphatase) technique. This method is very sensitive, produces a clear and distinct stain, and is particularly appropriate for blood and bone marrow prepara-
tions. Using the selected panel of monoclonal antibodies, we then came to the following results. In the group of 32 pts with CLL 31 of them had the characteristic profile of B-CLL. They were all positive with CD19, HLA-DR and CD20. They expressed SmIgM and 12 cases had expression of SmIgD. Monoclonality for kappa light chains was proved in 17 pts and for lambda light chains in 13 cases. All of them were CD5+, while FMC-7 and CD22 were positive in a minority of cases. Only one case of c-TCL was identified, the tumour cells being CD4+, CD7+, CD2+, CD3+, TCL αβ+, CD5-, CD8+ and B-cell Ag negative. Analysis in the group of 7 patients with HCL showed a distinct phenotype with B-cell associated Ag positivity like CD19, HLA-DR, CD22, and strong positivity of SmIgM and 7 cases as well as CD2+, CD11c+ and CD5-. Monoclonality for κ was seen in 4 pts and for λ light chains in 1 patient (2 of cases not tested). The Group of 5 pts with NHL consis-
ted of 2 cases with lymphoepithelioma with T-cell phenotype of cir-
culating tumor cells (one was CD4+, CD5+, CD8+, TCL αβ+). One case with lymphoproliferative lymphoma of the mature B-cell phenotype and one patient with centrolymphosarcoma of an immature B-cell origin with strong positivity of CALLA. One rare case of a cutaneous T-cell lymphoma was identified with phenotypic characteristic of Sézary syndrome: CD2+, CD3+, CD4+, CD8-, CD5+. The major clinical symptoms in essential thrombocythemia (ET) are related to bleeding or thrombosis. Thrombotic complications before establish-
ing the diagnosis and during the treatment were evaluated in twelve patients diagnosed as having ET in the period 1980-97. The diagnosis was assessed according to the strict criteria from the PSVG (persistent platelet count >600; HB <130 g/l or normal RBC mass; no Ph chromosome, no bcr-abl rearrangement or both; absent marrow fibrosis and no known cause for reactive thrombocythemia). The median age of the patients was 49 y (21-77); there were two males and 10 females pts. The mean platelet count at diagnosis was 1.112 (835-2.699), haemoglobin level 133 g/l (113-172) and WBC count 11.3 (4.4-22). All pts. had marked megalocytic hyperplasia in the bone marrow aspirates and biopsies. Neutrophil alkaline phosphatase mean (NAP) score was 265 (144-380). The thrombocytosis in these patients was (62%) two cases with splenic vein thrombosis and consecutive splenectomy (both males, age 33 y); a case with pelvic vein thrombosis and left ovariotomy (female, 21 y); two cases with myocardial infarction (females, age 39 and 44 y). Two additional thrombotic episodes were con-
firmed during the observation period (myocardial infarction and bilateral renal vein thrombosis with terminal renal failure). The mean observation period was 52 months (15-216). We report the results of treatment with busulphan (two pts) and hydroxyurea (9 pts; daily dose 500-1750 mg, sev-
eral courses (P=0.400) and two partial (<600) responders. The high platelet count in ET, at diagnosis or in uncontrolled treatment, is a high risk fac-
tor for developing thrombotic complications.

**PU-1203** Serum ferritin levels correlate with non-specific arthralgia

**Timmerkgolu A,** *Evic D*

*Akdeniz University Medical School, Antalya, *I*dr. M. U., First-Aid and Traumatology Hospital, Physical Therapy and Rehabilitation Center, Ministry of Health, Ankara, Turkey

Objective. Iron deficiency is a common finding especially in women in our country. Scrum ferritin level is the most reliable indicator of iron deficien-
ty. The association of iron deficiency in patients with chronic inflammatory disease is well-known. We investigated the correlation between serum fer-
ritin levels and nonspecific arthralgia in study group. Design and Methods. Two groups of women, one with the complaint of arthralgia (n=30) (mean age: 28.63±18.1) and another group of healthy women (n=30) were included in to the study. Patients were assessed by physical examination, radiodiagnostic methods and laboratory investigations. No evidence of any pathological results as the cause of arthralgia were found. Routine laboratory investi-
gations including total blood count, erythrocyte sedimentation rate, ASO, C-reactive protein, rheumatoid factor, urine analysis and the biochemistry were performed. Blood - urine cultures, the brucella antigens, hepatitis and immunologic markers were also examined. Serum iron concentration, total iron-binding capacity, ferritin, folic acid, B12 levels and mean corpus-

cular volume (MCV) of erythrocytes were determined in both groups. They were questioned at least 6 months before the diagnosis of arthralgia if there was nothing to obtain the cause. This could probably prevent unnecessary usage of NSAD.**

**PU-1204** Variant Ph’ translocation t(9;20)(22) in a patient with CML displays b2a2 chimeric gene. A case report

**Todoric B,** *Novak A,* *Krstulic A,* *Zastavnic D,* *Dordovic V.*

**Malesevic M**

Military Medical Academy, *Institute of Haematology - Clinical Center of Serbia, Institute for Nuclear Sciences ‘Vinica’, Belgrade, Yugoslavia

In 5-10% of CML cases Ph’ chromosome originates through a mechanism different than the classical (t9;21) translocation. If the segment from 22q is translocated to a chromosome other than 9 these are simple variant translocations. If more chromosomes are involved, that makes complex variant translocations. Participation of all chromosomes, with the exception of Y, in variant translocations has been reported. We report the case of a 45 year old male, CML patient, who presented with 205,000/mm³ white blood cells, 5% basophils, 4% eosinophils, 237,000/mm³ platelets and organomegaly. Cytogenetic analysis was done on undistributed bone mar-
row cells after direct preparation by HG-Banding technique. RT-PCR was per-
formed with the primers for bcr-abl sequence. All cells displayed variant translocation t(9;20)(22)(q34:q11) in which the Ph’ chromosome was ‘masked’ by translocation of 2q32 fragment. We examined the molecular conse-
quence of this variant translocation. RT-PCR analysis showed a M-
bcr break point producing the classic b2a2 rearrangement. It is not yet clear whether all molecular features of variant translocations are the same as in the classical t(9;22). Further investigations will answer the questions about the influence of small differences, reported by others, in MRK and BCR-
ABL protein product on leukaemogenic properties and clinical course.

**PU-1205** Polycythemia vera associated with chronic lymphocytic leukaemia

**Tomin D,** *Marisavljevic D,* *Basara N,* *Gotic M,* *Boskovic D,* *Rolovic Z**

*Institute of Haematology, Clinical Centre of Serbia, Belgrade, Yugoslavia

Simultaneous or sequential, but spontaneous occurrence of chronic lympho-

cytic leukaemia (CLL) and polycythemia vera (PV) is very unusual. Sometimes CLL occurs after PV treatment with radioactive P32 or alkylat-

ing agents. We analysed data from 2 patients (pts) with a diagnosis of CML after 10 and 15 yrs after PV diagnosis. Patient #1, male, 64 yrs old, treated with venessections for 6 yrs, radioactive P32 (one injection), 4 yrs after last therapy developed simultaneously the molecular features of chronic lympho-

eoblast progenitors (BFU-E and CFU-E, increased reticulocyte count, hyper-

menoneo of all marrow elements) and CLL (absolute lymphocytosis, B-CLL immunophenotype, nodular-interstitial infiltration of bone marrow biopsy, reduced number of haematopoetic progenitors). The patients’ plasma selectively inhibited growth of BFU-E and CFU-E progenitor cells of normal bone marrow. Patient #2, female, 49 yrs old, treated with venessections for 12 yrs, busulfan 2 months, developed signs of only CLL 3 yrs after last therapy (absolute lymphocytosis, B-CLL immunophenotype, nodular-inter-
sstitial lymphocyte infiltration in bone marrow biopsy with normal counts of other haematopoetic cells). Both pts were remarkable for the mild clinical course of the CML and PV (follow-up 4 and 2 yrs), suggesting the control between 2 malignant clones, possibly including some inhibitors due to CLL clone, which influenced-suppression of the PV clone (partial as in pt 2 or complete as in pt 2). Our results also confirm the value of haematopoietic culture tests (using pts cells and plasma in co-culture system) for diag-

nosis and further explanations of the pathogenesis of CML and PV.
Hepatitis G virus (HGV), is a new human RNA virus that is mainly transmitted by blood and blood transfusion associated products and its relation to liver disease is not clear. There is controversy about the role of viral infectious agents such as parvovirus and HGV in the pathogenesis of acquired aplastic anaemia. Aim. To investigate the frequency of HGV infection in patients with aplastic anaemia. Design and Methods. Serum samples were obtained from 12 patients with aplastic anaemia (median age: 9.5 years, 9 males and 3 females) who had been hospitalised between 1997 and 1998 in the Department of Haematology and Oncology, University of Ankara. Patients were tested for hepatitis serology (HBV, HCV, Parvovirus B 19) and coinfection were not detected but HGV was found in 1 patient (8.4%). Patients were treated with immunosuppressive therapy and monitored. Hepatitis G virus was not found in any of the volunteer controls (Boehringer Mannheim, Germany). Results. HBV, HCV, Parvovirus B 19 and confection were not detected but HGV was found in 1 patient (8.4%). There was no significant difference in the frequency of HGV between patients with aplastic anaemia and volunteer controls. Conclusions. HGV is not always innocent virus: based upon the present experience, here reported, the role of HGV in virus-induced hepatopathies as well as in the aetiology of aplastic anaemia still remains controversial.

**PU-1208** Immunologic phenotype of lymphoid cells in spleen and blood of patients with different non-Hodgkin's lymphomas

**Yuryevsky T, Tsiapaia O, Karoly M, Matian V, Lebed G, Eustakievich V, Eustakievich I, Loginsky V**

Research Institute of Blood Pathology & Transfusion Medicine, Lviv, Ukraine

Splenectomy remains an efficacious tool in the treatment of certain cases of NHL. We compared the immunophenotype of lymphoid cells from peripheral blood before splenectomy and from splenic tissue in 14 NHL patients. The primary involvement of spleen by lymphoma was the main reason for operation in 7 cases and the development of immune hemocytopenias - in the other 7 cases. In all patients mononuclear cells from blood as well as from splenic tissue had predominantly B-linear origin: HLA-Dr+, CD20+, CD22+, CD37+, CD72+, CD76+. The expression of CD20 and CD76 as splenic tissue had predominantly B-linear origin: HLA-Dr+, CD20+, CD22+, CD37+, CD72+, CD76+. The expression of CD20 and CD76 was not always as innocent virus: based upon the present experience, here reported, the role of HGV in virus-induced hepatopathies as well as in the aetiology of aplastic anaemia still remains controversial.

**PU-1209** 11q13 is a cytogenetically promiscuous site in haematological malignancies

**Wong KF**

Department of Pathology, Queen Elizabeth Hospital, Hong Kong, China

Objective. 11q13 translocation has been described in mantle cell lymphoma in the form of (11;14)(q13;q32), resulting in over-expression of the cyclin D1 gene. Recently, an association between 11q13 and AML has also been recognised. We report the occurrence of 11q13 translocation in both acute leukaemias and myelodysplastic syndrome. Methods. The cytogenetic findings of six patients with haematological malignancies and 11q13 abnormality were reviewed. They were diagnosed during the period 1993-1998, and included 3 AML, 1 RAEB, 1 T-Cell ALL and 1 common ALL. Immunohistochemical studies for cyclin D1 were performed on the marrow biopsies in 4 of cases using the DCS-6 antibody (Dakopatts, Copenhagen, Denmark). Results. The cytogenetic findings are summarised in Table 1. 11q13 abnormality was the sole change in 2 cases of AML. Several different chromosomes including chromosomes 7, 9, 10 and 17 were involved in the translocations with 11q13 in the 3 other cases. None of the 4 cases studied was immunoreactive for cyclin D1. Conclusions. Recurrent karyotypic aberrations are sometimes associated with specific subtypes of leukaemia. It is however unusual for the same chromosomal rearrangement to be found in leukaemia of different lineages. We have shown that 11q13 changes could be found in AML, ALL and MDS with different chromosomes serving as partners in the reciprocal translocations. Our results also suggest that 11q13 translocation is more commonly found in children with acute leukaemia. Furthermore, the absence of cyclin D1 expression suggests that the PRAD 1 gene is probably not involved in the pathogenesis of haematological malignancies despite cytogenetic involvement of 11q13.

**Table 1. Cytogenetic findings of haematological malignancies with 11q13 translocations**

<table>
<thead>
<tr>
<th>Case</th>
<th>Cytogenetic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/ AML</td>
<td>46;XX;+10(1.1)q11;p13(31.1);17q11.2(p12.1)</td>
</tr>
<tr>
<td>2/ AML</td>
<td>46;XX;+11(11.3)q13.3(11.3)</td>
</tr>
<tr>
<td>3/ AML</td>
<td>46;XX;+3(3q13.3)</td>
</tr>
<tr>
<td>4/ AML</td>
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</tr>
<tr>
<td>14/ AML</td>
<td>46;XX;+3(3q13.3)</td>
</tr>
</tbody>
</table>

**PU-1210** PSCT in a child with amegakaryocytic thrombocytopenia

**Yeulepik MA, Hazar V, Yegin O**

Adeniz University School of Medicine, Department of Pediatrics, Antalya, Turkey

Congenital amegakaryocytic thrombocytopenia is an extremely rare cause of neonatal thrombocytopenia. The syndrome appears to have an autosomal recessive inheritance pattern. Most of the patients will go on to complete aplasia, and many infants die because of infection or hemorrhage. Usually steroid therapy is not beneficial. Allogenic stem cell transplantation offers the only possibility for cure. Case Report. A 13 month old girl, suffred petechiae and ecchymoses on her body for a month. On physical examination, there is no pathologic findings except petechiae and ecchymoses. Her Hb was 13 g/dL, WBC: 5300/mm3, PLT: 80,000/mm3 on 120th day. We conclude that allogenic PSCT is a possible treatment for congenital amegakaryocytic thrombocytopenia.
PU-1211 Antimicrobial therapy in febrile neutropenic patients with pulmonary infiltrations

Zacharof AK, Petrogiannopoulos C, Zoumanis A, Choreuti E, Sakka M, Fragkaki I, Zacharof H
2nd Department of Medicine, Chemotherapy Unit and Haematology Laboratory, Hellenic Red Cross Hospital, Athens, Greece

Objective. We studied different empirical approaches to antimicrobial treatment of lung infiltrates in patients with neutropenia. Design and Methods. All patients with haematological malignancies and neutropenia (fever >38.5°C or higher) with associated newly diagnosed lung infiltrates were randomised for initial therapy with ampicillin plus aminoglycoside (Group A), third-generation cephalosporin plus aminoglycoside (Group B), or a double beta-lactam combination (Group C). Non-responders after 4 days of the above treatment were given empirical Fluconazole under strict observation. Results. Of 148 patients entered, 94.6% were evaluable. Complete response was obtained in 74.4% with no significant difference between treatment groups. Only 26.9% of patients achieved a complete response to antibiotic therapy without additional antifungal therapy. Fungi dominated in cases of microbiologically documented infections but were associated with a poorer outcome compared with bacterial pneumonias. Conclusions. Lung infiltrates in febrile patients with neutropenia represent a high risk of treatment failure. Systemic antifungal agents as first-line therapy, particularly in selected high-risk subgroups, might improve future treatment results.

PU-1212 Serum transforming growth factor-a in liver cirrhosis and hepatocellular carcinoma

Zahra MK, Badway ThS, Ismail SA,† El-Shazly SF
†Dep. Of Clinical Pathology and Tropical Medicine, Tanta Faculty of Medicine, Egypt

Objective. This work was conducted to study, the serum levels of transforming growth factor-alpha (TGF-α) in liver cirrhosis and hepatocellular carcinoma (HCC) associated with cirrhosis to throw more light on its clinicopathological significance. Design and Methods. Forty patients were classified histopathologically into a cirrhotic group (20 patients) or HCC associated with cirrhosis (20 patients). In addition, 20 apparently healthy individuals served as the reference group. Routine laboratory investigations for kidney function, stool, urine analysis and complete blood count, liver function profile, viral marker profile (hepatitis surface antigen, B core antibody, and hepatitis C antibody), serum levels of α-fetoprotein, ferritin and transforming growth factor-α (ELISA) were performed. Results. TGF-α was more significantly increased in cirrhotic patients; moreover it was significant increased in HCC than cirrhotic patients indicating the value of this marker in detection of HCC in cirrhotic patients. TGF-α was associated positively with HBs-Ag and IABc-Ab in the studied patients, however, HCV-Ab marker did not associate with TGF-α. TGF-α was the most sensitive marker (TGF-α 80%, α-fetoprotein 60% and ferritin 50%) in the detection of HCC in cirrhotic patients. Complementary usage of both TGF-α and α-fetoprotein raised sensitivity to 95% and complementary usage of TGF-α with ferritin raised sensitivity to 90%, while α-fetoprotein and ferritin raised sensitivity only to 75%. So diagnosis of HCC in cirrhotic patients can be made better by assaying TGF-α with either α-fetoprotein or serum ferritin. Conclusions. TGF-α is a more sensitive marker than α-fetoprotein and serum ferritin in early detection of HCC in cirrhotic patients. Complementary usage of TGF-α with either α-fetoprotein or serum ferritin improves sensitivity of these markers in detecting HCC in cirrhotic patients.