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Haematologica

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founded in 1920 by Adolfo Ferrata

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Haematologica publishes monthly Editorials, Original Papers, Case Reports, Reviews and Scientific Correspondence on subjects regarding experimental, laboratory and clinical hematology. Editorials and Reviews are normally solicited by the Editor, but suitable papers of this type may be submitted for consideration. Appropriate papers are published under the headings Decision Making and Problem Solving and The Irreplaceable Image.

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erized graphs, the diskette should contain these documents as well. Computer programs employed to prepare the above documents should be listed.

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Editorials should be concise. No particular format is required for these articles, which should not include a summary.

Original papers should normally be divided into abstract, introduction, materials and methods, results, discussion and references.

The section *Decision Making and Problem Solving* presents papers on health decision science specifically regarding hematologic problems. Suitable papers will include those dealing with public health, computer science and cognitive science. This section may also include guidelines for diagnosis and treatment of hematologic disorders and position papers by scientific societies.

Reviews provide a comprehensive overview of issues of current interest. No particular format is required but the text should be preceded by an abstract which should be structured as follows: background and objective, evidence and information sources, state of art, perspectives. Within review articles, **Haematologica** gives top priority to: a) papers on molecular hematology to be published in the section *Molecular basis of disease*; b) papers on clinical problems analyzed according to the methodology typical of *Evidence-Based Medicine*.

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tions will be accepted only in special cases). Letters and numbers should be large enough to remain legible (> 1 mm) after the figure has been reduced to fit the width of a single column. In preparing composite illustrations, each section should be marked with a small letter in the bottom left corner. Legends for illustrations should be typewritten on a separate page. Authors are also encouraged to submit illustrations as electronic files together with the manuscript text (please, provide what kind of computer and software employed).

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List all authors when six or fewer; when seven or more, list only the first three and add *et al*. Examples of correct forms of references follow (please note that the last page must be indicated with the minimum number of digits):

Journals (standard journal article,^{1,2} corporate author,³ no author given,⁴ journal supplement⁵):

1. Najfeld V, Zucker-Franklin D, Adamson J, Singer J, Troy K, Fialkow PJ. Evidence for clonal development and stem cell origin of M7 megakaryocytic leukemia. *Leukemia* 1988; 2:351-7.
2. Burgess AW, Begley CG, Johnson GR, et al. Purification and properties of bacterially synthesized human granulocyte-macrophage colony stimulating factor. *Blood* 1987; 69:43-51.
3. The Royal Marsden Hospital Bone-Marrow Transplantation Team. Failure of syngeneic bone-marrow graft without preconditioning in post-hepatitis marrow aplasia. *Lancet* 1977; 2:242-4.
4. Anonymous. Red cell aplasia [editorial]. *Lancet* 1982; 1:546-7.
5. Karlsson S, Humphries RK, Gluzman Y, Nienhuis AW. Transfer of genes into hemopoietic cells using recombinant DNA viruses [abstract]. *Blood* 1984; 64(Suppl 1):58a.

Books and other monographs (personal authors,^{6,7} chapter in a book,⁸ published proceeding paper,⁹ abstract book,¹⁰ monograph in a series,¹¹ agency publication¹²):

6. Ferrata A, Storti E. *Le malattie del sangue*. 2nd ed. Milano: Vallardi; 1958.
7. Hillman RS, Finch CA. *Red cell manual*. 5th ed. Philadelphia: FA Davis; 1985.
8. Bottomley SS. Sideroblastic anaemia. In: Jacobs A, Worwood M, eds. *Iron in biochemistry and medicine*, II. London: Academic Press; 1980. p. 363-92.
9. DuPont B. Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. *Proceedings of the third annual meeting of the International Society for Experimental Hematology*. Houston: International Society for Experimental Hematology; 1974. p. 44-6.
10. Bieber MM, Kaplan HS. T-cell inhibitor in the sera of untreated patients with Hodgkin's disease [abstract]. Paper presented at the International Conference on Malignant Lymphoma Current Status and Prospects, Lugano, 1981:15.
11. Worwood M. Serum ferritin. In: Cook JD, ed. *Iron*. New York: Churchill Livingstone; 1980. p. 59-89. (Chanarin I, Beutler E, Brown EB, Jacobs A, eds. *Methods in hematology*; vol 1).
12. Ranofsky AL. *Surgical operation in short-stay hospitals: United States-1975*. Hyattsville, Maryland: National Center for Health Statistics; 1978. DHEW publication no. (PHS) 78-1785, (Vital and health statistics; series 13; no. 34).

References to Personal Communications and Unpublished Data should be incorporated in the text and not placed under the numbered References. Please type the references exactly as indicated above and avoid useless punctuation (e.g. periods after the initials of authors' names or journal abbreviations).

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(indexed by Current Contents/Life Sciences and in Faxon Finder and Faxon XPRESS, also available on diskette with abstracts)



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Programma scientifico

Venerdì 19 maggio

- 8.00 Affissione poster**
- 8.30-10.00 Comunicazioni orali**
Sala plenaria Oral anticoagulant therapy
(in collaborazione con FCSA)
Sala A Platelets I
Sala B Arterial thrombosis: risk factors
Sala C Vascular and cellular haemostasis
- 10.30-12.00 Simposi**
Sala Plenaria Novità sull'eparina a basso peso molecolare
con la collaborazione di Rhone-Poulenc Aventis
Sala A L'impiego clinico del plasma fresco congelato:
moderne indicazioni e nuove sicurezze
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Laboratory
Sala A Millennium GIPSI
con la collaborazione di Bayer
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Arterial thrombosis: risk factors
Vascular and cellular haemostasis
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Il ruolo degli antagonisti del recettore
piastrinico per l'ADP
con la collaborazione di Sanofi Synthelabo
Sala A Il cuore come sorgente emboligena
con la collaborazione di ANMCO
Sala B La profilassi nell'emofilia
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Sabato 20 maggio

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Sala B Clinical aspects of inherited coagulation
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Sala plenaria Rischio tromboembolico nella fibrillazione
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con la collaborazione di Pharmacia Upjohn
Sala A Novità nel campo degli inibitori
con la collaborazione di Novo Nordisk
Farmaceutici
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con la collaborazione di Crinos
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Clinical aspects of inherited coagulation
disorders
Platelets II
Venous thromboembolism
Biochemistry and genetics of coagulation
factors
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Sala B Pregnancy: complications and hypercoagulability
Sala C Biochemistry and genetics of coagulation
factors
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von Willebrand
con la collaborazione di Grifols

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- 9.00-12.30 Simposio presidenziale**

XVI CONGRESSO NAZIONALE Siset
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 Programma scientifico

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The Italian Society for the Study of Thrombosis and Haemostasis

FOREWORD

The Italian Society for the Study of Thrombosis and Haemostasis (SISSET) was founded 30 years ago in Parma. Since then scientific Congresses have been held every two years, with Postgraduate Educational Courses organized in the intervening years. The active membership of the Society is relatively small (400-500 members), but participation at our scientific events is large and of high quality, reflecting the good standing of Italian "clotters" on the international stage. Since January 2000 *Haematologica* has become the official scientific organ of the Society and we are proud that such a high quality journal has given us the opportunity to join the prestigious scientific Societies that were already on board.

This issue of *Haematologica* features the 311 abstracts presented as oral communications or posters at the 16th SISSET Congress organized in Milan from May 18-21, 2000. It is the first Congress of the new Millennium and I am happy and honored to be Chairman of the Society at this time. Although I believe that many abstracts are original and of high scientific quality, I recognize that I might be biased in this respect. As usual, the scientific community at large will be the ultimate judge.

I take the opportunity to thank warmly the members of the SISSET Council who did choose the abstracts and prepared the scientific program (Chiara Cerletti, Giovanni Davì, Guido Finazzi, Gian Franco Gensini, Domenico Prisco, Elena Tremoli, and Francesco Violi, President elect of SISSET).

My gratitude also goes to Mario Cazzola and Michele Moscato of *Haematologica* and to Elena Zumbo for their precious and enthusiastic help during the preparation of this issue.

*P.M. Mannucci
President Italian Society for the
Study of Haemostasis and Thrombosis*



ORAL COMMUNICATIONS
Oral anticoagulant therapy

in collaboration with
Federazione Centri Sorveglianza Anticoagulati (FCSA)

CO-001

RECURRENT VENOUS THROMBOEMBOLISM AND BLEEDING COMPLICATIONS DURING ANTICOAGULANT TREATMENT IN PATIENTS WITH CANCER AND VENOUS THROMBOSIS

Prandoni P, Lensing AWA, * Piccioli A, Bernardi E, Bagatella P, Simioni P, Girolami B, Marchiori A, Scudeller A, Sabbion P, Noventa F, Girolami A

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A small proportion of patients with deep-vein thrombosis develops recurrent venous thromboembolic complications (VTE) or bleeding during anticoagulant treatment. These complications may occur more frequently if such patients have concomitant cancer. In order to assess the relative risk of recurrent VTE and bleeding during anticoagulation in cancer patients as compared with patients free from malignancies, 842 consecutive outpatients with confirmed symptomatic deep-vein thrombosis (of whom 191 with known cancer at entry) were prospectively followed for 1 year. Type and stage of cancer were classified as severe, moderately severe or less severe according to internationally accepted guidelines. Heparinisation and subsequent oral anticoagulation were given in therapeutic dosages, and duration of treatment depended on the presence of risk factors. Cancer was classified as severe in 34.0%, moderately severe in 23.6%, and less severe in 42.4% of patients. The 12-month cumulative incidence of recurrent VTE in cancer patients was 20.2% (95% CI, 14.3 to 26.1) versus 7.4% (95% CI, 4.1 to 10.3; $p < 0.001$) in patients without cancer, for a hazard ratio of 3.6 (95% CI, 2.2 to 5.9; $p < 0.001$). The 12-month cumulative incidence of major bleeding was 12.1% (95% CI, 7.3 to 17.6) and 5.2% (95% CI, 2.5 to 7.5; $p = 0.017$), respectively, for a hazard ratio of 2.1 (95% CI, 1.1 to 4.0; $p = 0.019$). Recurrence and bleeding were both related to cancer severity but could not be explained by under- or over-anticoagulation. We conclude that cancer patients with venous thrombosis are more likely to develop recurrent thromboembolic complications and major bleeding than those without malignancy. These risks increase with cancer severity. Optimisation of monitoring procedures for anticoagulant therapy has the potential to improve outcomes in only a small subset of patients.

CO-002

THE INR WITH THE MAJORITY OF CALIBRATED THROMBOPLASTINS IS VALID IN MONITORING WARFARIN THERAPY IN PATIENTS WITH LUPUS ANTICOAGULANTS

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Background. Though prothrombin time (PT) is seldom prolonged in patients with lupus anticoagulants (LA), the INR in patients with LA on oral anticoagulants (OAT) might be inappropriate because of the varied responsiveness of thromboplastin reagents to LA. A multicentre study was organised (i) to assess the responsiveness of different reagents to LA and (ii) to investigate the effects of LA on the PT-INR. *Design.* To increase the number of patients and to separate genuine effects of LA on PT-INR from artefacts due to incorrect ISI assignment and INR measurement it was decided (i) to collect patients' plasma in different Centres; (ii) to calibrate reagents at the co-ordinating Centre following WHO recommendations; (iii) to centralise the measurement of the INR for all frozen plasmas and (iv) to measure factor X (FX) amidolytic activity (known to be insensitive to LA). Each of 9 Centres was asked to collect plasma from the following groups: (1) patients with LA not on OAT; (2) patients with LA and on OAT (cases) and (3) patients without LA and OAT (controls). LA status was documented at each Centre. *Reagents.* Nine reagents, 3 from recombinant tissue factor (A-C); 1 from human placenta (D) and 5 from rabbit brain (E-I) were used. The number of patients studied was 67, 74 and 75 for group 1, 2 and 3. *Results.* Reagents responsiveness to LA. Mean PT ratios for group 1 were within the range of values for healthy subjects for all but one recombinant reagent (reagent A). LA interference on the INR. This was evaluated in three ways. (i) Between-reagent variabilities of the INR for cases and controls were compared on the assumption that they would have been equivalent if there was no effect of LA. The CV of the INR obtained with 9 reagents for cases (11.5%) was greater than that for controls (6.8%). However, the CVs for cases and controls (5.5% vs. 5.7%) were comparable after exclusion of results obtained with reagent A. (ii) INR obtained with individual reagents for cases and controls were compared to the overall mean on the assumption that reagents affected by LA would have recorded greater proportions of cases with INR deviations from the overall mean than controls. Reagent A recorded the highest proportion of cases with INR deviations from the overall mean. Finally, (iii) regression analyses of INR vs. FX activity for cases and controls was performed on the assumption that they would have been equivalent if there was no effect of LA. This analysis showed no substantial differences for all, but reagent A. In summary, all the above approaches indicates that

reagent A is indeed affected by LA. **Conclusions.** (i) The extent of LA interference on the PT-INR does not seem to be such to cause concern for the vast majority of reagents. (ii) New reagents, especially those derived from recombinant tissue factor should be checked to assess their responsiveness to LA before they are used to monitor OAT.

CO-003

ASSESSMENT OF PATIENT ABILITY TO SELF-ADJUST THE ORAL ANTICOAGULANT DOSE: A MULTICENTRE STUDY ON HOME USE OF A PORTABLE PROTHROMBIN TIME MONITOR (COAGUCHECK)

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Self-testing and self-monitoring with portable prothrombin time (PT) monitors has been shown to be feasible and safe. However the ability acquired by patients on chronic oral anticoagulant therapy (OAT) to self-adjust their dose has not been properly evaluated. **Aims of the study.** To evaluate the following: 1) the ability for dose self-adjustment acquired by patients on chronic OAT; 2) the accuracy and reliability of a portable PT monitor (Coaguchek, Roche Diagnostics, Germany) for home use; 3) the integration of Coaguchek into routine patient care of anticoagulation clinics. **Experimental design.** Nested case-control study in four centres of the Italian Federation of Anticoagulation Clinics (FCSA). **Study subjects.** Seventy-eight subjects on stable OAT for at least 6 months (47 men, 31 women, age range: 18-75 years) were selected on a volunteer basis and enrolled after passing an abbreviated mental test and providing informed consent. After three instruction sessions on the use of Coaguchek, subjects performed the PT test at home, communicated the INR results to the Centre and suggested the dose adjustment and date for next control as they thought appropriate. However, they were requested to follow the prescription given by the Centre. **Controls** (78 subjects) matched for age (± 5 years), sex and therapeutic range with the cases, were selected among those who attended the anticoagulation clinics and managed by usual care. **Results.** When compared with the dose prescribed by the Clinic, the dose suggested by warfarin and acenocoumarol users was equal to or within $\pm 6\%$ of the mean weekly dose in 80% and 82% of suggestions, respectively. The proportion of monitor INRs that agreed with laboratory INRs as being within, above or below the therapeutic range was 79% and 76% in the instruction and surveillance phase, respectively. Time spent in the therapeutic range during the study was the same (80%) for cases and controls. **Conclusions.** Our data indicate that well selected and trained patients on chronic anticoagulant therapy can acquire a satisfactory ability to self-adjust of OAT dose.

CO-004

SAFETY AND EFFICACY OF ORAL ANTICOAGULATION FOR THE TREATMENT OF VENOUS THROMBOEMBOLISM IN PATIENTS WITH OR WITHOUT MALIGNANCY

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Whether a course with oral anticoagulants, treatment of choice of secondary prophylaxis in patients with venous thromboembolism (VTE), is sufficiently safe and effective in VTE patients with malignancy is still matter of debate. The present study analysed the anticoagulation courses recorded in 95 patients with malignancy who were treated for a VTE; their results were compared with those obtained in 733 VTE patients without malignancy. All patients were included in the ISCOAT study and were prospectively followed-up since commencement of anticoagulation. Based on 744 patient-years of treatment and follow-up, the rates of major (5.4% vs 0.9%), minor (16.2% vs 3.6%) and total (21.6% vs 4.5%) bleeding were statistically significantly higher in cancer patients compared with patients without cancer. Bleeding was also a more frequent cause of early anticoagulation withdrawal in patients with malignancy (4.2% vs. 0.7%; $p < 0.01$; RR 6.2 (95%CI 1.95-19.4). There was a trend towards a higher rate of thrombotic complications in cancer patients (6.8% vs. 2.5%; $p = 0.058$; RR 2.5 (CI 0.96-6.5)) but this did not achieve statistical significance. In the group of patients with cancer, the bleeding rate was high across the different INR categories and was independent of the temporally associated INR value. In contrast, the bleeding rate was increased only with INR values greater than 4.5 in the group of patients without cancer. The rate of thrombotic events was significantly higher in both cohorts when the INR was less than 2.0. In conclusion, patients with malignancy treated with oral anticoagulants have a higher rate of bleeding and possibly an increased risk of recurrent thrombosis compared with patients without malignancy. Safer and more effective anticoagulant therapy is needed for this challenging group of patients.

CO-005

P450 CYP2C9 POLYMORPHISM IN PATIENTS ON ORAL ANTICOAGULANT TREATMENT

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Genetic polymorphism of P450 (CYP2C9*1 wild-type allele) allows the distinction of patients on war-

farin (W) in normal metabolisers (NM) and poor metabolisers (PM). Patients with the allelic variants CYP2C9*2 (Cys144, Ile359) and CYP2C9*3 (Arg144, Leu359) have a decreased W clearance. Subjects included in the study: a) 68 PM patients [46 W and 22 acenocoumarol (A)] with oral anticoagulant treatment (OAT) median dose =8.75 (range 2.5-10); b) 81 NM patients (51 W and 30 A) with OAT median dose =21.25 mg/week (range 12.5-61.52) matched by age, sex, drug and INR. All the patients had normal liver function tests; no concomitant drugs interfering with OAT were given; c) 150 controls unrelated to the patients. The three haplotypes were identified by PCR and appropriate enzyme restriction digestion.

Frequencies Alleles	PM (68)		NM (81)		Controls (150) %
	W %	A %	W %	A %	
P2C9*1	45	66	73	84	78
P2C9*2	25	9	18	8	16
P2C9*3	30	25	9	8	6
<i>Genotypes</i>					
P2C9*1*1	26	42	54	70	58
P2C9*2*2	4	-	2	3	-
P2C9*3*3	11	4	-	-	2
P2C9*1*2	20	14	25	10	32
P2C9*1*3	17	36	13	17	8
P2C9*2*3	22	4	6	-	-

P2C9 Alleles	Patients		O.R.	95% CI
2* and 3* vs 1*	PM W	NM W	3.63	1.98-6.67
	PM A	NM A	2.58	1.03-6.50
	PM	NM	3.3	2.00-5.44
2* vs 1* and 3*	PM W	NM W	0.6	0.30-1.21
	PM A	NM A	0.9	0.23-3.60
	PM	NM	0.6	0.34-1.17
3* vs 1* and 2*	PM W	NM W	4	1.77-9.12
	PM A	NM A	3.66	1.17-11.4
	PM	NM	3.7	2.05-7.69

The allele CYP2C9*3 is more frequent in PM vs NM independently of the type of treatment ($p < 0.001$). Warfarin subgroup: the difference between PM vs NM is still significant ($p < 0.001$). Acenocoumarol subgroup: the difference between PM and NM is significant ($p = 0.04$) PM vs NM with 1 or 2 allelic variants: 69% vs 38%; OR=3.61 (95% CI 1.82-7.14). *Conclusions.* These data confirm that CYP2C9*3 and *2 regulate warfarin and acenocoumarol metabolism.

CO-006

MORE FREQUENT INR CONTROLS ARE NEEDED AFTER INFLUENZA VACCINATION IN ELDERLY PATIENTS ON LONG-TERM ANTICOAGULANT THERAPY

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The influenza vaccination is recommended for elderly subjects and for those of any age who have medical conditions that place them at high risk of complications from influenza. Among patients on long-term oral anticoagulant therapy, several subjects need annual influenza vaccination. Previous findings suggested the safety of influenza vaccination for patients on oral anticoagulant therapy (OAT), however conflicting results on the effects of warfarin were reported. We assessed the effect of influenza vaccination on anticoagulation levels in 73 patients, 42 males and 31 females, attending an anticoagulation clinic from July 1998 to February 1999. The indications for OAT were: prosthetic heart valve ($n = 39$), venous thromboembolism ($n = 7$), atrial fibrillation ($n = 9$), heart valve disease ($n = 5$), coronary artery disease ($n = 7$) and arterial disease ($n = 6$). The analysis was performed by Rosendaal's method. All subjects were on stable long-term OAT for at least six months. We compared the patients' anticoagulation levels with those of a control group of 70 patients, matched for sex, age and therapeutic INR target level. Both groups were observed during the same period to rule out seasonal interference. No differences in the anticoagulation levels were found in patients and in control group during the three months before and after the vaccination. In patients older than 70 years, but not in the control group, we observed a reduction of anticoagulation intensity in the month after the vaccination: the time below the range was significantly longer (10% before and 27% after, $p = 0.001$) and time spent in the target range was decreased (67% before and 53.5% after, $p = 0.08$). These data persisted three months after vaccination (67% before and 58% after, $p = 0.053$ and 10% before and 2.5% after, $p = 0.009$ respectively). No haemorrhagic or thrombotic complications were observed during the follow-up period. Our results confirm the safety of influenza vaccination in patients on OAT, but the reduced effect of warfarin in the elderly subjects suggests the need for more frequent INR controls in the three months after vaccination in these patients.

CO-007

GENETIC MODULATION OF ORAL ANTICOAGULATION WITH WARFARIN AND BLEEDING RISK

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Oral anticoagulation for the prevention and treatment of patients with arterial and venous thromboembolic disorders is one of the most employed therapies in clinical practice. The cytochrome P450 CYP2C9 metabolises a large number of clinically important drugs, including warfarin. Gene variants within the CYP2C9 locus, CYP2C9*2 and CYP2C9*3

alleles, have been associated with hyper-responsiveness to small doses of warfarin and a higher bleeding complication rate. In a cohort of 180 patients followed up at one specialised clinic from the start of the anticoagulation with warfarin, we investigated whether CYP2C9 gene variants affected doses of drug prescribed to acquire the target anticoagulation intensity. In addition, the incidence of bleeding complications among carriers of the different haplotypes was assessed. The adjusted dose required of warfarin was higher among patients with the CYP2C9*1 haplotype (5.6 mg) than among patients carrying the CYP2C9*2 (4.7 mg; $p=0.007$, Scheffé's test) or the CYP2C9*3 haplotype (4.0 mg; $p<0.001$, Scheffé's test). Carriers of CYP2C9*2 and/or CYP2C9*3 haplotypes had a significantly higher incidence of minor bleeding episodes (12.3 per 100 person-years) than that (3.8 per 100 person-years) observed among patients with the CYP2C9*1 haplotype (rate ratio: 3.29; 95% CI: 1.90-5.69). The occurrence of bleeding complications was independently and significantly more frequent among patients with the CYP2C9*2 and/or the CYP2C9*3 haplotype than in carriers of the CYP2C9*1 haplotype (OR: 2.62; 95% CI: 1.13-6.12). CYP2C9 gene variants modulate the anticoagulant effect of the dose of warfarin prescribed. In addition, the incidence of bleeding complications in patients carrying CYP2C9*2 and CYP2C9*3 haplotypes was about four-fold higher than that in non-carriers. In the present setting, the contemporary prescription of other drugs did not interfere with the effect of CYP2C9 gene variants. In addition, the exclusion of 67 patients who were taking additional drugs did not change results. Present data confirmed a significant role of gene variants within the CYP2C9 gene locus in the modulation of the anticoagulant effect of the dose of warfarin prescribed. CYP2C9 genotyping may be helpful to identify patients with high risk of bleeding complications.

CO-008

EFFECT OF COMPUTER-AIDED MANAGEMENT ON QUALITY OF TREATMENT IN PATIENTS ON ORAL ANTICOAGULANTS: A PROSPECTIVE, RANDOMISED, MULTICENTRE TRIAL

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Objective. To investigate whether a computer-based decision support system can improve the laboratory quality of treatment in patients on oral anticoagulant (OA) treatment in comparison with a similar group followed by experienced doctors. **Design.** Prospective, randomised, collaborative trial. **Setting.** Five specialized anticoagulation clinics in Italy associated with the Italian Federation of Anticoagulant Clinics (FCSA). **Patients.** Two separate sets of patients; one in the first three months "stabilisation

phase" ($n=335$), and one during the "maintenance phase" of treatment with warfarin or acenocumarol ($n=916$, 774.7 patient-years). **Intervention.** Use of a computerised system, including algorithms able to suggest OA dosing and to schedule the follow-up visits (computer-aided dosing), or use of the same computerised system but without such algorithms (controls). **Main Outcomes.** A) Stabilisation phase: percentage of patients reaching a stable state (three consecutive checks in range with at least one week between each) during each of the first three months. B) maintenance phase: percentage of time spent within the therapeutic range. **Results.** Patients in the computer-aided dosing group achieved a stable state in a significantly shorter time than controls ($p<.001$), and they spent a longer time within the therapeutic range during the maintenance phase ($p<.001$). The favourable effect of computer-aided dosing was mainly due to the reduction of the time spent below the therapeutic range. This reduction was associated with an increase of mean INR value, of OA doses administered, and with a reduction of the number of visits (i.e. of laboratory controls) per patient, all these differences being statistically significant ($p<.001$). The computer-aided dosing was very efficient in the maintenance phase, since it gave suggestions in about 2/3 of visits, with the large majority of these suggestions accepted by the experienced doctor in charge. **Conclusion.** The prescription algorithms were effective in improving the laboratory quality of OA treatment both in the stabilisation and in the maintenance phases, and was associated with a reduction of the number of laboratory controls needed to maintain patients in the scheduled therapeutic ranges.

CO-009

ORAL ANTICOAGULATION THERAPY IN ELDERLY PATIENTS ATTENDING AN ANTICOAGULATION CLINIC FOR ATRIAL FIBRILLATION IS ASSOCIATED WITH A LOW RATE OF BLEEDING COMPLICATIONS

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Oral anticoagulation therapy (OAT) is indicated in elderly patients with atrial fibrillation (AF) but, due to the commonly reported high bleeding risk, the application of this guideline is limited. In an observational study evaluating the incidence of OAT complications in 601 out-patients attending a Florence anticoagulation clinic (AC), we found a low rate of bleeding complications (major bleeding 0.84% pt-yrs, minor bleeding 6.34% pt-yrs). Here we report the experience of our AC from July 1995 to December 1999 on OAT AF patients. We studied the frequency of bleeding and thromboembolic events in 146 patients with AF (mean age 70.7 yrs) followed for a mean time of 22 months (230 pt-yrs). The time spent within, below and above therapeutic range was 69%,

14% and 15% respectively. The frequency of major bleeding and of major thromboembolic events was 0.84% and 1.8% pt-yrs respectively and no fatal event was observed. In conclusion, a low incidence of complications was maintained during a prolonged follow-up. In particular, in patients with AF even if older than the total general population attending our AC (70.7 yrs vs 64.6 yrs), a lower rate of bleeding events (0.46% pt-yrs vs 0.84% pt-yrs) was observed. These results suggest that in the clinical setting of an AC the risk of bleeding complications is acceptably low in old patients with AF. If these data are confirmed in larger studies they would add an important piece of information on the real-life risk of bleeding in elderly patients with AF on long-term OAT attending an AC.

ORAL COMMUNICATIONS

Platelets I

CO-010

INCREASE OF PLATELET-LEUKOCYTE AGGREGATES DURING THE LATE ASTHMATIC REACTION (LAR) IN PATIENTS WITH ALLERGIC ASTHMA

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Several clues point to an involvement of platelets in allergic asthma, especially in the development of the inflammatory changes associated with bronchial hyperresponsiveness. An activation of circulating leukocytes in asthmatics has also been shown but no data are available on the possible relationship between platelet and leukocyte activation. The late asthmatic reaction (LAR) is a clinical model of allergen-induced bronchial inflammation. Aim of our study was to assess the effects of allergen-challenge in asthmatic subjects on platelet-leukocyte interactions in whole blood. Eight mild asthmatic patients were challenged with either diluent or the specific allergen. During the LAR (8 hours after challenge) the percentage of leukocytes positive for platelet markers, as assessed by cytofluorimetry in circulating blood, was higher than the value obtained pre-challenge or the value obtained 24 hours post-challenge (pre=22.7%±3; LAR=53%±9.8; 24 hours post=26%±3.5; pre vs LAR p<0.05; LAR vs 24 hours p<0.05). In contrast, 8 hours after challenge with saline no increase of platelet/leukocyte aggregates was observed (26%±9). The platelet fluorescence ratio showed similar changes although it did not achieve statistical significance (pre=5.7±2.08, LAR=8.94±3.6; 24 hours=4.7±1.1, p>0.5). The expression of P-selectin on circulating platelets during the LAR was significantly lower than the basal and the 24 h post value (pre=100; LAR=57.5±7; 24 hours=170±52; control day=108±29, pre vs LAR p<0.01).

PAC-1 binding and LIMP expression did not change significantly after allergen challenge. In conclusion, our results show an increase of platelet-leukocyte aggregates during LAR. The fact that at same time we observed a decrease of P-selectin expression on platelets may be explained by the sequestration of activated platelets in platelet/leukocyte aggregates. In fact, the formation of these aggregates is dependent on P-selectin expression. In asthma, leukocytes contribute to bronchial inflammation and platelets may facilitate leukocyte rolling, adhesion and migration into the bronchial tissue and enhanced leukocyte accumulation at the inflammatory site.

CO-011

IDIOPATHIC THROMBOCYTOPENIC PURPURA IN THE ELDERLY: CLINICAL COURSE AND RESPONSE TO THERAPY

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Idiopathic thrombocytopenic purpura (ITP) is often diagnosed in the elderly. From published data, questions arose about some subjects: age cut off, bleeding tendency, clinical evolution, therapeutic approach (timing, drugs, doses). We have retrospectively analysed 183 patients (M/F = 0.90, med. age 71 yrs, range 65-87), affected by ITP diagnosed from 1981 to 1998. At diagnosis, mean platelet (PLT) count was $55.6 \times 10^9/L$ (SD 38.6, range 1-138 $\times 10^9/L$); PLT number was $<50 \times 10^9/L$ in 94/183 (51%) patients. Bleeding symptoms (WHO 1-3) were present in 73/183 (40%). Mean follow-up (F.U.= observation time until last control or unfavourable event) was 39 months (range 2-173). In 113/183 (62%) (M=63, F=50, M/F=1.26, med. age 70 yrs, range 65-85, mean PLT number: $79 \times 10^9/L$, range 23-138 $\times 10^9/L$) only serial controls were planned because no remarkable bleeding symptoms were present and PLT count was at haemostatic levels. During the F.U., however, in 11/113 (9.7%) therapy was started (3-48 months from diagnosis), because of either in a decrease PLT number, or occurrence of bleeding symptoms; in 4/113 (3.5%) evidence of a primary disease (RAEB 1, SMPCr 1, APA syndrome 2) was found (2-84 months from diagnosis); in 1/113 (0.8%) PLT decrease was considered as EDTA-related. 97/113 (86%) patients remained in F.U. without therapy (mean 33.5 months, range 6-144): in 5 of them (5.2%) spontaneous normalisation of PLT count was recorded. In 70/183 (38%) patients (M=24, F=46, M/F=0.52, mean PLT count: $18 \times 10^9/L$, SD $15.6 \times 10^9/L$) therapy was started at diagnosis because of either a low PLT count or bleeding symptoms. During F.U. 5 of them (7%) developed a malignancy (solid neoplasia in 3, malignant haematological disease in 2 [CLL, CMML]) 3-146 months after diagnosis. In the 81 patients submitted to therapy, the therapeutic approach was as follows: in 77 low-dose prednisone (PDN) (mean daily dose: 0.4 mg, SD 0.31), in 4 azathioprine, at a daily dose of 100 mg. Among the PDN treated patients (evaluable cas-

es: 75), complete response was obtained in 22 (29.3%), partial response in 32 (42.7%), no response in 24 (32%). Forty patients (53.3%) had a persistent, partial or complete responses. No response was reached with azathioprine. From our survey, some issues arose: 1) age cut-off at 65 yrs is a reasonable choice; 2) serial controls over time appear to be suitable in asymptomatic patients with mildly decreased PLT number; 3) only patients with very low PLT count or with bleeding symptoms need treatment; 4) PDN at low doses is able to ensure persistent responses in more than 50% of cases, while azathioprine does not appear to be effective; 5) in non-responders, maintenance therapy with PDN is able to control bleeding tendency; 6) observation on follow-up of untreated patients may reveal a possible primary disease.

CO-012

PULSED HIGH-DOSE DEXAMETHASONE AS FIRST-LINE THERAPY IN IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background. Corticosteroids are the "gold standard" therapy for idiopathic thrombocytopenic purpura (ITP), but dose, timing and way of administration are not still so well defined. Recently, the use of high-dose (HD) dexamethasone in ITP patients (pts) has given conflicting results. **Patients and methods.** We evaluated the effect of HD dexamethasone (40 mg/day i.v. for 4 sequential days every 28 days) in 28 untreated patients (F15-M13, median age 31, range 20-58 yrs). Therapy was planned if platelet (plt) count was $\leq 20 \times 10^9/L$ and/or if bleeding symptoms were present. At the beginning of treatment plt count was: $x = 10.6 \times 10^9/L$, median $9 \times 10^9/L$, range 2-25; 25 pts had bleeding symptoms. Patients were scheduled to receive 4-6 therapy courses according to the response. **Results.** One patient stopped dexamethasone after 1 therapy course because of systemic hypertension. In 3 pts therapy was discontinued after 3 courses: 2 pts obtained a complete response ($plt \geq 150 \times 10^9/L$) and refused further therapy courses; 1 pt developed a very important anxiety syndrome. Thus, 24 pts are valuable for response to therapy. The first evaluation was performed 28 days after the 4th course and showed: 1) In 15 pts (62.5%) persistent complete response, already reached after 2 courses; 2) In 5 pts (20.8%) partial response ($50 \leq plt < 150 \times 10^9/L$); 3) In 4 pts (16.7%) no response ($plt < 50 \times 10^9/L$). Nine patients stopped administration of further therapy courses: 6 because of side effects (fluid retention, cutaneous rash, anxiety, gastric pain, visual alteration); 2 pts because of low compliance to therapy; 1 non-responding patient was splenectomised because of bleeding syndrome. The second evaluation, in the last 15 pts, was performed 28 days after the 6th course with the following results: 1) In 11 pts complete response; 2) In 2 pts partial response; 3) In 2 pts no response. At present,

median follow-up is of 14 months (range 6-33). Twelve pts are off-therapy with a normal platelet count, 4 off-therapy pts are partial responders, 2 have normal plt levels after splenectomy, 6 are non-responding and low-dose prednisone is necessary to maintain a safe plt count. **Conclusion.** Sixteen pts (66.6%) are responding to pulsed HD dexamethasone and presently off-therapy. Therapy is fairly well-tolerated and seems able to stop bleeding symptoms so that we believe that dexamethasone could be a successful emergency therapy. Most patients had a response already with 4 therapy courses. In future studies it would be advisable to reduce the number of therapy courses. This approach seems to give favourable results, but longer experience will be required to compare this therapy with conventional treatment for untreated ITP patients.

CO-013

FACTORS INFLUENCING POST-TRANSFUSIONAL PLATELET INCREMENT IN 87 CONSECUTIVE PATIENTS GIVEN BONE MARROW TRANSPLANTATION. A PROSPECTIVE STUDY

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Several factors responsible for a poor efficacy of platelet transfusion have been identified in different clinical settings, but little is known about their role in patients receiving bone marrow transplantation (BMT). In order to gain further information on this argument, we evaluated the effectiveness of 551 leukodepleted platelet transfusions by post-transfusion (16-hour) corrected count increments (CCI) in 87 consecutive children receiving allogeneic BMT for haematological malignancies (72 pts) or non-malignant disorders (15 pts). Mean CCI was 5.9 ± 8.3 in the whole population (CCI < 0 in 17% of pts, 0-5 in 39%, 5-10 in 24%, > 10 in 20%), but was significantly lower in CML (3.4 ± 5.0) and MDS (2.0 ± 3.6). CCI was higher for platelets obtained by apheresis from single donors as compared with pooled platelet concentrates (6.3 ± 8.3 vs 3.5 ± 8.6 , $p = 0.012$). Clinical factors associated with poor transfusion efficacy were fever $> 38^\circ C$ (CCI 5.2 ± 6.7 vs 7.0 ± 10.3 , $p = 0.012$), splenomegaly (3.2 ± 5.0 vs 6.5 ± 8.9 , $p = 0.003$), serum antibodies to CMV and/or anti-CMV therapy (3.2 ± 6.0 vs 6.6 ± 8.5 , $p = 0.0002$), therapy with vancomycin (1.9 ± 5.3 vs 6.2 ± 8.7 , $p = 0.005$) or amphotericin B (4.9 ± 6.7 vs 6.6 ± 9.2 , $p = 0.019$). HLA-class I and/or platelet-specific antibodies were observed in 13 pts (anti-HLA: 7 pts, anti-GPIIb-IIIa: 5 pts, anti-GPIa-II: 4 pts) and were associated with low CCI (anti-HLA: 3.4 ± 5.9 vs 6.9 ± 8.8 , $p = 0.029$, anti-GPIIb-IIIa: 1.5 ± 4.2 vs 6.8 ± 8.8 , $p = 0.050$, anti-GPIa-IIa: 1.2 ± 4.1 vs 6.7 ± 8.8 , $p = 0.046$). All patients with antibodies against GPIIb-IIIa or GPIa-IIa were HPA-1 a/a or HPA-5 a/a respectively, and the genotype HPA-1

a/a was associated *per se* with a lower CCI (5.3 ± 7.6 vs 7.6 ± 10.8 , $p=0.013$). A multivariate analysis considering all factors associated with poor response to platelet transfusion at univariate analysis was performed. It showed that the strongest predictive factors of transfusion failure were the presence of anti-platelet antibodies (both against HPA and HLA), positivity of anti-CMV antibodies and vancomycin therapy. In conclusion, an inadequate response to platelet transfusion was observed after BMT in more than 50% of cases. Non-immune factors (some of which iatrogenic) were involved in poor response to platelet transfusions in the large majority of cases, but the most severe refractoriness to platelet transfusions was observed in pts with anti-platelet antibodies.

CO-014 UNEXPECTED HIGH INCIDENCE OF PORTAL VEIN THROMBOSIS IN YOUNG PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA

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Portal vein thrombosis (PVT) is considered a rare, albeit characteristic, complication of chronic myeloproliferative disorders (CMDs). Optimal management and outcome is uncertain, especially in younger patients who are generally considered at low risk for vascular events. However, in a recent update of thrombotic complications occurring in our patients with MPDs, an unexpectedly high incidence of PVT in young subjects with essential thrombocythaemia (ET) was found. In the last four years, we have observed 4 cases of PVT in 51 consecutive patients with ET aged less than 60 (incidence 1.96% pt-yr). They were 2 males and 2 females, aged 32, 35, 48 and 54 years. Three patients were previously asymptomatic and untreated, whereas one female, aged 54 ys., was given hydroxyurea (HU) for an obstructive arteriopathy of the toes. Platelet counts at the onset of PVT were $372,000/\text{mm}^3$ (on HU), $415,000/\text{mm}^3$, $590,000/\text{mm}^3$ and $579,000/\text{mm}^3$. In all patients, the presenting symptom of disease was abdominal pain, often associated with spleen enlargement and reduction of platelet count. Diagnosis was achieved by colour Doppler ultrasound and/or computerised tomography. Our treatment protocol included: a) unfractionated i.v. heparin to maintain the APTT ratio between 1.5 and 2.5, followed by long-term warfarin with a PT INR target range of 2.0 - 3.0; b) hydroxyurea, to maintain platelet counts below $400,000/\text{mm}^3$. Complete recovery was achieved in all patients and no recurrence was observed after a median follow-up of 32 months (range 2 - 46). Our findings suggest the following practical considerations: a) PVT is more common than expected in young patients with ET; b) an high grade of clinical suspicion should be maintained in these patients and every episode of abdominal discomfort, especially if associated with apparent spleen enlargement and/or

platelet count reduction should be aggressively diagnosed and treated; c) immediate and long-lasting anticoagulant and cytoreductive treatment with hydroxyurea was associated with a favourable clinical outcome.

CO-015 EFFECT OF ANTI- β 2-GLYCOPROTEIN I AND ANTI-PROTHROMBIN ANTIBODIES ON PLATELET ACTIVATION

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Antiprothrombin and anti- β 2-glycoprotein I (α β 2-GPI) antibodies belong to the family of antiphospholipid antibodies (aPL), which are associated with clinical events such as arterial and/or venous thrombosis. However, the precise mechanisms responsible for thrombosis remain unclear. Recently, it was hypothesised that, as a consequence of an initial damage, anionic phospholipids are exposed on the surface of platelets, endothelial cells and trophoblasts. These potentially reactive phospholipids promote the cofactor-mediated binding of different aPL, which concentrate on the cell surface, bind to the cellular Fc γ receptors and induce strong thrombosis-promoting modifications (Arnout, *Thromb and Haemost* 1996; 75: 536-41). To test this hypothesis we studied the effect of purified aPL on the activation of washed platelets isolated from healthy donors. Platelet activation was evaluated by flow cytometric analysis using anti-CD62P monoclonal antibody directed against activation-dependent granule-external protein (PADGEM or GMP-140). Results were expressed as fluorescence index (FI), that is the product of MFI (mean fluorescence index) and the percentage of positive platelets. We tested the effects of affinity purified IgG anti- β 2-GPI antibodies from 11 patients: all preparations showed anti-cardiolipin activity in ELISA-assay; 10 of them showed anticoagulant activity in phospholipid-dependent coagulation tests. In the absence of β 2-GPI, none of the preparations was able to induce activation of resting platelets, whereas in the presence of a physiological concentration of β 2-GPI (0.2 mg/mL) one sample only showed this ability. When platelets were pre-stimulated with sub-threshold thrombin concentrations (up to 0.025 U/mL), six preparations were able to enhance platelet activation in a concentration-dependent, but in an apparently β 2GPI-independent way. The ability of anti- β 2-GPI antibodies preparations to enhance platelet activation was not correlated with either their immunological and anticoagulant properties, or with the clinical history and laboratory data of the patients. Subsequently, we evaluated the activity of 9 preparations of anti-prothrombin antibodies, none of which was able to activate resting platelets or significantly augment platelet activation either alone or in the presence of prothrombin and calcium. In conclusion, these data suggest that the enhancement of platelet activation

might represent one of the possible pathogenetic mechanisms of thrombosis in a minority of aPL-positive patients.

CO-016

SEVERE HIV-RELATED THROMBOCYTOPENIA: SPLENIC IRRADIATION vs SPLENECTOMY

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Chronic thrombocytopenia is a frequent complication of HIV-infection occurring in around 20-55% of patients in advanced stage of disease and 10% of AIDS-free HIV-infected patients. Severe, symptomatic thrombocytopenia is much less frequent (1.5-5% of HIV-infected patients) but major haemorrhagic complications, including deaths from intracranial or visceral bleeding, are not rare (4-5% of these patients during the course of the disease). These patients require active treatment but a variable percentage of them are refractory to most of the conventional or experimental drugs and represent a serious therapeutic dilemma. Splenic irradiation has been proposed as an alternative to splenectomy in severe HIV-related thrombocytopenia refractory to medical treatment in order to avoid the surgical and immunologic risks of splenectomy, but no comparative studies have assessed these two procedures. We have carried out a retrospective, multicentre, chart-review trial with independent extraction of data from patient records. Twelve HIV-positive patients with symptomatic (all with epistaxis and easy bruising, 2 with rectal bleeding, 2 with menorrhagias), severe ($<25 \times 10^9/L$), long lasting (≥ 24 months) thrombocytopenia, refractory to at least two previous medical treatments, underwent splenectomy (6 patients) or low-dose splenic irradiation (10 Gy in 5-10 fractions over 1-3 weeks) (6 patients). End points were the platelet count, total lymphocytes, CD4-positive and CD8-positive T-cell counts and percentages and HIV-viral load, measured at baseline and at 3 and 6 months after treatment. Low-dose splenic irradiation did not increase the platelet count at 3 and 6 months (basal: 13 ± 7 ; 3 months: 11 ± 14 ; 6 months: $16 \pm 16 \times 10^9/L$). Splenectomy increased significantly the platelet count at 3 and 6 months (basal: 8 ± 3 ; 3 months: 198 ± 58 ; 6 months: $192 \pm 46 \times 10^9/L$); the difference between the two treatments was significant at 3 and 6 months. In splenic irradiated patients a decrease of total CD4- and total CD8-positive T-cells was observed while these parameters increased significantly in the splenectomised group with a significant difference at 6 months in favour of splenectomised. No changes in viral load were observed in either group. Follow-up at 12 months is available for the 6 splenectomised patients (platelet count: $196 \pm 59 \times 10^9/L$) and for one of the irradiated patients (platelet count: $30 \times 10^9/L$). One patient not responding to splenic irradiation was later (after 7 months) splenec-

tomised with a subsequent rise of platelets up to $300 \times 10^9/L$ (16 months later). Splenic irradiation is ineffective and potentially detrimental to the immunologic status for patients with severe, long-lasting, HIV-related thrombocytopenia refractory to medical treatment while splenectomy is safe and effective and does not induce immunologic impairment.

CO-017

THROMBOXANE A₂ ACCOUNTS FOR TRANSIENT MYOCARDIAL ISCHAEMIA FOLLOWING INJECTION OF THE INFLAMMATORY PEPTIDE fMLP IN THE RABBIT

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Injection of fMLP (30 nmol/kg) in the jugular vein of the rabbit induced marked granulocytopenia and thrombocytopenia. Electron microscopy showed activated and aggregated polymorphonuclear leukocytes (PMN) and platelets in the capillaries and venules of the lungs but not of the heart. Activation of circulating cells was accompanied by the production of LTB₄ (peak levels at 1 min following fMLP injection: 25.0 ± 4.8 ng/mL), LTE₄ (peak levels at 3 min: 141.1 ± 17.0 ng/mL) and TXA₂ (peak levels at 1 min: 29.4 ± 4.5 ng/mL). In all the animals (6 out of 6) fMLP evoked ischaemic electrocardiographic changes: within the first minute of infusion a profound depression of the ST segment and inversion of the T wave was observed at lead II. Mean aortic pressure and heart rate fell to 67.6 ± 6.2 and $82.4 \pm 1.7\%$ of the basal levels at 3 and 10 min after fMLP, respectively. All these alterations were transient and disappeared within 30 min after fMLP infusion. The role of eicosanoids in mediating fMLP-dependent cardiovascular derangement was investigated by using 5-lipo-oxygenase, cyclo-oxygenase and thromboxane synthase inhibitors. Intravenous infusion of BAY X1005 (0.2 mg/kg/min for 60 min before fMLP), a specific inhibitor of 5-lipo-oxygenase did not modify either cell recruitment or cardiovascular alterations. In contrast, aspirin (50 mg/kg iv 45 min before fMLP) prevented electrocardiographic alterations in all (5 out of 5) treated animals, partially reverted hypotension and bradycardia (72.0 ± 7.6 and $94.8 \pm 4\%$ of the basal values, respectively) but did not modify PMN and platelet recruitment. Aspirin abolished TxA₂ (peak level 1.9 ± 0.5 ng/mL) and partially reduced LTB₄ (peak level 15.0 ± 5.1 ng/mL) as well as LTC₄ (peak level 102.0 ± 31.1 ng/mL). Similarly to aspirin, ridogrel (20 mg/kg, iv, 10 min before fMLP), a thromboxane synthase and receptor inhibitor, prevented electrocardiographic alterations and bradycardia ($94 \pm 5.4\%$ of the basal value) but did not improve and even worsened fMLP-induced hypotension ($52 \pm 05\%$ of basal value). Ridogrel suppressed TxA₂ (peak level 1.3 ± 0.1 ng/mL) and reduced LTs synthesis (peak levels 14.3 ± 1.3 and 97.4 ± 13.8 ng/mL for

LTB₄ and LTC₄, respectively). Moreover ridogrel blocked lung platelet but not PMN sequestration. These results indicate that TxA₂, synthesised following inflammatory cell interaction with platelets, is the major player of a transient myocardial ischaemia in the absence of a thrombotic coronary occlusion.

CO-018

GLANZMANN'S THROMBOASTHENIA: IDENTIFICATION OF MUTATIONS IN 10 OF 16 PATIENTS

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Glanzmann's thromboasthenia (GT) is an autosomal recessively inherited bleeding diathesis. Quantitative or qualitative abnormalities of the platelet allbb3 integrin (also known as the glycoprotein complex IIb-IIIa) have been shown to be responsible for this disorder. Mutations within the genes that code for allbb3 subunits have been described in GT patients. Understanding of platelet allbb3 integrin functions is crucial for the knowledge of platelet aggregation and normal haemostasis. To elucidate the molecular basis of GT, we have investigated 16 GT patients looking for gene variations within the allbb3 genes and in 10 of them we have found 16 candidate point mutations. Isolation of DNA and PCR analysis were carried out according to standard procedures. An 18 mL volume of blood was drawn into 2 mL of 3.8% sodium citrate. For DNA extraction, peripheral blood leukocytes were separated by sedimentation and incubated overnight at 37°C in a digestion buffer (100 mM NaCl 10 mM Tris-HCl, 25 mM EDTA, 1% SDS) containing 0.1 mg/mL of proteinase K. The nucleic acid was isolated by phenol/chloroform extraction and ethanol precipitation. Amplification of all coding regions of allb and b3 genes and intron/exon boundaries was achieved using sense and antisense oligonucleotides. Complete coding sequences of allb and b3 genes and intron/exon boundaries were screened for mutations in 16 GT patients. Fifteen abnormal SSCP patterns were found. All abnormal patterns cosegregated with the GT phenotype and did not appear in SSCP patterns from normal subjects and therefore are likely to be pathogenetic mutations. Amplification products that showed abnormal electrophoretic mobilities were then sequenced. All except one belong to the allb gene. We found 15 different mutations of the allb gene in 9 patients and 1 mutation of the b3 gene in a patient. Twelve missense mutations, two insertions of a single nucleotide, and two potential splicing mutations were found. The mutation detected

within the b3 gene was missense. Overall, in 11 out of the 16 patients investigated a possible pathogenetic mutation. Further investigations are needed, since mutations identified provide *in vivo* experimental models that offer a unique opportunity to unravel the role of specific regions of allbb3 integrin components.

ORAL COMMUNICATIONS

Arterial thrombosis: risk factors

CO-019

HAEMOSTATIC EVALUATION OF CARDIOVASCULAR RISK IN HIV PATIENTS

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Background. The introduction of highly active anti-retroviral therapy (HAART), that includes the use of protease inhibitors (PI), in the treatment of HIV infected patients has dramatically reduced the incidence of death among this population. HAART treated patients may be at risk of atherosclerosis, and present evidence of endothelium dysfunction. The risk of development of cardiovascular disease may increase as a consequence of the body composition and metabolic changes known as "lipodystrophy", related to PI use. In the last 2 years there has been an increase in reports of cardiovascular events in HIV subjects, possibly related to the introduction of HAART. The objective of this study is to evaluate the haemostatic parameters of cardiovascular risk in patients treated and untreated with HAART regimen containing PI. **Method.** Twenty-seven HIV-subjects divided into naive HAART untreated (11 patients) and HAART treated (16 patients) were enrolled in this study. Haemostatic parameters of cardiovascular risk (fibrinogen, FVII) as well as a marker of endothelial injury (thrombomodulin -TM-) were evaluated. Carotid duplex coded ultrasound colour scan was performed. **Results.** The two groups were comparable for age, CD4 cell count and generic factors of cardiovascular disease (smoking, alcohol use, blood pressure). Mean HIV-RNA load was 95,334 copies/mL in untreated and 913 copies/mL in PI treated patients. PI treatments in combination regimens lasted a mean of 30 months (range: 20-41). Mean TM (55.8 ng/mL in untreated and 54.6 ng/mL in treated patients) was increased in both groups with respect to normal values. Among haemostatic parameters of cardiovascular risk, FVII was higher in treated patients than in untreated (130% and 93 % respectively, p<0.05). Intimal thickening was found both in treated and untreated patients, but was more evident in the first group, being 0.83 and 0.54 mm respectively (p<0.01). **Com-**

ments. Our data underline an increased haemostatic activation and intimal thickening in both treated and untreated HIV patients. PI treated patients show an increase in FVII and fibrinogen as well as increased intimal thickening that may represent an adjunctive risk for cardiovascular disease. In patients at high risk, cardiovascular prophylaxis could be indicated. More detailed studies are needed to address this point.

CO-020 GENETIC RISK FACTORS FOR ISCHAEMIC STROKE

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Background. The role of thrombophilic polymorphisms as risk factors for ischaemic stroke is debated. Our study was aimed to evaluate the risk associated with two common thrombophilic polymorphisms (factor V Leiden and G20210A mutation in the prothrombin gene) and the risk associated with two polymorphisms that are possible causes of hyperhomocysteinaemia. **Patients and methods.** We investigated 234 patients (M/F 106/128) with a history of ischaemic stroke before 65 years documented by CT or NMR scan; the mean age at the thrombotic event was 38 years (median 39, range 2 to 63). In 142 of them the thrombotic event occurred before 50 years of age and in the absence of acquired risk factors (smoking, hypertension, diabetes, dyslipidaemia, oral contraceptive intake). A control group of 483 healthy individuals (M/F 256/227, mean age 45 years, median 44, range 7 to 93) was also investigated. All individuals were genotyped for the presence of factor V Leiden (FV-L), the G20210A mutation in the prothrombin gene (FII-A), the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene, and the T833C/68bp insertion in the cystathionine-β-synthase (CBS) gene. **Results.** Among the patients we found 8 heterozygotes for FV-L, 22 heterozygotes for FII-A, 2 double carriers of FV-L and FII-A, 2 homozygotes for FII-A; 41 individuals were homozygous for the MTHFR polymorphism and 31 were heterozygous for the CBS polymorphism, 6 of them were double carriers of the MTHFR and the CBS polymorphisms. In the control group 11 were heterozygous for FV-L, 1 homozygous for FV-L, 13 heterozygous for FII-A; 80 individuals were homozygous for the MTHFR polymorphism and 63 were heterozygous for the CBS polymorphism, 14 of them were double carriers of the MTHFR and the CBS polymorphisms. No significant increase in risk was found associated with factor V Leiden, homozygous MTHFR polymorphism, heterozygous CBS polymorphism, double carriership of MTHFR and CBS polymorphisms either in the overall patient group or in the selected group of patients below 50 years of age and without acquired risk factors. The risk associated with the G20210A mutation was 4.5-fold higher in the overall patient group (95% CI 2.3 to 9.0). Among

the younger patients with no risk factors the risk associated with the G20210A mutation was 3.3-fold higher (95% CI 1.5 to 7.5). **Conclusions.** The G20210A mutation in the prothrombin gene is associated with an increased risk of ischaemic stroke before 65 years of age.

CO-021 BIOAVAILABILITY OF VITAMIN E IN RELATION TO FOOD INTAKE IN HUMANS

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Vitamin E is a lipid-soluble, chain breaking antioxidant that has been studied in experimental and clinical models to assess its effectiveness in reducing the atherosclerosis process. An important issue related to the clinical use of vitamin E is the choice of optimal dosage, that should be based on pharmacodynamic studies. Here we report a human supplementation study that aimed at investigating the bioavailability of synthetic vitamin E in relation to meals. We enrolled twenty healthy volunteers (12 females and 8 males, mean age 31.2 years) who were randomly assigned to receive 300 mg of synthetic vitamin E before (group A) or after meals (group B). In each subject plasma vitamin E concentration was determined at baseline and at seven days and fifteen days of vitamin E supplementation. Plasma vitamin E levels were determined by high-performance liquid chromatography (HPLC) and were expressed in terms of lipid ratio (vitamin E/cholesterol+triglycerides mg/g). Data were expressed as mean ± standard deviation. Plasma alpha-tocopherol levels were significantly increased in both groups after 7 and 15 days of vitamin E supplementation (group A: 5.81±0.99, 8.28±1.81, and 8.08±0.57 mg/g; group B: 5.60±1.20, 9.95±2.35, and 10.74±1.56 mg/g); nevertheless, after 15 days, the increase was significantly higher in group B than in group A (+91.8% vs +39.0%, p<0.05). This study suggests that intestinal absorption of vitamin E and, as a consequence, its bioavailability, is favoured by food ingestion. This finding may be crucial for future use of vitamin E in clinical studies and for correct evaluation of its effectiveness in reducing the atherosclerosis process.

CO-022 A C 807T SUBSTITUTION IN THE PLATELET COLLAGEN RECEPTOR INTEGRIN α2β1 GENE IS A GENETIC RISK FACTOR FOR STROKE AT A YOUNG AGE BUT NOT FOR MYOCARDIAL INFARCTION

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Background. Glycoprotein Ia (α_2 integrin) is a subunit of the heterodimeric membrane complex (GPIa/IIa) that mediates platelet adhesion to collagen. Several nucleotide sequence variations of GPIa have been described. Recently a C/T substitution at nucleotide 807 and a G/A substitution at nucleotide 873 were identified. These polymorphisms are linked to the expression density of the collagen receptor. The GPIa T807/A873 allele causes a higher expression, enhancing platelet binding to collagen. This might produce a genetic predisposition for the development of thrombotic complications. Since the two polymorphisms are in high linkage disequilibrium we analysed the C807T substitution in patients with stroke and myocardial infarction. **Patients.** In this case control study the genotypes of the GPIa C807T polymorphism were compared in stroke patients <50 years of age (n=70, 22 males and 48 females) with sex and age matched controls (n=125, 62 males and 63 females), and in myocardial infarction patients (n=177, 163 males and 14 females) with sex and age matched controls (n=147, 135 males and 12 females). **Results.** In patients <50 years with stroke, the T allele was over-represented compared with controls ($p < 0.01$, odds ratio 1.8; 95% CI 1.3-6.9). The genotype distributions between cases and controls are as follows: 29% CC, 44% CT, 27% TT for cases and 38% CC, 52% CT, 10% TT for controls with odds ratio 1.15 (95%CI, 0.6-2.26) for CT vs CC and odds ratio 3.8 (95%CI, 1.6-9.3) for TT vs CC. In patients with myocardial infarction there was no statistically significant difference between cases and controls, (odds ratio 0.84 95% CI, 0.61-1.15) for C vs T. **Conclusion.** Our results indicate that the GPIa T807 genotype might be an inherited risk factor for the development of stroke in patients <50 years of age, but does not represent a risk factor for patients with myocardial infarction at a young age.

CO-023

PLATELET GLYCOPROTEIN RECEPTOR IIIA POLYMORPHISM PLA1/PLA2 AND VASCULAR RISK: A META-ANALYSIS

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Platelet membrane glycoprotein IIb/IIIa plays a major role in platelet adhesion and aggregation. The gene encoding the GP IIIa shows a common polymorphism PIA^1/PIA^2 that has been associated with vascular disease in some but not all reports. **Aim.** To assess the role of glycoprotein IIb/IIIa polymorphism in vascular risk. Studies were identified by MEDLINE searches, hand searching of cardiology, epidemiology, and genetic journals, and abstract books from 1996 to date. Population based studies with a clear description of the control group and of the relationship between PIA^2 allele and vascular risk were included (43 out of 46 identified studies). The following information was extracted: size and type of cohort,

type of events, range of age, odds ratio (OR) with 95% confidence intervals (95% CI), genotype distribution, allele frequency, and degree of adjustment for confounders. The general variance-based method was used to combine data from different studies. A total of 30 studies for coronary artery disease, 6 for restenosis after revascularisation and 7 for cerebrovascular disease were identified, for a total of 8994 cases and 13666 controls. No difference in PIA^2 allele frequency in controls (ranging from 0.11 to 0.15) was found among different studies grouped according to setting country. In coronary artery disease, the overall OR was 1.10 (95% CI: 1.03 to 1.18), and was higher in subjects younger than 60 years (OR=1.22; 95%CI: 1.06 to 1.39). The OR for PIA^{A2A2} genotype in comparison with ($PIA^{A1A1} + PIA^{A1A2}$) genotype was 0.95 (95% CI: 0.74 to 1.22). The overall effect on thrombotic events after revascularisation procedures (for a total of 649 patients with adverse events and 3905 patients with no complications) was 1.31 (95% CI: 1.09 to 1.56), while that for cerebrovascular disease was 1.11 (95% CI: 0.95 to 1.30). Funnel plot of the estimate of the logarithm of odds ratio against sample size shows that studies of different size seemed to be scattered equally above and below the pooled estimate. An overview of the currently available studies supports a role of PIA^2 allele of the platelet membrane glycoprotein IIIa gene on the risk of coronary thrombosis. The association was particularly evident before the age of 60 and in thrombotic complications after stenting procedures. No effect was found on cerebrovascular disease.

CO-024

WINE CONSUMPTION AND VASCULAR RISK: A META-ANALYSIS

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The existence of an inverse association between moderate alcohol consumption and vascular risk is well established. Several reports giving individual relative risks attributable to consumption of wine, beer or spirits have been published. However, the relative benefit from specific types of alcoholic beverages remains to be assessed. An overview of the literature on selected studies providing specific information on wine consumption in relation to vascular risk, may help in evaluating the possible specific benefit attributable to wine, which is the alcoholic beverage of choice of Mediterranean populations. The aim of this study was to quantify the relative risk of vascular events for wine drinkers in comparison with non-drinkers, through a meta-analysis of available data. Studies were identified by a MEDLINE search for epidemiological investigations of alcohol and vascular risk published after 1965, and supplemented through citations in review articles and journals dedicated to

tracking alcohol related-research. The following information was extracted: size and type of cohort, type of events, relative risks (RR) with 95% confidence intervals (95% CI), and degree of adjustment for confounders. The general variance-based method was used to combine data from different studies. Out of the 50 studies on the association between alcohol consumption and vascular events, 11 were identified reporting RR for wine consumption, for a total of 9201 cases and 159,537 controls. The overall effect was 0.70 (95%CI: 0.66 to 0.75; 11 studies), indicating a 30% reduction in the risk of fatal or non fatal vascular events for wine drinkers in comparison with non-drinkers. Subgroup analysis was performed according to type of cohort, as well as type of event. The overall effect from the four prospective studies was 0.66 (95%CI: 0.62 to 0.71) while for the case-control studies it was 0.83 (95%CI: 0.74 to 0.93; 7 studies). Overall RR for non-fatal myocardial infarction was 0.86 (95%CI: 0.76 to 0.97; 6 studies), and it was 0.66 (95%CI: 0.61 to 0.70; 5 studies) when studies with a combined endpoint (fatal or non-fatal cardiovascular or cerebrovascular events) were considered. An overview of the currently available studies supports the protective role of wine consumption against the risk of vascular events. The association is independent of the type of cohort as well as of the endpoint considered.

CO-025

-511 C/T PROMOTER POLYMORPHISM OF INTERLEUKIN 1- β GENE AND RISK OF PREMATURE MYOCARDIAL INFARCTION

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Inflammation may play an important role in the initiation and progression of atherothrombosis. Interleukin-1 β (IL-1 β) enhances the production of fibrinogen, c-reactive protein and other inflammatory mediators, which have been associated with the risk of ischaemic coronary disease. Several polymorphisms in interleukin genes have been related with the risk of inflammatory disease. We studied the association of polymorphisms at position -511 and +3953 of the IL-1 β gene with the risk of myocardial infarction at young age. One hundred and fifty-eight patients with myocardial infarction before the age of 45 (males) or 50 years (females) were frequency-matched for age and sex with 153 healthy controls selected from the general population. Subjects carrying the CC genotype of -511 promoter polymorphism showed a 3 times higher risk of myocardial infarction (OR=3.01; 95% CI: 1.31-6.92), after adjustment for conventional risk factors. Odds ratios

increased with the genotypes in the following order: TT (OR=1) <CT<CC in univariate (p=0.006) and multivariate (p<0.0001) analysis. Multivariate linear regression indicated a significant association between the -511CC genotype and the levels of fibrinogen both in cases and in controls. Subjects with the C allele had higher levels of fibrinogen than TT homozygotes. No association was found between +3953 of the IL-1 β gene and the risk of myocardial infarction. Our findings show that IL-1 β gene polymorphism is associated with myocardial infarction at young age and support the relevance of inflammation in the pathogenesis of myocardial infarction.

CO-026

POLYMORPHISMS OF COAGULATION FACTOR VII GENE ARE ASSOCIATED WITH A REDUCED RISK OF PREMATURE MYOCARDIAL INFARCTION IN MEN

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Genetic variants of coagulation factor VII (FVII), associated with low FVII levels have been shown to reduce the risk of myocardial infarction (MI). However, these findings were not confirmed in other studies. A functional polymorphism has been described in the promoter of FVII. One hundred and fifty-eight patients with MI before the age of 45 (males) or 50 years (females) were frequency matched for age and sex, with healthy controls selected from the general population. The frequency of the rare allele P10 of -323 promoter polymorphism in controls was 0.19 (95% CI: 0.15 to 0.23), significantly higher than in patients with juvenile MI (0.14, 95% CI: 0.10 to 0.18; $\chi^2=3.1$, df=1, p=0.07). A noteworthy sex heterogeneity led us to analyse men and women separately. The frequency of P10 allele was significantly lower in male patients than male controls (0.12, 95% CI: 0.08-0.17 vs 0.20, 95% CI: 0.16-0.26; $\chi^2=5.7$, df=1, p=0.02). Genotype distribution was in HW-equilibrium and significantly different between cases and controls (p=0.05, Fisher exact test). Due to the low number of subjects homozygous for the P10 allele, we concentrated our analysis on the combined group P0/P10+P10/P10. This last group had a statistically significant half-reduction in the risk of familial MI as compared with carriers of the P0/P0 genotype (OR=0.45, 95% CI: 0.23 to 0.86, after adjustment for confounders). Our findings suggest that the promoter polymorphism of FVII gene may influence the risk of MI at young age in males and suggest a different impact of this polymorphism on the risk of MI in males and females.

CO-027 EFFECTS OF ACUTE HYPERGLYCAEMIA ON PLATELET ACTIVATION IN TYPE II DIABETES

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Ischaemic cardiovascular disease represents the first cause of mortality and morbidity in type II diabetes. Several studies have shown platelet abnormalities in diabetes, on the other hand strict metabolic control over several days normalises increased platelet activation *in vivo*. Moreover, *in vitro* experiments have shown that hyperglycaemia can facilitate platelet activation. This has led to the hypothesis that hyperglycaemic peaks can precipitate thrombotic events in diabetics. However, no studies have assessed whether *in vivo* hyperglycaemia induces an acute condition of platelet hyperreactivity in diabetics. We have carried out a cross-over, randomized, double-blind study in 10 patients with type II diabetes to study the effects of 4 hrs acute hyperglycaemia (13.9 mmol/L, 250 mg/dL) or euglycaemia (5.5 mmol/L, 100 mg/dL), maintained with a glucose clamp technique, on *in vivo/in vitro* platelet function. Blood samples were collected just before and at the end of the glycaemic clamp for the measurement of shear stress-induced platelet activation (O'Brien's method), circulating activated platelets (cytofluorimetry of P-selectin) and plasma vonWillebrand factor (vWF-ELISA). Bleeding time and the appearance of activation antigens on the surface of platelets present in the bleeding time blood were also measured. Before the eu- and hyper-glycaemic clamps, fasting blood glucose was 7.5 ± 1.3 mmol/L (135 ± 23 mg/dL) and 7.7 ± 1.4 mmol/L (142 ± 26 mg/dL), respectively; glycaemic levels were kept at ± 0.3 mmol (± 5 mg/dL) of the desired level throughout the clamps. In both hyper- and euglycemic studies plasma insulin was maintained at the physiological post-prandial concentration (~ 50 mU/mL). The expression of P-selectin on circulating platelets was not changed after the eu- and hyperglycaemic clamp while in the bleeding time blood it showed a significant increase after the hyperglycaemic clamp (post vs pre: 1st min = $+42.7 \pm 14.7\%$; 2nd min = $+87.1 \pm 22.2\%$; 3rd min = $+137.4 \pm 45.6\%$; $p < 0.05$), but not after the euglycaemic clamp (post vs pre: 1st min = $+23 \pm 17.5\%$; 2nd min = $-24.4 \pm 41.5\%$; 3rd min = $-72.3 \pm 50.3\%$). Shear stress induced platelet activation was not affected by the euglycaemic clamp while it increased significantly after hyperglycaemic clamp (closure time = 49.4 ± 1.9 sec. vs 41 ± 1.8 , $p < 0.005$; platelets retained 20-40 sec = $81.9 \pm 3.5\%$ vs $87.1 \pm 3.2\%$, $p < 0.005$). Finally, plasma vWF decreased slightly after euglycaemic clamp (pre $114.7 \pm 14.9\%$, post $77.5 \pm 6.3\%$ $p < 0.05$) while it increased strikingly after the hyperglycaemic clamp (pre $99.2 \pm 13.2\%$, post $161.2 \pm 11.7\%$ $p < 0.01$). In conclusion, our data demonstrate that acute, short-term hyperglycemia induces an increased activation of platelets exposed to high shear stress conditions *in vitro* (filtration

method) or *in vivo* (bleeding time). This effect is probably largely related to the increased levels of vWF in the circulation. Acute, short term hyperglycaemia in type II diabetics may precipitate vascular occlusions by facilitating platelet activation.

ORAL COMMUNICATIONS Vascular and cellular haemostasis

CO-028 FLUVASTATIN REDUCES AORTIC TISSUE FACTOR EXPRESSION IN CHOLESTEROL-FED RABBITS

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Pathological studies performed both in animals and in human have identified tissue factor (TF), associated with cells and the extracellular debris within the atherosclerotic plaque, as a candidate molecule responsible for the thrombogenicity associated with plaque rupture. A variety of antithrombotic strategies based on the inhibition of thrombin formation and/or activities are presently under evaluation. We have previously shown that several HMG CoA reductase inhibitors, e.g. fluvastatin and simvastatin, are able to downregulate the expression of tissue factor in human endothelial cells and macrophages. The effect of these drugs is prevented by mevalonate and by geranylgeraniol, indicating that an intact isoprenoid pathway is required for TF expression. In order to investigate whether the protective effect of the drugs is *in vivo* present also, we examined the effect of 4 week- fluvastatin treatment on TF expression in the vessel wall as well as in circulating monocytes of cholesterol-fed rabbits. Two groups of New Zealand male rabbits were fed a 1% cholesterol diet for 4 weeks (HC). One of the group received fluvastatin 5 mg/kg/day together with the hypercholesterolaemic diet (F-HC). At sacrifice, blood was withdrawn in order to test TF activity of mononuclear cells and the aorta was processed for immunohistochemical studies. Crosssections of aortic arch of rabbits fed a 1% cholesterol diet revealed intimal thickening which was significantly reduced in F-HC rabbits. Immunostaining for TF was pronounced in the intima as well as in the media and adventitia of HC rabbits. Staining of adjacent sections for RAM11 revealed the presence of a pronounced amount of macrophages in the intimal layer as well as in the media. Significant reductions in the TF positive areas were observed in F-HC rabbits, which derived from smaller TF-positive areas in the intimal and media layers, with no change in that of the adventitia. Similarly, a significant reduction in the RAM11 positive area was observed. No effect of fluvastatin was observed on TF expression in circulating monocytes isolated from either HC or F-HC rabbits.

CO-029**TISSUE FACTOR EXPRESSION AND APOPTOSIS INDUCED BY OXIDISED LOW DENSITY LIPOPROTEINS ON HUMAN UMBILICAL ENDOTHELIAL CELLS ARE PREVENTED BY ANTIOXIDANTS**

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The oxidative modification of low density lipoproteins (LDL) and endothelial expression of tissue factor (TF) and fibrinolytic proteins are key events in the pathogenesis of atherosclerosis. In this study we evaluated the effect of oxidised LDL on the expression of TF, plasminogen activator inhibitor type 1 (PAI-1) on human umbilical vein endothelial cells (HUVEC). The hypothesis that oxidised LDL functions as a pro-oxidant signal was also evaluated by studying the effect of different radical-scavenging antioxidants on the expression of these proteins. Exposure of LDL to HUVECs in the presence of 2.5 μM Cu^{2+} for 16 hours resulted in a dose-dependent increase in TF, PAI-1 expression with a maximal effect at 50 $\mu\text{g}/\text{mL}$ of LDL. PAI-1 secretion increased two fold after 8 hours incubation, whereas TF expression required 16-18 hours (five fold increase) following MAP kinase activation. The increase of TF is concomitant with the appearance of an apoptotic process, as assessed by TUNEL and cytofluorimetric analysis. Pretreatment with different antioxidants (vitamin E 100 μM and probucol 50 μM) prevented TF expression together with the apoptotic phenotype, whereas PAI-1 release was not affected. The ability of antioxidants to reduce TF expression was further confirmed in experiments performed in HUVECs incubated for 6 hours with 5 ng/mL $\text{TNF}\alpha$. $\text{TNF}\alpha$ -induced TF expression (10 fold stimulation) was reduced by 50% by vitamin E (100 μM). These *in vitro* findings suggest that oxidised LDL can induce the expression of different proteins and processes involved in the pathogenesis of atherosclerosis in endothelial cells. In addition procoagulant protein and apoptotic phenomena can be prevented by pretreating either the LDL or the cells with radical-scavenging antioxidants.

CO-030**PROCOAGULANT AND FIBRINOLYTIC PROPERTIES OF ENDOTHELIAL CELLS ARE MODULATED BY DEFIBROTIDE**

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Defibrotide (DF), a polydeoxyribonucleotide derived from mammalian tissues, has proven effective in resolving (30%) severe hepatic veno-occlusive disease (VOD), a life-threatening complication of high dose chemo/radiotherapy regimens for stem cell transplantation. VOD is characterised by endothelial injury, inflammation and fibrosis leading to occlu-

sion of small hepatic venules. To understand the mechanisms of the beneficial effects of DF in VOD, we evaluated the impact of DF on some endothelial cell (EC) hemostatic properties. Specifically, we studied the effect of DF on the expression of tissue factor (TF) and fibrinolytic proteins (PAI-1 and t-PA) by EC obtained from macrovascular (HUVEC) and microvascular (HMEC-1 cell line) beds. We compared the response of the two EC types to DF in the presence or absence of a proinflammatory stimulus, i.e. LPS. HUVEC and HMEC-1 were treated with DF (25-200 $\mu\text{g}/\text{mL}$) \pm 10 $\mu\text{g}/\text{mL}$ LPS at 37°C. After 4 h incubation, cell lysates were tested for: a) TF activity (TF:Act), measured by the recalcification assay of normal human plasma [results expressed as rabbit brain thromboplastin units (URBT)/10⁵cells], and b) TF antigen (TF:Ag), measured by ELISA (results expressed as pg/10⁵cells). Conditioned media, collected for up to 72 h incubation, were used to quantify PAI-1 and t-PA antigens (PAI-1:Ag and tPA:Ag) by ELISA, and t-PA activity (t-PA:Act) by chromogenic assay. The results (mean \pm SD) show that DF alone did not significantly influence TF expression by both EC; whereas DF was able to affect LPS-induced TF:Act expression in HMEC-1 only, in a dose-dependent fashion. At the dose of 200 $\mu\text{g}/\text{mL}$, TF:Act of HMEC-1 was 44.7% reduced compared to LPS alone (DF+LPS vs LPS: 41.63 \pm 7.12 vs 75.34 \pm 10.1 URBT/10⁵ cells, $p < 0.01$). Similar results were obtained by TF:Ag assay (DF+LPS vs LPS: 344 \pm 45.4 vs 479 \pm 80.8 pg/10⁵ cells, $p < 0.05$). The results of fibrinolysis assays show that DF counteracted LPS-induced increase in PAI-1:Ag expression by both EC types in a dose- and time-dependent way: after 72 h incubation, 200 $\mu\text{g}/\text{mL}$ DF reduced by 25.7% and 46% PAI-1:Ag levels, in HMEC-1 and HUVEC, respectively. Differently, DF did not significantly affect t-PA:Ag levels both in the presence and absence of LPS, but inhibited the LPS-induced decrease in t-PA:Act expression by both EC types in a dose- and time-dependent way. In conclusion, this study shows that DF can interfere with TF expression at the microvascular site, and reduces the antifibrinolytic action of LPS on both macro- and microvascular EC. These phenomena suggest a protective role of DF against proinflammatory insults on vascular endothelium.

CO-031**RETINOIDS MODULATE THE HAEMOSTATIC PROPERTIES OF MACRO- AND MICRO-VASCULAR ENDOTHELIAL CELLS**

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All-trans retinoic acid (RA) is a cyto-differentiating agent that induces complete remission in patients with acute promyelocytic leukaemia, and resolves the severe coagulopathy of this disease. RA exerts multiple actions on haemostasis. Many of the effects of RA are mediated via specific nuclear receptors (RAR α , β and γ). To characterise the interaction of RA with endothelial cells (EC) better, in this study we evalu-

ated the effects of RA and three selective RAR α -, β - and γ -agonists (Am580, CD2019 and CD437, respectively) on the fibrinolytic and procoagulant properties of macrovascular [human umbilical vein EC (HUVEC)] and microvascular EC [human microvascular EC line-1 (HMEC-1)]. EC were treated for up to 24 h with retinoids (from 10⁻⁹ to 10⁻⁶M) in the presence or absence of TNF α (100 U/mL). Conditioned media were used to quantify PAI-1 and t-PA antigens (PAI-1:Ag and t-PA:Ag) by ELISA, and t-PA activity (t-PA:Act) by chromogenic assay. Cell lysates were tested for: TF activity (TF:Act), measured by the recalcification assay of normal human plasma; and TF antigen (TF:Ag), measured by ELISA. The results show that RA treatment significantly ($p < 0.05$) increased t-PA:Ag in a dose-dependent fashion both in HUVEC and HMEC-1. Similar effects were obtained with Am580, while CD2019 had minor and CD437 no effects on both cell types. RA, Am580 and CD2019 were also able to counteract the TNF α -induced decrease in t-PA:Ag in HUVEC and HMEC-1. In addition, in both EC types, RA and Am580 significantly ($p < 0.05$) increased the t-PA:Act and prevented the decrease in t-PA:Act induced by TNF α . PAI-1:Ag levels were slightly increased in HMEC-1 by all retinoids; no significant effects were observed in HUVEC. Further, retinoids did not modify the TNF α induced-increment of PAI-1:Ag either in HMEC-1 or HUVEC. Analysis of procoagulant activity showed that in both HUVEC and HMEC-1, RA and Am580 significantly inhibited TNF α -induced TF expression (both measured as TF:Act and as TF:Ag), whereas CD2019 and CD437 were less effective. In conclusion, this study demonstrates that RA increases the antithrombotic potential of both macro- and microvascular EC, by inducing t-PA, without affecting PAI-1, and by inhibiting TNF α -induced TF expression. The effects of Am580 and CD2019 on the same parameters suggest a major role of RAR α and RAR β receptors in these phenomena.

CO-032

ENHANCED RELEASE OF HYDROXYL RADICAL FROM LEUKOCYTES OF PATIENTS WITH HYPERCHOLESTEROLAEMIA

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Patients with hypercholesterolaemia show evident oxidative stress but the underlying mechanism has never been investigated. We developed a new method to assess the leukocyte release of hydroxyl radical (OH^o) in whole blood and evaluated whether OH^o release is enhanced in subjects with elevated serum cholesterol. Leukocyte OH^o release was measured in 19 subjects with serum cholesterol >240 mg/dL and in 19 subjects matched for sex and age, with serum cholesterol <240 mg/dL. Leukocyte OH^o release was evaluated by an HPLC analysis of 2,3 and 2,5 dihy-

droxybenzoates, two stable compounds deriving from salicylic acid interaction with OH^o. Leukocyte OH^o release was measured upon citrated whole blood stimulated with N-formyl-methionyl-leucyl-phenylalanine. In order to verify whether a cause-effect relationship exists between cholesterol and leukocyte OH^o release, 8 subjects with hypercholesterolaemia were given pravastatin 40 mg/daily, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, for 8 weeks. Patients with hypercholesterolaemia showed a significantly higher leukocyte OH^o release ($p = 0.0004$) compared with control. A significant direct correlation was observed between leukocyte OH^o release, serum total cholesterol (Rho=0.71; $p = 0.0001$) and LDL-cholesterol (Rho=0.69; $p = 0.00008$). In those subjects treated with pravastatin, we found a significant decrease of leukocyte OH^o release (-63%; $p = 0.003$) coincidentally with a significant decrease of serum total- and LDL-cholesterol -23%; $p = 0.0005$ and -29%; $p = 0.0001$). *In vitro* studies excluded a direct antioxidant effect of pravastatin. This study shows that in hypercholesterolaemic patients oxidative stress may be dependent upon enhanced release of oxygen free radicals by leukocytes.

CO-033

SIMVASTATIN INHIBITS THE MONOCYTE EXPRESSION OF PRO-INFLAMMATORY CYTOKINES IN PATIENTS WITH HYPERCHOLESTEROLAEMIA

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Simvastatin, an inhibitor of 3-hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, reduces cardiovascular events in patients with myocardial infarction and hypercholesterolaemia. As it has been suggested that this effect could be due to an anti-inflammatory activity, we tested this hypothesis in patients with hypercholesterolaemia. Sixteen patients with polygenic hypercholesterolaemia were randomly allocated to diet ($n = 8$) or diet plus 20 mg/day simvastatin ($n = 8$) for 8 weeks. Before and at the end of the treatment period lipid profile and monocyte expression of tumor necrosis factor α and interleukin 1 β were measured. At baseline no difference in lipid profile or monocyte expression of tumor necrosis factor α and interleukin 1 β was observed between the two groups. In patients allocated to diet alone no change in lipid profile or monocyte expression of tumor necrosis factor α and interleukin 1 β was seen. In patients with diet plus simvastatin significant decreases of total cholesterol (-27%, $p < 0.02$), low density lipoprotein-cholesterol (33%, $p < 0.02$) and monocyte expression of tumor necrosis factor α (-49%, $p < 0.02$) and interleukin 1 β (-35%, $p < 0.02$) were observed. At the end of the treatment period patients treated with simvastatin had lower cholesterol and monocyte tumor necrosis factor α and interleukin 1 β than patients assigned to diet alone.

This study suggests that simvastatin possess anti-inflammatory activity *via* the inhibition of proinflammatory cytokines tumor necrosis factor α and interleukin 1 β expressed by monocytes.

CO-034

POLYMORPHONUCLEAR ADHESION TO IL-1 β -ACTIVATED HUVEC: E-SELECTIN STIMULATES TYROSINE KINASE-DEPENDENT ADHESIVENESS OF β 2-INTEGRINS

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Selectins and β 2-integrins co-operate in an adhesion cascade that allows polymorphonuclear (PMN) recruitment at the site of inflammation and thrombosis. Using soluble recombinant E-selectin, E-selectin-transfected CHO cells (CHO-E) and HUVEC, we investigated the role of E-selectin in promoting β 2-integrin-dependent homologous PMN aggregation, as well as adhesion to CHO-E and HUVEC. A double colour cytofluorimetric assay was used to evaluate the formation of mixed cell conjugates in sheared (1000 rpm) cell suspensions (PMN:HUVEC or CHO ratio=5). In parallel, the extent of PMN recruitment in homologous or mixed cell conjugates was evaluated by optical count of non-adherent PMN. Soluble E-selectin-IgG-chimera dose-dependently stimulates homologous PMN aggregation: 50% of PMN formed aggregates after 2 min of shear in the presence of 20 μ g/mL of E-selectin. PMN aggregation triggered by E-selectin was blocked by an antibody against β 2-integrins and was accompanied by tyrosine phosphorylation of a prominent protein of 110 kD (P110). The latter effect was significantly decreased by β 2-integrin blockade. A specific inhibitor of src tyrosine kinases, PP1, dose-dependently (1-20 μ g/mL) inhibited PMN aggregation as well as P110 phosphorylation triggered by soluble E-selectin. Similarly to the soluble protein, E-selectin expressed on CHO cells promoted a β 2-integrin-dependent PMN recruitment. After 2 min of shear 25 \pm 1.5% of PMN adhered to CHO-E by a β 2-integrin-dependent mechanism, as indicated by complete inhibition elicited by anti- β 2-integrin antibodies. Phosphorylation of P110 accompanied PMN adhesion to CHO-E cells and PP1 blocked both P110 phosphorylation and PMN recruitment on CHO-E cells. The effect of PP1 suggests that a src kinase-dependent pathway is involved in β 2-integrin function triggered by E-selectin. PMN did not adhere to resting HUVEC while they did so when HUVEC were prestimulated for 4 h with rIL-1 β . After 2 min of shear, mixed cell conjugates involved 82 \pm 2% of HUVEC and 76 \pm 1% of PMN. PMN recruitment on HUVEC was reduced to 15 \pm 2% and 30 \pm 3% of the control by an anti-CD18 or a combination of anti-CD11a and CD11b antibodies, respectively. PMN

adhesion to HUVEC was accompanied by tyrosine phosphorylation of P110. Pretreatment of PMN with the non-specific tyrosine kinase inhibitor genistein or with PP1 prevented both P110 phosphorylation and PMN recruitment on HUVEC. In the presence of 25 μ M PP1, PMN recruitment was reduced to 30 \pm 6.4% of the control. This study demonstrates that E-selectin promotes src-kinase-dependent adhesiveness of the β 2-integrins and suggests that this mechanism may be relevant for PMN recruitment by IL-1 β -activated HUVEC.

CO-035

TISSUE FACTOR SYNTHESIS BY ACTIVATED MONOCYTES AND ENDOTHELIAL CELLS IS UPREGULATED BY PENTRAXIN PTX3

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PTX3 is a novel inflammatory acute phase reactant, which belongs, together with C-reactive protein and serum amyloid P component, to the family of the pentraxins. PTX3 is synthesised by monocytes and endothelial cells following exposure to agents such as IL-1 β , TNF- α and bacterial endotoxin (LPS). Monocytes and endothelial cells, exposed to a variety of agents, synthesise tissue factor (TF), the transmembrane glycoprotein that, upon binding to activated factor VII, initiates the coagulation cascade. We decided to evaluate whether PTX3 could modulate TF in human monocytes and endothelial cells. For this purpose, mononuclear cells (MN), obtained from peripheral blood of healthy donors, were incubated with highly purified PTX3. At the end of incubation, cells were disrupted by 3 cycles of freeze and thaw and procoagulant activity was assessed by a one-stage clotting time. In basal conditions, MN, with or without PTX3, do not express any procoagulant activity. By contrast, PTX3 enhances procoagulant activity from MN stimulated by LPS. The activity is attributable to TF, since it is completely abolished by an anti-TF monoclonal antibody. The enhancement is dose and time-dependent: a peak is reached within 6 hours with a concentration of 5 μ g PTX3/mL. The amplification in activity is paralleled by an increase in TF antigen, as assessed by ELISA. The enhancement of TF activity requires mRNA synthesis, as assessed by reverse transcriptase PCR, and translocation of the transacting factor c-Rel/p65 into the nucleus, as determined by EMSA (electro mobility shift assay). The increase in activity is specific for LPS, since in the presence of other TF-inducing agents, such as IL-1 β , and TNF- α , PTX3 is not effective. To investigate whether PTX3 was effective also on endothelial cells, human umbilical vein endothelial cells (HUVEC) were incubated with LPS in the presence or absence of purified PTX3. Similarly to MN, PTX3 enhanced TF expression induced by LPS in

a dose-dependent way. In contrast with the results obtained with MN, PTX3 was effective also when IL-1 β and TNF- α were used instead of LPS, suggesting a different regulatory mechanism for these cells. A dependence upon mRNA synthesis and c-Rel/p65 translocation was observed in all instances. These results suggest a novel biological role for PTX3: this molecule could modulate cell-mediated fibrin deposition which is a common feature of the inflammatory response.

CO-036

ANGIOTENSIN II UPREGULATES TISSUE FACTOR EXPRESSION BY HUMAN MONOCYTES

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We have recently shown that angiotensin converting enzyme (ACE) inhibitors downregulate the synthesis of tissue factor (TF), the essential cofactor for the initiation of blood coagulation, in monocytes. TF plays a leading role in arterial thrombosis which occurs following atherosclerotic plaque fissuring and can be expressed by monocytes following stimulation by several agents. The TF-inhibitory action of ACE inhibitors could, at least in part, explain their efficacy in reducing the rates of death, myocardial infarction, and stroke in patients with high-risk factors for cardiovascular disease. In addition to TF, monocytes express several components of the renin-angiotensin system (RAS), which could be involved in TF modulation. Because losartan, a competitive inhibitor of the angiotensin II (Ang II) receptor AT1, reduces TF synthesis at a rate similar to that exerted by ACE inhibitors, we decided to investigate the possibility that Ang II could play a role in monocyte TF expression. To test this hypothesis, mononuclear leukocytes (MN), obtained from healthy volunteers by Lymphoprep sedimentation, were incubated for different time intervals at 37°C with or without endotoxin (LPS), in the presence or in the absence of Ang II. At the end of incubation, cells were disrupted by 3 cycles of freeze-thaw, and tested for procoagulant activity by a one-stage clotting assay. Exposure of MN to LPS resulted in synthesis and expression of TF, while Ang II by itself could not induce TF synthesis. By contrast, the LPS-induced TF activity was significantly enhanced (2-3 fold) when Ang II was present during the incubation. The effect is dose and time-dependent: a peak is reached within 6 hours with a concentration of 10 μ g Ang II/mL. Preliminary results indicate that translocation of the transacting factor c-Rel/p65 into the nucleus is required, as determined by EMSA (electro mobility shift assay). Because Ang II was not an inducer and required LPS in order to affect TF expression, we hypothesised that LPS could induce AT1 synthesis or activation of an inactive receptor in monocytes. To answer this question,

reverse transcriptase PCR experiments were performed. Resting MN expressed the Ang II mRNA expected band. LPS stimulation did not affect Ang II mRNA synthesis, suggesting that LPS was responsible for a change in affinity of AT1 for its ligand. Radioreceptor assays are currently underway to corroborate this hypothesis. These data indicate a thrombogenic role for Ang II, supporting the usefulness of ACE inhibitors for anti-ischaemic purposes, and suggesting a cardioprotective role for AT1 receptor antagonists as well.

ORAL COMMUNICATIONS

Hyperhomocysteinaemia

CO-037

CYSTEINE LEVELS ARE A RISK FACTOR FOR VENOUS AND ARTERIAL THROMBOSIS AND ARE NOT AFFECTED BY METHIONINE LOADING

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Preliminary data indicate that cysteine (Cy) levels are higher in patients with venous thrombosis but no data are available about their role in arterial thrombosis. In order to investigate this issue we assayed Cy and homocysteine (Hcy) levels in 150 patients with venous and arterial thrombosis in fasting state and after methionine loading and we compared them with 100 healthy subjects as controls. Cy and Hcy plasma levels were determined by HPLC with fluorimetric detection. Cy and Hcy levels were significantly higher in patients than in controls (Cy: 220 mmol/L (100-341) vs 198 mmol/L (99-331); $p < 0.01$ / Hcy: 16.0 mmol/L (9-69) vs 7.5 mmol/L (1-28.5); $p < 0.001$). After the oral methionine loading Cy levels were not significantly different than in the fasting state either in patients or in control subjects (Pts: 213.5 mmol/L (72-368); Ctrl: 195 mmol/L (100-341). Hypercysteinaemia and hyperhomocysteinaemia were defined at the 95th percentile of the control group: we calculated odds ratios adjusted for age and sex. Out of 150 patients 25 (17%) had Cy levels above 311 mmol/L and 7 (4.6%) had post-methionine Cy levels above 300 mmol/L compared with 5% in the control group. The adjusted odds ratio was 3.7 (95% C.I. 1.4 - 9.9; $p < 0.005$) for fasting Cy levels and 0.93 (95% C.I. 0.3-2.8; ns) for post-methionine cy levels. Out of the 150 patients 60 (40%) had fasting Hcy levels above 18.3 mmol/L and post-methionine Hcy levels above 31.9 mmol/L compared with 5% in the control group. The adjusted odds ratio was 9.0 (95% C.I. 4.0-20.0; $p < 0.0001$) for fasting Hcy levels and 14.8 (95% C.I. 6.6-33.1; $p < 0.0001$)

for post-methionine Hcy levels. After correction for each other the odds ratios for fasting hypercysteinaemia was 3.0 (95% C.I. 1.1-8.1; $p < 0.05$) in the fasting state, 0.98 (95% C.I. 0.2-3.9; ns) after methionine loading; the odd ratio for hyperhomocysteinaemia was 8.1 (95% C.I. 3.6-18.1; $p < 0.0001$) in the fasting state and 12.1 (95% C.I. 6.0-24.5; $p < 0.0001$) after methionine loading. Thirteen patients and 2 control subjects had both elevated Cy and Hcy plasma levels (OR 4.4; 95% C.I. 1.0-20.0; $p < 0.05$). Our data demonstrate that elevated Cy levels play a role as a risk factor for arterial thrombosis and confirm that they are a risk factor for venous thrombosis.

CO-038

EVALUATION OF METHODS FOR HOMOCYSTEINE MEASUREMENT: RESULTS OF A MULTICENTRE STUDY

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After the demonstration that moderate hyperhomocysteinaemia is associated with thrombosis, many haematological laboratories are becoming interested in total homocysteine (tHcy) measurement. This prompted us to organise a collaborative study to investigate the performance of methods used in this setting. Two pairs of pooled plasma (pools) were prepared at the co-ordinating Centre (A-B and C-D). tHcy levels were normal in pools A-B and high in pools C-D. The concentrations of tHcy were similar but not identical within each pair. Aliquots were taken from each pool to prepare sets of 100 samples (coded from 1 to 100). Each set consisted of 25 replicates for each pool. Samples were frozen at -70°C and shipped in dry ice to 16 laboratories with sets of common frozen aqueous standards. Participants were asked to measure (in blind) tHcy with their methods and standards. Measurements had to be performed in 5 sessions, each including 20 samples in a random order. Results were sent to the coordinating Centre both as raw readings and as tHcy concentrations. The following methods were used: high pressure liquid chromatography (HPLC)-derived methods in 12 laboratories (home made in 10 and commercial in 2); enzyme immuno assays (EIA) in 4 laboratories (manual in 2 and automated in 2) and capillary electrophoresis (CE) in one. One laboratory used 2 methods. Results for paired pools (A-B and C-D) were analysed by the t test to assess for the ability to discriminate similar but not identical concentrations. Results for each pool were used to assess within-laboratory reproducibility and between-laboratory comparability. All laboratories, except 1 using CE and 2 using home-made HPLC, were able to discriminate similar tHcy levels in the normal range (pool A-B). Ten laboratories (4 using home-made HPLC, 2 commercial HPLC, 2 automated EIA, 1 manual EIA and 1 CE) were able to discriminate similar high tHcy levels (pool C-D). Within-laboratories

reproducibility expressed as CV ranged from 2.0% to 27.2% (home-made HPLC); from 7.5% to 12.8% (commercial HPLC); from 13.7% to 30.0% (manual EIA); from 2.1% to 4.0% (automated EIA) and from 9.3 to 22.7% (CE). Between-laboratories comparability expressed as CV was 14.1% and 13.9% for pools A and B and 15.6% and 14.5% for pools C and D. These values were considerably lower (CV values $< 6.8\%$) when a common plasma standard was used for calculation of tHcy concentration, while the use of a common aqueous standard failed to achieve the necessary harmonisation. In conclusion, performance characteristics of the EIA-based automated method compare favourably with the well-established HPLC-based methods. It is simpler and more suitable for use in general haematological laboratories. Comparability of results in different laboratories is still a problem. The establishment of an international plasma standard would be of help to standardise tHcy measurement across laboratories.

CO-039

LOW PLASMA LEVELS OF VITAMIN B6 ARE INDEPENDENTLY ASSOCIATED WITH AN ELEVATED RISK OF DEEP-VEIN THROMBOSIS

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Background. 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). **Aim.** In the present case-control study we investigated whether plasma levels of B vitamins that are involved in Hcy metabolism are associated with an elevated risk of DVT. **Methods.** We compared 397 patients with DVT of the lower extremity (M/F, 194/203; median age, 42y, range 10-80y) with 585 healthy controls (M/F, 243/342; 44y, 13-77y). The plasma levels of folate, vitamin B12 and vitamin B6 were measured. The following risk factors for DVT were also looked for: resistance to activated protein C; antiphospholipid syndrome; deficiencies of antithrombin, protein C, protein S; high fasting and PML plasma levels of tHcy; mutations G1691A of the factor V gene and G20210A of the prothrombin gene. No patient had overt neoplastic, liver, renal or autoimmune disease. **Results.** The prevalence of high fasting levels and/or PML increases of tHcy was higher in cases (67/391, 17%) than controls (48/583, 8%; OR 2.3, 95% CI: 1.5-3.6). The mean (\pm SD) plasma levels of folate, B12 and B6 were 6.5 ± 3.8 ng/mL, 430 ± 224 pg/mL and 36.5 ± 34.6 nmol/L in patients and 6.7 ± 3.5 , 420 ± 202 and 40 ± 33.5 in controls ($p > 0.05$). Fasting levels and PML increases of tHcy correlated negatively with vitamins. The odds ratios (95% CI) for DVT associated with quartiles (Q) of vitamin levels (adjusted for age, sex, fasting and PML tHcy, other vitamins, other risk factors of DVT) were:

	Q1	Q2	Q3	Q4
Folate	0.7 (0.5-1.2)	0.6 (0.4-0.9)	0.8 (0.5-1.2)	1
Vitamin B12	0.8 (0.5-1.2)	0.7 (0.5-1.1)	0.8 (0.5-1.2)	1
Vitamin B6	1.8 (1.2-2.8)	1.9 (1.3-3.0)	1.0 (0.6-1.6)	1

Conclusion. The study confirms that high tHcy plasma levels are associated with an elevated risk for 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 of DVT.

CO-040 MEASUREMENT OF TOTAL HOMOCYSTEINE LEVELS IN PLASMA: COMPARISON OF THE IMX IMMUNOASSAY WITH AN ESTABLISHED HPLC-METHOD

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Objective. To compare the performance of a commercially available IMx immunoassay with that of a reversed-phase HPLC-method for plasma total homocysteine (tHcy) measurement. **Methods.** The levels of tHcy before and after an oral methionine loading (PML) were measured in 135 healthy subjects and 39 patients with previous episodes of arterial or venous thrombosis. The IMx method is based on fluorescence polarisation immunoassay (FPIA) technology. The HPLC-method includes derivatisation with ABD-F and post-column fluorescence detection. **Results.** There was a good correlation between the measurements obtained with the two methods ($r^2=0.96$, $p<0.05$). The mean levels of tHcy measured with the IMx immunoassay tended to be slightly higher than those with the HPLC-method both in the fasting state (mean difference = 0.8 mmol/L) and PML (5.3 mmol/L), but the PML difference only was statistically significant ($p<0.001$). The imprecision was very low for both methods: the within-run coefficient of variations (CV) were 2.1% and 1.2%, for basal and PML values, with the HPLC method, and 1.9% and 1.4% with the IMx immunoassay; the between-run CV were 4.6% and 2.7% with HPLC and 2.5% and 2.2% with the IMx immunoassay. In the recovery experiments, the equations of the regression analysis of expected (y) vs observed (x) tHcy levels were $y=-2.31 + 1.05x$, $r^2=0.998$ for HPLC and $y=-0.98 + 1.01x$, $r^2=0.998$ for the IMx immunoassay. The frequency of thrombotic patients with fasting tHcy levels >95th centile of normal distribution was 38% with HPLC and 35% with IMx; the same frequencies for PML tHcy increments above fasting levels were 69% with HPLC and 58% with IMx. **Conclusion.** The IMx method for tHcy measurement compares well with an established HPLC-method. It could, therefore, be used for routine tHcy measurement.

CO-041

INTERMEDIATE HYPERHOMOCYSTEINAEMIA AND THE 844INS68 MUTATION OF THE CYSTATHIONINE β -SYNTHASE (CBS) GENE

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Hyperhomocysteinaemia (HHcy) has been reported to be a graded risk factor for arterial and/or venous thrombosis. However, a major determinant of HHcy, the thermolabile methylenetetrahydrofolate reductase (MTHFR) mutation, is not consistently associated with an increased risk of thrombosis. The Hordaland Homocysteine Study showed that the thermolabile MTHFR mutation is a major determinant of intermediate HHcy (tHcy ≥ 40 mmol/L) in the presence of marked reductions in folate and vitamin B12 plasma levels. However, the interaction of thermolabile MTHFR with folate levels – as observed in the control population – did not account for the tHcy levels observed in subjects with intermediate HHcy. We investigated whether the association of thermolabile MTHFR with the CBS 844ins68bp insertion might be responsible for intermediate HHcy in the Hordaland population. The table shows Mantel-Haenszel odds ratios (M-H OR, stratified for gender) for intermediate HHcy contributed by MTHFR/CBS genotypes in 69 subjects with intermediate HHcy and in 314 age-matched controls.

MTHFR	CBS 844	Intermediate	Controls	M-H OR	95% C.I.
677TT	ns68	HHcy cases			
-	-	15 (21.7%)	258 (80.9%)	1	-
-	+	4 (5.8%)	31 (9.7%)	2.13	0.45-10.2
+	-	43 (62.3%)	29 (9.1%)	25.9	14.2-47.4
+	+	7 (10.1%)	1 (0.4%)	85.8	27.0-272.4

Gender, age, folate, vitamin B12, the MTHFR/CBS genotype, and the interaction of the MTHFR genotype with folate explained 35% of the variation in fasting tHcy levels in control subjects. In subjects with intermediate HHcy, 13% of the variation in tHcy levels was explained by gender and vitamin B12 levels. Low folate and vitamin B12 levels ($p=0.0001$), MTHFR thermolability ($p=0.0001$) and association of the latter with the CBS insertion ($p=0.004$) distinguished subjects with intermediate HHcy from control subjects. These data confirm previous findings that the CBS 844ins68 mutation is a major determinant of fasting HHcy – and a risk factor for thrombosis – only when associated with MTHFR thermolability.

CO-042**DETERMINANTS OF POST-METHIONINE LOAD TOTAL HOMOCYSTEINE LEVELS**

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We evaluated total homocysteine (tHcy) levels 2 and 6 hours after an oral methionine load (D-L methionine, 0.1g/Kg b.w.) in 284 patients with a history of venous and/or arterial thromboembolism screened for fasting tHcy, MTHFR thermolability (677TT), and the 844ins68 insertion of the cystathionine β -synthase (CBS) gene. Plasma vitamin B₁₂, B₆ and folate were also measured. Results of post-methionine load tHcy determinations were expressed as Δ PML at 2 and 6 h and as the area under curve (AUC = Δ PML(2h) + (Δ PML(2h) + Δ PML(6h)) \times 2). With respect to 95th percentiles in a control population of 70 subjects PML hyperhomocysteinaemia was detected in 6.8% (Δ PML(2h)), 14.0% (Δ PML(6h)), and 11.6% (AUC) of patients. tHcy levels according to the MTHFR and CBS genotypes are reported in the table.

	Nihil (n = 186)	CBS 844ins68 (n = 20)	MTHFR 677TT (n = 58)	MTHFR + CBS (n = 20)
Fasting	9.2 \pm 4.9	7.8 \pm 1.5	19.7 \pm 28.8	21.8 \pm 23.4
Δ PML(2h)	10.0 \pm 5.7	9.6 \pm 4.8	12.8 \pm 11.5	9.2 \pm 5.9
Δ PML(6h)	16.6 \pm 10.0	19.9 \pm 1.6	21.9 \pm 19.5	17.9 \pm 7.9
AUC	63.5 \pm 36.0	68.8 \pm 35.0	82.7 \pm 72.4	64.8 \pm 30.0

In addition to fasting tHcy, patients with MTHFR thermolability had PML tHcy levels higher ($p < 0.01$) than the remaining patients. However, fasting tHcy explained 6.5% of the variation in Δ PML(2h), 25.5% of the variation in Δ PML(6h) and 19.3% of the variation in AUC. Determinants of PML tHcy were evaluated in a generalised linear model including gender, age, genotype, vitamin levels and fasting tHcy as predictor variables. Only gender ($p < 0.02$) and fasting tHcy ($p < 0.0001$) were independent predictors of PML tHcy, explaining 15.4%, 31.7% and 26.0% of the variation in Δ PML(2h), Δ PML(6h) and AUC respectively. These data suggest that the gene abnormalities evaluated in this study may influence PML tHcy only as a result of their effects on fasting tHcy levels.

CO-043**USEFULNESS OF VITAMIN B6 DETERMINATION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN THE INTERPRETATION OF PLASMA HOMOCYSTEINE LEVELS**

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Vitamin B₆ as pyridoxal-5'-phosphate (PLP) is the coenzyme involved in the trans-sulphuration pathway of atherogenic amino acid homocysteine (Hcy). Little is known about the effect of vitamin B₆ deficiency on circulating homocysteine concentrations and whether a vitamin B₆ deficiency may contribute to an abnormal fasting tHcy and abnormal tHcy post-methionine loading (PML) test. In recent years a large number of methods have been developed for assay of B₆ vitamins in plasma by HPLC with post-column derivatisation, but they were unsuitable for routine clinical practice. The aim of this study was: 1) to develop a new assay for plasma vitamin B₆ determination by HPLC suitable for routine use; 2) to determine the influence of PLP, folates, vit. B₁₂ on tHcy and tHcy post-methionine loading (PML) in a cohort of free-living apparently healthy subjects in Northern Italy (Bologna). *Methods.* A pre-column derivatisation and separation by HPLC was employed. The eluted peaks were monitored by a fluorometric detector set at Ex 300 nm/ Em 400 nm. The PLP Rt was 3.03 min. Apparently healthy subjects (n=97; 53 men, 44 women, age range: 14-94 y) were selected from the general population in the area of Bologna, Italy. ML-test: L-methionine at the dose of 100 mg /Kg b.w. was administered orally in fruit juice. tHcy levels were measured according to Araki and Sako method (1987). Folate and vit. B₁₂ serum levels were measured by automated chemiluminescence assay (Chiron Diagnostics, East Walpole, MA, USA). *Results.* The intra-assay CV was 3% and the inter-assay CV was <5%. The plasma PLP limit of quantification was 5 nmol/L. The PLP values in healthy people were 30-80 nmol/L (10th and 90th percentiles; n=100). A significant negative correlation was found between between tHcy and folate, vit. B₁₂ and PLP. In a multivariate analysis vit. B₁₂ and PLP in women explained 15 % of PML variance. *Conclusions.* Simplicity, reproducibility and stability of utilised materials make our PLP method suitable for clinical routine. Multivariate analysis shows vitamin B₆ deficiency may contribute to an abnormal fasting tHcy and abnormal tHcy post-methionine loading (PML).

CO-044**ROLE OF EMERGING METABOLIC RISK FACTORS IN PREMATURE CORONARY ARTERY DISEASE**

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In young patients the evidence of atherosclerotic coronary artery disease (CAD) is not always discernible, so highlighting the potential importance of prothrombotic risk factors. New emerging haemostasis-related risk factors have been associated with CAD. Hyperhomocysteine (Hcy) is associated with a higher risk of cardiovascular events and high plasma levels of lipoprotein (a) (Lp(a)) confer an increased

risk of premature CAD. The aims of this study were: 1) to evaluate the prevalence of high Hcy and Lp(a) plasma levels in young patients with CAD; 2) to investigate the association between the C677T polymorphism in the gene coding for methylenetetrahydrofolate reductase (MTHFR) and Hcy in these patients. We studied 70 Italian patients (age: male <50, female <55 years) with angiographically documented CAD, when they first developed symptoms, and 70 apparently healthy subjects. Hcy plasma levels were significantly higher in patients than in controls both in the fasting state [12.1 mmol/L (1.9-45.8) vs 7.5 mmol/L (1-28); $p=0.00017$] and after a methionine loading test [36.6 mmol/L (9.7-66.6) vs 25.2 mmol/L (16.4-38.9); $p=0.00009$]. Hyperhomocysteine was diagnosed in 48/70 patients, with a prevalence of 68.7%. The Odds Ratio (OR) for CAD at young age associated with hyperhomocysteine was 7.8 (95% CI 2.13-28.7; $p=0.001$). This result was confirmed after adjustment for family history of CAD, hypertension, hypercholesterolaemia, smoking habit, and elevated Lp(a) plasma levels (OR 7.2 95% CI 1.5-33.1; $p=0.01$). In 21/70 (30%) patients we documented Lp(a) plasma levels >300 mg/L. Lp(a) plasma levels were not significantly higher than in controls. At univariate analysis the OR for CAD at young age associated with elevated Lp(a) plasma levels was 5.9 (95% CI 1.2-29; $p=0.03$), but this result was not confirmed (OR 0.60 95% CI 0.05-6.5; $p=0.5$) at multivariate analysis. Both Lp(a) and Hcy levels were elevated in 14/70 (66%) patients [OR for CAD at univariate analysis 10.9 (95% CI 1.4-84.7; $p=0.02$); this result was confirmed at multivariate analysis (OR 17.4 95% CI 1.6-183; $p=0.01$). The distribution of the MTHFR genotype was not different in patients and controls (patients: +/+ 27%; +/- 54%; -/- 19%; controls: +/+ 18%; +/- 56%; -/- 26%). In conclusion, our data indicate that in young patients with CAD, both fasting and post loading hyperHcy but not MTHFR polymorphism plays an important role as a prothrombotic risk factor, suggesting the need to investigate Hcy in all patients with CAD at young age. The meaning of high Lp(a) levels remains controversial, but the contemporary presence of elevated Lp(a) and Hcy levels has an additive effect as prothrombotic risk factors.

CO-045 PLASMA HOMOCYSTEINE AND VENOUS THROMBOEMBOLISM IN THE ACTIVE POPULATION

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Background. Several studies have suggested that increased levels of plasma homocysteine (Hcy) could be associated with venous thromboembolism (VTE). However, there are few population-based studies about the impact of hyperhomocysteinaemia of this disorder on the public health. **Aim of the study.** To demonstrate that hyperhomocysteinemia is a risk factor for thromboembolism in the general population. **Methods.** We performed a case-control study within

the VITA Project framework. In this study, carried on from 1993 to 1997 in a sample of 15,109 randomly recruited people aged 18-65 years, 116 subjects with VTE were identified by a validated methodology (questionnaire + Doppler ultrasound). Total plasma homocysteine (t-Hcy) was measured by HPLC with fluorescence detection using t-nitrobutylphosphine to generate free thiols followed by ABD-F derivatisation in 109 subjects with VTE and in 336 healthy controls randomly chosen from the same study population. Hyperhomocysteinaemia was defined as plasma tHcy levels above the 97.5 percentile of the control group. **Results.** Mean tHcy value was 8.8 $\mu\text{mol/L}$ (± 3.5 $\mu\text{mol/L}$ SD); the 97.5 percentile of tHcy in controls was 17.1 $\mu\text{mol/L}$. Men had significantly higher levels of homocysteine than women (9.9 \pm 4.1 $\mu\text{mol/L}$ vs 7.9 \pm 2.6 $\mu\text{mol/L}$, $p<0.01$); however, this difference did not require different reference intervals for men and women. Of the 336 controls, 12 (3.5 percent) had plasma tHcy levels above the 97.5 percentile of the control distribution. tHcy levels above the 97.5 percentile of the control distribution were observed in 7 subjects with thromboembolism (6.4 percent). After adjustment for sex and age, the odds ratio for VTE in hyperhomocysteinaemia was 2.2. **Conclusions.** We found that homocysteine is a risk factor for venous thrombosis in the general population, with a relative risk of VTE recurrence two times greater in subjects with hyperhomocysteinaemia than in those without hyperhomocysteinaemia.

ORAL COMMUNICATIONS Antiphospholipid antibody syndrome

CO-046 SOME PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME EXPRESS HITHERTO UNDESCRIBED ANTIBODIES TO CARDIOLIPIN-BINDING PROTEINS

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Contrary to infective anticardiolipin (aCL) antibodies, autoimmune aCL antibodies react with phospholipids (PL) mainly via binding to the plasma glycoprotein cofactor $\beta 2$ -Glycoprotein I ($\beta 2\text{GP1}$). Other antigenic targets of antiphospholipid antibodies comprise prothrombin, protein C, protein S, high-molecular weight kininogen and annexin V. While there is a well-documented link between the risk of thrombosis and the presence of $\beta 2\text{GP1}$ -dependent anticardiolipin antibodies, the pathological impact of other antiphospholipid antibodies is less clear. By means of cardiolipin affinity-chromatography, we

isolated and identified 3 CL-binding proteins, complement component C4, complement factor H and a kallikrein-sensitive glycoprotein, and tested for the presence of autoantibodies against these proteins in patients with antiphospholipid syndrome (APS), systemic lupus erythematosus (SLE) and other autoimmune diseases. High titers of autoantibodies to C4 as compared to age- and sex-matched healthy controls were present in 2 of 16 patients with APS, and weak titers were found in 2 of 16 patients with SLE and in none of 16 patients with other autoimmune diseases. Autoantibodies to complement factor H were found in 3 APS, 2 SLE and none of the other autoimmune patients. Autoantibodies to kallikrein-sensitive glycoprotein were detected in 5 APS patients, 1 SLE patient, and 1 patient with another autoimmune disease. A close relationship between these antibodies was found. This behaviour might be explained by the fact that autoantibodies often arise, in grouped or linked, from a common macromolecular complex. This hypothesis might be appropriate for the 3 described CL-binding proteins, given the observation that some patients, with APS syndrome, harbored antibodies against all 3. Although we do not yet understand the role of this new class of antibodies to PL-binding proteins and their clinical relevance, our data underline the fact that the family of 'antiphospholipid antibodies' is expanding. Systematic screening of these antibodies in a large number of patients with APS could provide useful information on their significance and utility in this clinical setting.

CO-047

ANTIBODIES TO TISSUE TYPE PLASMINOGEN ACTIVATOR IN PLASMA FROM PATIENTS WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME

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Background. A reduction of fibrinolysis has been described in association with thrombosis in primary antiphospholipid syndrome (PAPS). The presence of anti-tissue type plasminogen activator (t-PA) antibodies, which has never been evaluated in PAPS, could potentially induce fibrinolytic defects related to thrombosis. **Patients and Methods.** We measured anti t-PA antibodies and anti-fibrin-bound t-PA antibodies, as a possible cause of hypofibrinolysis, in 39 patients with PAPS compared with 39 controls matched for gender and age. We also evaluated the differences in anti t-PA antibodies between patients without previous thrombosis (20 patients) and patients with previous episodes of thrombosis (19 patients: deep vein thrombosis in 9, ischaemic stroke in 6, arterial leg thrombosis in 1, hepatic vein thrombosis in 1, thrombophlebitis in 1 and cerebral venous thrombosis in 1). Anti t-PA antibodies were measured by an enzyme linked immunosorbent assay

(ELISA) and anti t-PA fibrin-bound antibodies were measured by a solid phase-fibrin immunoassay (SOFIA). **Results.** Out of 39 patients with PAPS, three had high plasma levels of anti t-PA IgG measured by ELISA (12 U/mL; 18 U/mL and 36 U/mL respectively; [normal subjects = 4+2 U/mL]). These 3 patients had had a previous episode of thrombosis (2 patients with DVT and 1 patient with ischaemic stroke). Four other patients had positive results for antibodies directed against fibrin bound t-PA measured by SOFIA (9.5 mOD/min; 6.6 mOD/min; 6.1 mOD/min; 5.3 mOD/min [normal controls 3.3 + 0.9]); one patient had had a previous episode of DVT and one had thrombocytopenia. Patients with ischaemic stroke had higher plasma levels of anti t-PA IgG (6.5+1.9 U/mL; mean + SD) than patients without thrombosis (3.1+2.0 U/mL; p=0.027). **Conclusions.** Our data show that in patients with PAPS the highest levels of anti t-PA antibodies are present in subjects with previous thrombotic events. The discrepancy in the results obtained with two methods of detection of anti-t-PA antibodies, ELISA and SOFIA, indicates a different interaction of the antibodies with the t-PA molecules, which are directly bound to polystyrene plates in ELISA and bound to fibrin as a bridging molecule in SOFIA.

CO-048

ASSOCIATION BETWEEN ANTIPHOSPHOLIPID ANTIBODIES AND LYMPHOMAS: A CASE-CONTROL STUDY

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Hodgkin's and non-Hodgkin's lymphomas were registered in one prospective observational survey of patients with antiphospholipid (aPL) antibodies, even though they are not typical manifestations of the antiphospholipid syndrome. To establish the strength of this association, we performed a case-control study on 100 consecutive patients with different lymphomas. There were 58 males and 42 females, aged 15-86 years, median 58.5 years. Eighty patients suffered from non-Hodgkin's lymphoma and the other 20 from Hodgkin's disease. The control group was represented by 100 age- and sex-matched apparently healthy subjects, without a history of autoimmune, and neoplastic diseases. All cases and controls were analysed for the presence of aPL antibodies. Lupus anticoagulants (LA) were detected according to the criteria of the SSC Subcommittee of Standardisation of Lupus Anticoagulant/Phospholipid-Dependent Antibodies. IgG and IgM aCL antibodies were measured by ELISA; the results were expressed in GPL and MPL units. Values exceeding 15 GPL and MPL units were considered abnormal. Twenty-six per cent of patients and 8% of controls had evidence of aPL (p=0.0007, OR 4.04, 95% C.I. 1.73-9.95). LA was detected in 7 patients and 1 control (p=0.0304, OR 7.45, 95% C.I. 0.9-61.7); 23 patients had increased IgG aCL titres (one of them also had increased IgM aCL) vs 7 controls (p=0.015,

OR 3.97, 95% C.I. 1.62-9.75). aPL-positive patients were similar to aPL-negative cases with respect to age, sex, type and staging of lymphomas. During follow-up the 2 groups of patients did not differ in terms of response to chemo-radiotherapy, number of relapses or death; conversely, the incidence of thrombosis was significantly higher in aPL-positive patients (12% patients/year) than in aPL-negative cases (1.8% patients/year). One aPL-positive patient died before remission could be evaluated; all the other cases reached complete remission of lymphoma: at that time aPL antibodies had disappeared from all of them. In conclusion, aPL antibodies are associated with lymphomas. Their determination is useful to identify patients at high risk of developing thrombotic complications, but not to predict treatment outcome and disease prognosis.

CO-049

THE WAPS (WARFARIN IN ANTIPHOSPHOLIPID SYNDROME) STUDY: UPDATE AND PRELIMINARY RESULTS

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The WAPS study is a prospective, randomised clinical trial aimed at assessing the risk/benefit ratio of high-dose oral anticoagulation in patients with antiphospholipid antibodies (aPLs) and thrombosis. General design Patients with aPLs (lupus anticoagulant and/or moderate-high titres of anticardiolipin antibodies) and previous arterial or venous thrombosis will be randomised between high-dose warfarin (PT INR 3.0-4.0) (intervention group) or conventional therapy (aspirin for arterial thrombosis, low-dose warfarin, PT INR 2.0-3.0, for venous thrombosis) (control group). Asymptomatic patients and those with a absolute indication or contraindication to high-dose warfarin will be followed in a parallel observational arm. Current status. Nine countries (Italy, Norway, Poland, Argentina, Belgium, Bulgaria, France, Germany, Czech Republic) including 42 hospitals agreed to participate. So far 394 patients (M/F 109/285; median age 39 yrs. range 14-82) have been enrolled: 107 in the randomised and 287 in the observational arm. Reasons for excluding patients from the trial and including them in the observational arm were: asymptomatic: 120 cases; excessive bleeding risk: 51; absolute indication for high-dose warfarin: 44; refusal: 72. Median follow-up is now 12 months (range 0-27). Adherence of randomised patients to the prescribed therapeutic range Actual PT INR of the 107 randomised patients was assessed at 3, 6, 12 and 24 months of follow-up. Median (and range) values of patients randomised in the 'high-dose' group (assigned range 3.0-4.0) were 3.1 (1.7-4.4); 3.4 (2.3-4.9), 3.1 (1.7-5.0) and 3.1 (1.0-4.3), whereas in the 'conventional' group (assigned range 2.0-3.0) they were 2.3 (1.7-3.7), 2.4 (1.6-3.0) and 2.7 (1.6-3.9) and 2.5 (2.1-2.9), respectively. *Ad inter-*

im analysis of safety. An *ad interim* analysis of bleeding, thrombotic and fatal complications was carried out for safety reasons: 22 bleeding (incidence 6.88% pt-yr), 19 thrombotic (5.94% pt-yr) and 6 fatal events (1.8% pt-yr) have been registered in the total population of 394 enrolled patients. The type and incidence of complications was consistent with those expected. The end of the study is foreseen, three years after starting, in December 2000.

CO-050

SENSITIVITY AND SPECIFICITY OF LUPUS ANTICOAGULANTS AND ANTICARDIOLIPIN ANTIBODIES TOWARDS ARTERIAL AND VENOUS THROMBOSIS

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Arterial and venous thromboses are the most common and clinically relevant complications of the antiphospholipid Syndrome (APS). The role of lupus anticoagulants (LA) and anticardiolipin antibodies (aCL) as risk factors of thrombosis, however, has not been clearly established. Therefore, we reviewed 12 prospective, 4 "nested" case-control, 4 cross-sectional, 3 case-control, and 3 retrospective (with an adequate follow-up) studies, published between 1991-1999 on 4.394 patients. Twenty-six studies reported on aCL in 4.218 patients. Enrolment criteria were: cerebral ischaemic stroke (n=1240), deep vein thrombosis/pulmonary embolism (n=946), systemic lupus erythematosus (SLE) (n=817), aCL and/or LA positivity (n=715), myocardial infarction (n=500). The sensitivity of aCL antibodies for arterial and/or venous thrombosis ranged from 10 to 34%; the specificity from 81 to 98%. Seven out of 14 studies reported a significant association with arterial or venous thrombosis (down to p=0.0013); the Odds Ratio (OR) for thrombosis ranged from nonsignificant to 4.882. LA were investigated by 12 studies in 1.608 patients. Enrolment criteria were: aCL and/or LA positivity (n=663), deep vein thrombosis/pulmonary embolism (n=463), SLE (n=427), cerebral ischaemic stroke (n=55). The sensitivity of LA for arterial and/or venous thrombosis ranged from 8.5 to 14%; the specificity from 98 to 100%. The association with thrombosis ranged from p=0.016 to p=0.0004; the OR ranged from 7.3 to 10.7. Seven studies analysed the various coagulation and immunoassays employed to detect LA and aCL antibodies for their association with thrombosis in 645 patients. The strongest relationship was described for the dRVVT (p ranged from 0.02 to 0.001; sensitivity for thrombosis approximately 60%; specificity 83-93%). In conclusion, despite the heterogeneity of the reported studies - in terms of design, enrolment criteria, laboratory methodology - LA detection, rather than aCL measurement, seems to be of help to identify patients at particularly high risk of thrombosis.

CO-051**LUPUS ANTICOAGULANTS AND THROMBOSIS: PREDICTIVE VALUE OF DIFFERENT COAGULATION ASSAYS**

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The dilute Russell's viper venom time (dRVVT) and the kaolin clotting time (KCT) are two of the most commonly used coagulation tests for the detection of lupus anticoagulants (LA). The dRVVT seems better than the KCT in identifying LA-positive patients at risk of thrombosis. However, this relationship is greatly influenced by both the source of reagents and the instrumentation employed to carry out the assays. Therefore, 4 dRVVTs and 1 KCT were performed in our 2 centres and compared for their retrospective correlation with the thrombotic complications of 72 LA-positive patients. Clinical history was positive for thromboembolic events in 44 of them (61%). The clotting tests performed in this study were: a "home-made" dRVVT assay, DVV test (American Diagnostica), Bioclot LA (Biopool), LA screen and Kaoclot (Gradipore). Despite the good inter-laboratory and intra-assay degree of correlation, coagulation profiles generated by comparison of the KCT ratio with the ratios of the various dRVVTs correlated with the thrombotic history of the patients only when the "home-made" dRVVT was used. The analysis of each test showed a significant association between arterial and/or venous thrombosis and LA screen ($p=0.0019$), DVV test ($p=0.0043$), and Bioclot ($p=0.0255$), and the "home-made" dRVVT ($p=0.0503$) in one centre. This last assay was also significantly associated with thrombosis in the other centre ($p=0.0139$). When venous and arterial thrombosis were considered separately, DVV test was statistically associated with venous thrombosis in both centres ($p=0.0076$ and $p=0.0187$, respectively), and LA screen in one centre ($p=0.0303$) but not in the other one; no correlations were found with arterial thrombosis. Kaoclot was not correlated with thrombosis in either centre. Similarly, no association with thrombosis was found with a "home-made" KCT performed in one of the 2 centres. The prevalences of IgG and/or IgM anticardiolipin (aCL), anti β_2 -glycoprotein I (a β_2 -GPI), and antiprothrombin (aPT) antibodies were 74, 86, and 85%, respectively. Increased titres of IgG aCL were associated with arterial thrombosis ($p=0.0375$), whereas IgM a β_2 -GPI were associated with venous thrombosis ($p=0.0433$). In conclusion, these data suggest that the dRVVT, rather than other coagulation or ELISA tests, seems able to identify LA-positive patients at high thrombotic risk.

CO-052**GENETIC AND ANTIPHOSPHOLIPID ANTIBODIES-RELATED THROMBOPHILIA IN JUVENILE ISCHAEMIC STROKE. A REPORT ON 139 CASES**

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Background. Genetic thrombophilia is frequently associated with venous occlusion but its association with arterial thrombosis is not consistent whilst the antiphospholipid syndrome (APS), an acquired thrombophilia, is characterised by venous and/or arterial thrombosis, the latter involving mainly the cerebral district. *Patients and Methods.* We assessed genetic thrombophilia and the presence of APS in 139 patients with juvenile ischaemic stroke (74 M, 65 F, median age 39 years, range 4-50) and in 431 normal subjects. Fifty-two patients (37%) were smokers, 24 (17%) hypertensive, 11 (8%) hyperlipidaemic, 2 (3%) obese and 14/66 (21%) women were on oral contraception; more than one risk factors were present in 28 (20%) patients. All patients were submitted to the following screening for thrombophilic factors: antithrombin (AT), protein C, protein S, activated protein C resistance (aPCr), factor V Leiden (FVL), prothrombin A20210, TT677 genotype of MTHFR, lupus anticoagulant (LA) and anticardiolipin antibodies (aCL). Ischaemic strokes were confirmed by CT scan and/or nuclear magnetic resonance imagining. *Results.* Among the natural anticoagulants, AT deficiency was found in only 1 case (0.7%). The prevalence of aPCr matching FVL was 8.6% (12/139) in patients and 5% (21/431) in controls (NS); the prevalence of prothrombin A20210 was 7.2% (10) in patients and 4.7% (20) in controls (NS); the prevalence of TT677 genotype of MTHFR was 21.6% (30) in patients and 18% (78) in controls (NS). A primary APS was observed in 5 (4%) patients (all with LA+/aCL+ pattern). Combined genotypes were found in 5 (4%) patients (FVL+A20210 n=1, A20210+TT677 n=2, FVL+TT677 n=2); 2 patients with APS also had a thrombophilic genotype (TT677 and A20210 respectively). There was history of venous thromboembolism in 9 (6.5%) patients of whom 5 with thrombophilic genotypes and 1 with APS+TT677 mutation. *Conclusions.* In juvenile ischaemic stroke, natural coagulation inhibitors defects are rare. FVL, prothrombin A20210 and TT677 of MTHFR mutations are not as common as in patient with deep vein thrombosis. Nevertheless patients with these genotypes have an increased risk of venous thromboembolism. APS is confirmed as a severe acquired thrombophilic state.

CO-053**ROLE OF ANTIPHOSPHOLIPID ANTIBODIES AND INHERITED THROMBOPHILIC GENOTYPES IN UPPER LIMB VENOUS THROMBOSIS: STUDY OF 34 CASES**

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Background. Deep vein thrombosis mainly affects iliac and femoral veins with or without concurrent inferior caval thrombosis. Upper limb deep vein thromboses (ULDVT) are not so frequent and are usually associated with anatomical abnormalities or central venous catheter positioning (CVC). **Aims.** We investigated the role of antiphospholipid antibodies and inherited thrombophilic genotypes in the pathogenesis of ULDVT. **Patients and Methods.** Thirty-four patients with ULDVT, documented by phlebography or ultrasound, (15 males, 19 females, median age 34 years, range 15-71) were submitted to screening of thrombophilic factors: protein C and antithrombin (Chromogenic assay), total and free protein S (ELISA), activated protein C resistance (Chromogenic), lupus anticoagulant (LA) (aPTT sensitive, KCT, DRVVT), anticardiolipin antibodies (aCL) (MELISA Byk Gulden). The presence of factor V Leiden, A20210 mutation of prothrombin (PTHR) and genotype TT677 of the methylene tetrahydrofolate reductase (MTHFR) was also investigated. **Results.** Anatomical abnormalities or previous trauma were present in 7 (20%) cases, intense physical stress in 1(3%), CVC in 1 (3%); ULDVT occurred post-operatively in 2 further cases (6%) and in 3 patients (9%) taking oral contraceptives. No patients had protein C, protein S or antithrombin deficiencies. Three patients (9%) were heterozygous for FVL, 2 patients (6%) for A20210 PTHR and 11 (32%) had TT677 MTHFR genotype. In 2 patients (6%) a diagnosis of primary antiphospholipid syndrome could be made (pattern LA+/aCL- in 1 and LA-/aCL+ in 1). Associated genotypes were present in 4 (12%) cases: FVL+ TT677 MTHFR in 2, FVL+A20210 PTHR in 1, A20210 PTHR + TT677 MTHFR in 1). In the patient with FVL+A20210 PTHR mutations the deep vein thrombosis recurred in the lower limbs. **Conclusions.** One or more thrombophilic genotypes were present in 47% of our patients with ULDVT and 6% had primary antiphospholipid syndrome. Inherited thrombophilia and antiphospholipid syndrome must be included in the screening of patients with ULDVT.

CO-054**DIFFERENT SPECIFICITIES OF ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS WITH DEEP VENOUS THROMBOSIS**

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Antiphospholipid antibodies (aPL) are a heterogeneous family of autoantibodies associated with a clinical syndrome characterised by thrombo-occlusive events. Anticardiolipin antibodies (aCL) are currently the best characterised and standardised. However, aPL, rather than being a single or a homogeneous group of autoantibodies, constitute a heterogeneous family. Antibodies against phospholipid other than cardiolipin have been less studied. We have addressed the prevalence and the possible clinical significance of aPL other than aCL in deep venous thrombosis. Among 103 patients with a documented diagnosis of deep venous thrombosis in one leg consecutively referred for a thrombophilic work-up, we measured by ELISA, in presence of human β 2-GPI, circulating levels of antibodies against 4 different antiphospholipid antigens: cardiolipin (aCL-h), phosphatidic acid (aPA), phosphatidyl inositol (aPI), phosphatidyl serine (aPS). In addition, the titre of aCL was also measured using bovine β 2-GPI (aCL-b) in 99 patients. A sample was considered positive when the value detected was greater than 5 SD above the mean of values measured in 121 control subjects randomly selected. Thus, a value higher than 12.0 (aCL-b), 19.25 (aCL-h), 14.53 (aPA), 17.77 (aPI), and 17.55 (aPS) were considered positive. Overall, 23 patients (22.5%) displayed positive values. A positivity for aCL-b was identified in 15 subjects (15.2%), whereas 19 (18.4%) individuals carried a significant titre of aCL-h, 16 (15.5%) aPA, 16 (15.5%) aPI, and 17 (16.5%) aPS. Nine samples were positive for all the aPL. Fifteen subjects were positive for all aPL measured in the presence of human β 2GPI. A major difference was observed between aCL-b and other aPL. In addition, antibodies against β 2-GPI were measured in all patients. A titre above 26.52 (mean plus 5SD) was considered positive. Nine patients (8.7%) had levels above the cut-off. All subjects who had anti β 2-GPI carried at least one of the following aPL: aCL-h, aPA, aPI, or aPS. Then, patients carrying the factor V Leiden (19; 18.6%) or the factor II A20210 (14; 13.7%) mutation were excluded. In the remaining patients (71), a positive aPL value was observed in 14 cases for aCL-b (out of 67; 20.9%), in 17 for aCL-h (23.9%), in 15 for aPA (21.1%), in 14 for aPI (19.7%), and in 16 for aPS (22.5%). Finally, 9 patients (12.7%) had a positive anti β 2-GPI value. Overall, 19 patients (26.8%) had a positive value. Thus, in patients with deep venous thrombosis, a high prevalence of aPL was observed, aCL measured in the presence of human β 2-GPI showing the highest prevalence. Different non-aCL aPL did not show an independent possible clinical significance.

ORAL COMMUNICATIONS

Von Willebrand factor and disease

CO-055

THE 20-YEAR (1978-1998) NATURAL HISTORY OF VON WILLEBRAND'S DISEASE IN ITALY: A MULTICENTRE RETROSPECTIVE ANALYSIS ON DIAGNOSIS AND THERAPY IN 1,234 PATIENTS

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Introduction. Von Willebrand's disease (vWD) is the most frequent congenital bleeding disorder. vWD is very heterogeneous and correct diagnosis and treatment may sometimes be difficult. Despite the high prevalence of vWD, there are only a few reports on large studies on vWD patients. **Aims of the study.** To evaluate the retrospective natural history of vWD in Italy (>1,000 cases) and the need for FVIII/vWF concentrates, we developed a simple computerised programme. **Methods.** The programme was devised to collect, among many other parameters, specific information on: 1) vWD types with age-sex distribution and type of bleeding episodes; 2) non-transfused vWD cases who are responsive to desmopressin (DDAVP); 3) the amount of FVIII/vWF concentrates used. Twenty-three Italian Haemophilia Centres received the programme in February 1997. They were requested to make the diagnosis of type 1, 2, 3 vWD or 3 heterozygous following the criteria of the ISTH-SSC on vWD. **Results.** A total of 1,547 cases were sent from 16/23 Centres: 313 cases (20%) were not considered because of incomplete laboratory data or because enrolled by another Centre. A descriptive analysis of some of the data on the 1,234 cases (expressed in %) are as follows:

vWD (n=1234) types	1=63.5;	2=25.5;	3=5.3;	heteroz=5.7;	Total (100 %)
Number of families	246	96	36	36	404
Age (> 18 years)	83	85	73	86	83
Sex (female)	59	57	52	53	57
Mucosal bleeding	63	65	71	29	64
Hematomas or Hemarthrosis	14	15	33	7	15
DDAVP-test (done vs pos)	77/49	76/47	30/17	26/14	70/48
Using DDAVP only	18	13	3	7	15
Never transfused	80	62	15	97	73
DDAVP+transfusions	5	4	0	0	4
FVIII/vWF conc. only	3	7	18	0	5
FVIII/vWF conc. +transf.	12	27	67	3	18

Conclusions. Our data confirm that vWD is mostly diagnosed in young adults (83%) and occurs mainly in women (57%). Mucosal bleeding (64%) is more frequent than haematomas or haemarthrosis (15%) but

does not require transfusions in 73% of cases. DDAVP can be effective in 48% but was used in only 15% of cases. FVIII/vWF concentrates with or without blood components are used in 23% of all patients; with type 3 (85%), type 2 (34%) and type 1 (15%) vWD.

CO-056

RISTOCETIN COFACTOR ACTIVITY AND COLLAGEN BINDING ASSAY NORMALISED WITH VON WILLEBRAND FACTOR ANTIGEN FOR A RAPID DIAGNOSIS OF TYPE 2 VON WILLEBRAND'S DISEASE: RESULTS FROM A STUDY IN 72 PATIENTS COMPARING HOMEMADE VERSUS COMMERCIAL ASSAYS

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Background. Despite the multifunctional sites of von Willebrand factor (vWF), only one basic assay has been widely used for diagnosis of von Willebrand's disease (vWD) for the last 20 years: the ristocetin cofactor (vWF:RCo) activity which measures the capacity of plasma vWF to bind to its platelet receptor (GPIb-IX). More recently, the vWF collagen binding assay (vWF:CBA) has also been used in vWD diagnosis. **Aims of the study.** To test the potency of two "homemade" assays for vWF:RCo and vWF:CBA versus two commercial kits and to evaluate the role of vWF:RCo/Ag and vWF:CBA/Ag ratios <0.7 for a rapid diagnosis of type 2 vWD. **Patients and methods.** vWD cases (n=72), included all the main subtypes, except type 3 and 2 N vWD. We compared the "homemade" vWF:RCo (agglutination test with formalin-fixed platelets) with the "vWF ACTIVITY" by Murdock *et al.* and the "homemade" vWF:CBA (collagen type I) with IMMUNOZYM vWF:CBA (collagen type III). **Results.** Data of each assay were normalised using their ratios with the vWF:Ag by ELISA. Results were analysed by the paired Student's t test and significant differences (*p<0.05; **p<0.01; ***p<0.001) with the "homemade" vWF:RCo are reported:

Individuals (n)	vWF:RCo Home made	vWF:Activity Commercial kit	vWF:CBA (I) Home made	vWF:CBA (III) Commercial kit
Normals (27)	0.92 ± 0.19	0.89 ± 0.17	0.97 ± 0.20	0.98 ± 0.16
Type 1 P.N. (15)	0.98 ± 0.17	1.06 ± 0.24	1.08 ± 0.13	1.04 ± 0.10
Type 1 P.L. (6)	0.89 ± 0.14	0.93 ± 0.07	0.95 ± 0.17	0.89 ± 0.19
Type 1 P.D. (7)	0.39 ± 0.29	0.85 ± 0.24*	0.79 ± 0.43*	0.88 ± 0.07*
Type 2 A (9)	0.14 ± 0.16	0.85 ± 0.33***	0.10 ± 0.10	0.46 ± 0.25***
Type 2 B (15)	0.54 ± 0.19	0.89 ± 0.20***	0.12 ± 0.16***	0.66 ± 0.28**
Type 2 M-Vic (14)	0.65 ± 0.25	1.07 ± 0.33***	0.80 ± 0.34*	0.93 ± 0.17**
Type 2 M (6)	0.40 ± 0.16	0.85 ± 0.50*	0.82 ± 0.42*	0.82 ± 0.13*

Conclusions. The "homemade" vWF:RCo is still the most sensitive assay to characterise vWD patients with defective vWF (vWF:RCo/Ag<0.70 in types 1 PD, 2A, 2B, 2M-Vic and 2M) while vWF:ACTIVITY is not sensitive at all. Both the vWF:CBA assays can identify types 2A and 2B with abnormal multimers but not

type 2 M vWD: type 1 collagen seems to be more sensitive. Therefore, we can conclude that the vWF:RCo/Ag and vWF:CBA/Ag ratios might be useful for a rapid diagnostic screening of the different types 2 vWD, before using multimeric analysis.

CO-057

RESPONSE TO DESMOPRESSIN IN PATIENTS WITH CLINICALLY SEVERE TYPE 1 AND 2 VON WILLEBRAND'S DISEASE: RESULTS OF A MULTICENTRE EUROPEAN STUDY

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Introduction. In von Willebrand's disease (vWD), the main goals of treatment are to correct the dual defect of haemostasis caused by a reduced or abnormal von Willebrand factor (vWF), i.e. prolonged bleeding time (BT) and deficiency of factor VIII coagulant activity (FVIII:C). Desmopressin (DDAVP) has diminished the need for transfusion in many patients, but DDAVP is ineffective in type 3 and in some clinically severe cases of types 1 and 2 vWD. **Aims of the study.** To evaluate the biological response to DDAVP in clinically severe type 1 and 2 vWD patients; to define the proportion of DDAVP-unresponsive cases who require plasma-derived FVIII/vWF concentrates. **Patients and methods.** Five Haemophilia Centres (Milan, Malmö, London, Frankfurt and Lille) participated in this study. **Inclusion criteria.** All hereditary type 1 and 2 vWD with a severe bleeding history (more than one episode of severe blood loss in their life) and at least one of the following laboratory parameters: BT >15 min; vWF:RCo <10 IU/dL; FVIII:C <20 IU/dL. **Exclusion criteria:** type 3 and type 2 B cases; acquired vWS; age <12 and >65 yrs; cardiovascular diseases and epilepsy; previous reactions to DDAVP. **Protocol.** A 0.3 mg/kg i.v. infusion was given with BT and FVIII/vWF tested before and 0.5, 1, 2, 4 hours after DDAVP. **Definition of response to DDAVP:** patients who, after 2 hours, showed increases of baseline levels of FVIII:C and vWF:RCo of at least three fold, with levels of at least 30 IU/dL and BT of 12 min or less. **Results.** Among a total of 957 vWD patients followed by the five Haemophilia Centres, 116/957 (12 %) satisfied the inclusion criteria and 67/957 (7%) were enrolled after giving their informed consent. Median (range) of the basal values of BT and FVIII/vWF activities together with cases with positive (%) DDAVP response are reported here:

all vWD (n = 67) types:	1 (n=38)	2A (n=21)	2 M (n=4)	2 N (n=4)
BT (min)	17 (7->30)	12 (9->30)	11.30 (6-15)	5.30 (5-8)
FVIII:C (U/dL)	30 (7-115)	34 (4-56)	37 (16-77)	12 (7-24)
vWF:RCo (U/dL)	26 (<6-63)	12 (<6-30)	11(<6-12)	50 (29-82)
DDAVP response (%)	9/38 (23.7)	3/21 (14.3)	4/4 (100)	2/4 (50)

Conclusions. Our data demonstrate that only 18/67 (27 %) of clinically severe type 1 and 2 vWD patients are responsive to DDAVP; the response rate is different according to vWD subtypes. Therefore, a DDAVP infusion test should be always performed in clinically severe vWD patients to exclude the need for replacement therapy with concentrates.

CO-058

MOLECULAR CHARACTERISATION OF A MULTIETHNIC GROUP OF 21 PATIENTS WITH TYPE 3 VON WILLEBRAND'S DISEASE

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Type 3 von Willebrand disease (VWD) is a rare autosomal disorder characterised by unmeasurable levels of von Willebrand's factor (VWF) and severe haemorrhagic symptoms. We studied a multiethnic group of 21 patients, from India (n=6), Iran (n=9) and Italy (n=6), to identify the molecular defects and to evaluate genetic heterogeneity among these populations. Twenty-four different gene alterations were identified, 20 were novel, not having been previously described. As expected the majority of the mutations found cause null alleles. Eleven were nonsense mutations (Q218*, W222*, R365*, R373*, E644*, Q706*, S1338*, Q1346*, Y1542*, R1659*, E2129*), 4 small deletions (437Gdel, 2679Cdel, 6431Tdel, 8491-8499del), 3 possible splice site mutations [lvs9(-1)g→a, lvs29(+10)c→t, lvs40(-1)g→c], 3 candidate missense mutations (C275S, C2174G, C2804Y), 2 small insertions (7375Cins, 7921Cins) and 1 large gene deletion. The latter mutation was associated with the development of alloantibodies to VWF, but this complication was also found in a patient homozygous for a nonsense mutation (Q1346*). Due to the ethnic origin of the patient many were the result of consanguineous marriages and so were homozygous for the mutations found (19/21). Our results indicate that molecular defects responsible for type 3 VWD are present in the entire VWF gene (from exons 3 to 52), but there is no prevalent and common gene defect in these 3 populations.

CO-059

BLOOD-BORNE VIRAL INFECTIONS IN PATIENTS WITH VON WILLEBRAND'S DISEASE: PREVALENCE AND CLINICAL CORRELATION

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Patients with von Willebrand's disease (vWD) are a unique clinical model of post-transfusion viral infec-

tions in that they have been most exposed to blood transfusions or cryoprecipitate and less frequently to large-pool factor VIII/von Willebrand factor (FVIII/vWF) concentrates, which in the past carried a high risk of viral infections. The aim of this study was to assess the prevalence of the major blood borne viruses (HIV, HBV, HCV and HGV) and the relationship with liver disease in a cohort of 112 consecutive patients with vWD. **Methods.** HBsAg, anti-HBs, anti-HIV, anti-HCV and anti-HGV were tested with immunoenzymatic assays. Serum HCV-RNA and HGV-RNA were detected by nested reverse transcription (RT)-PCR using primers of the 5' non-coding and NS3 region, respectively. HCV was genotyped by LiPA HCV (Innogenetics, Belgium) and quantified by bDNA 2.0 (Chiron Co, CA). **Patients.** The 112 patients (48 males, 64 females, mean age + SD: 41+17) were affected by vWD of type 1 in 62, type 2 in 34 and type 3 in 16. Thirty-nine patients had never been transfused and 73 had been infused with plasma, whole blood and/or factor VIII/vWF concentrates (62 before 1987). **Results.** Among the 73 treated patients, anti-HIV was present in only one patient, and HBsAg in one other. HCV infection was present in 43 (59%, 4 treated after 1987), 35 (81%) of whom were HCV-RNA positive. The genotype distribution was type 1a in 9, type 1b in 9, type 2a/c in 8 and other types in the remaining 9; the levels of viraemia ranged from <0.2 to 36 MEq/mL. Eight (11%) patients were HGV-RNA positive, while in 17 (23%) anti-HGV indicated a recovery from past infection. Serum ALT activity was persistently or intermittently high in 21 (29%), 20 of them were HCV-RNA positive. Conversely, none of the 39 untreated patients was HCV or HIV infected, while 2 (5%) were HBsAg positive and 2 showed active or past HGV infection. All had normal ALT levels. **Conclusions.** In comparison with haemophilic patients, who have been exposed life-long to more infectious large-pool concentrates, our patients with vWD showed a sporadic risk of infection with HIV and a lower prevalence of HCV, while HGV and HBV were similarly distributed. Biochemical evidence of liver damage was present only in HCV infected patients. This setting becomes a unique model to study the national history of HCV without HIV co-infection.

CO-060

TYPE 2M VON WILLEBRAND'S DISEASE CHARACTERISED BY ABNORMAL VON WILLEBRAND FACTOR MULTIMER PATTERN

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We report a von Willebrand's disease (VWD) variant characterised by low plasma and platelet von Willebrand factor (VWF), impaired ristocetin-induced VWF binding to platelet glycoprotein Ib (GPIb), and abnormal VWF multimer pattern not associated with the absence of large forms. A cytosine

to thymidine transition at nucleotide 4120 in exon 28 of the VWF gene was found; this mutation introduces a cysteine at the codon for arginine 611 of mature VWF, and was already described and included in type 2A VWD. This variant was transmitted as autosomic dominant trait, present in mother and son, and gave rise to mild bleeding symptoms. In addition to the decrease in factor VIII (FVIII) and VWF levels, ristocetin-induced platelet aggregation (RIPA) was almost absent, and VWF ristocetin cofactor activity (VWF:RCo) was significantly more decreased than VWF antigen. The patients also showed a mild defect in VWF collagen binding activity (VWF:CBA). Plasma VWF multimers were characterised by the absence of the normal discontinuous multimer organisation which was replaced by a diffuse smear, especially detectable in the large forms. Moreover, together with a decrease in all VWF multimers, especially large forms, there was no limit in the size of large components. This picture was emphasised by DDAVP infusion, which also increased FVIII and VWF levels, and normalised VWF:CBA, but not GPIb-dependent VWF functions, such as RIPA and VWF:RCo. Based on these findings, we conclude that the abnormal multimer pattern and the defective GPIb-VWF interaction must be considered unrelated events, and therefore the defect in GPIb does not depend on the decrease in large VWF multimers. Hence, we include this variant in type 2M VWD, focusing on the abnormality in GPIb-dependent VWF functions. We suggest that this defect depends on the mutation in the GPIb binding domain of VWF, rather than the abnormal VWF multimer pattern; instead, the abnormal VWF multimerisation could result from the introduction of a new cysteine residue in the amino-terminal region of VWF, which might perturb the cysteine-mediated VWF multimerisation.

CO-061

NINE ADDITIONAL FAMILIES WITH TYPE 2 M VON WILLEBRAND'S DISEASE VICENZA FROM VICENZA AREA SHOW SEGREGATION OF THE G2470A (M740I) AND THE G3864A (R1205H) MUTATIONS IN THE VON WILLEBRAND FACTOR GENE WITH THE ABNORMAL PHENOTYPE

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We have demonstrated that the two original families with von Willebrand's disease (vWD) Vicenza show the co-segregation of a G3864A (R1205H) and a G2470A (M740I) substitution in exon 27 and 17 of the von Willebrand factor (vWF) gene, respectively, with the laboratory and clinical phenotype. We sequenced exon 17 and 27 of the vWF gene in nine families with vWD and similar phenotypes, for a total of 29 affected members and 11 normal relatives. All the affected members showed similar laboratory phenotypes (low FVIII/VWF measurements and presence of supra-normal vWF multimers in plasma, normal platelet vWF content). In all the affected members

the two mutations were present, whereas none of the normal relatives showed evidence of any of the mutations. Going back five generations, only two of these new families were related to the previously reported families. In conclusion, vWD type 2 M Vicenza is more frequent than previously believed. This report confirms that the two candidate mutations co-segregate with the abnormal phenotype and that this rare pattern is peculiar to the families living in the Vicenza area.

CO-062

USE OF THE COLLAGEN BINDING ASSAY IN THE ANALYSIS OF TYPE 2 VON WILLEBRAND'S DISEASE

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Von Willebrand factor collagen binding assays (VWF:CBA) were performed on a group of patients previously diagnosed with Type 2 von Willebrand's disease (VWD). Patients with type 2 VWD have reduced VWF:RiCo/VWF:Ag ratios (<0.7). VWF:CBA levels are directly proportional to the presence of the high molecular weight (HMW) multimers. This has led to the suggestion that the VWF:CBA may be used as an alternative to VWF functional assays. We have studied 32 patients with previously characterised VWD including 25 patients with type 2M VWD, 6 with type 2A VWD, one with type 2B VWD and 20 normal controls. All patients and controls were analysed for FVIIIc (one stage clotting assay), VWF:Ag (ELISA), VWF:CBA (ELISA) using human type III collagen (Sigma), and VWF:RiCo activity using fresh washed platelets. Results for CBA were calculated as a ratio of VWF:Ag/VWF:CBA, for normal controls, ratios ranged from 0.7 to 1.3. In patients with type 2M VWD, VWF:Ag/VWF:CBA ratios ranged from 0.9 to 1.5, whilst in patients with type 2A/2B VWD, the VWF:Ag/VWF:CBA ratios ranged from 2.0 to 43. The VWF:CBA allows discrimination of VWD patients who lack collagen binding as a result of the loss of the HMW multimers. In our patient group with type 2M VWD, all VWF:RiCo/VWF:Ag ratios were below 0.7 and all VWF:Ag/VWF:CBA ratios fell within the normal range reflecting full collagen binding. In conclusion, these data demonstrate that the VWF:CBA is inadequate at differentiating type 2M VWD and probably should not be considered as a replacement assay for VWF:RiCo.

CO-063

VON WILLEBRAND FACTOR CLEAVING PROTEASE: CHANGES IN HEALTH AND DISEASE

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It has been recently recognized that thrombotic thrombocytopenic purpura (TTP) is due to the congenital or acquired deficiency of a metalloprotease that cleaves VWF physiologically. The deficiency of the VWF cleaving protease reduces or abolishes the removal from plasma of supranormal VWF multimers, that aggregate platelets intravascularly and thereby cause the thrombotic microangiopathy typical of TTP. Although the behavior of the VWF cleaving protease has been extensively studied in TTP and in the hemolytic uremic syndrome, there is little information in other physiological and pathological conditions. Using a recently developed method based on the preferential binding of large VWF multimers to collagen, we measured the protease in four groups of healthy individuals of both sexes in the age groups 20-35, 36-50, 51-65 and over 65. While there was no difference between men and women, lower values were found in the elderly groups compared with groups of younger age (mean values expressed in % of average normal plasma (96+22 vs 102+27)). To evaluate whether the cleaving protease is synthesized in the liver, 18 patients with decompensated liver cirrhosis (Child C) were studied: mean values were definitely lower than in normal individuals (43+28 vs 102+27). To evaluate whether the protease behaves as an acute phase reactant, it was measured in individuals with inflammatory states defined by serum levels of C reactive protein higher than 5 mg/dL. Data obtained so far in 10 patients would indicate that the VWF cleaving protease behaves as a negative acute phase reactant, being low in most patients (median value 55%, range 31-84). These data provide preliminary information on the behavior of the VWF cleaving protease in health and disease.

ORAL COMMUNICATIONS

Diagnosis and treatment of arterial thrombosis

CO-064

ANTITHROMBOTIC ACTION OF MAGNESIUM SULPHATE IN A RAT MODEL OF ARTERIAL THROMBOSIS

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Magnesium deficiency is associated with a high frequency of cardiac arrhythmias, hypertension and sudden ischaemic myocardial death. Administration of magnesium has been shown to decrease arterial blood pressure but a controversy still exists regarding its usage in the treatment of myocardial infarction. In the present study we have investigated the *in vivo* effects of intravenous magnesium administration in a rat model of carotid thrombosis. Whatman filter paper, saturated with 30% ferric chloride (FeCl₃), was

applied to the surgically exposed common carotid artery of anaesthetised Sprague-Dawley male rats. Thrombus formation was recorded as time for loss of carotid artery blood flow, monitored by the application of a Doppler flow probe connected to a flow meter (Transonic T106). Infusion of magnesium sulphate (MgSO_4) 5 min before and during the application of FeCl_3 significantly reduced thrombus formation in a dose-dependent manner (13.4 ± 0.49 min for control; 20 ± 2.6 min and no occlusion for MgSO_4 0.15M and 0.3M, respectively). This effect was related to magnesium ions, since similar data were obtained by infusing of MgCl_2 at the same concentration. Late infusion (7 min after FeCl_3 application) of MgSO_4 (0.6M) delayed, but did not inhibit thrombus formation (13.1 ± 0.6 min versus 24.6 ± 0.9). The effect of MgSO_4 was accompanied by a slight reduction of platelet aggregation and no modifications of coagulation or fibrinolytic parameters. In contrast whole blood clotting time was markedly prolonged (tail transaction method). These data may provide a rationale for the use of magnesium as an antithrombotic agent but suggest, however, that correct timing of administration is critical to obtain an efficient effect.

CO-065

GENE COAGULATION ABNORMALITIES AND CEREBRAL INFARCTION

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The nature of haematological disorders in different stroke subtypes remains uncertain. Recently, it has been recognised that arterial cerebral thrombosis is also associated with polymorphisms of genes of the natural anticoagulant system and vascular vessel wall. In 164 referred young cases, 90 men and 74 women, median age 33 years (range 19 to 48), with a history of ischaemic stroke and in 164 age frequency-matched apparently healthy individuals from the same ethnic background, we investigated whether inherited prothrombotic gene defects were more frequent. Factor V Q506 allele was found in 7 patients compared to 2 in the control group ($X^2=1.7$, $p=0.19$). Factor II A20210 allele was found in 17 patients (in 1 in a homozygous state) and 3 controls ($X^2=7.9$, $p=0.005$). The C 677 T transition in the methylenetetrahydrofolate reductase gene was found in 72 in a heterozygous state and in 39 in a homozygous state. Genotype frequencies in control subjects were 58 ($X^2=0.8$, $p=0.3$) for heterozygotes and 18 for homozygotes ($X^2=5.8$, $p=0.01$). PAI-1 4G allele was detected in 120 patients and in 77 healthy controls

($X^2=5.4$; $p=0.01$). The D allele in the ACE gene polymorphism was present in 134 patients and 92 controls (4.2 ; $p=0.03$). Our findings demonstrate that polymorphisms of genes of the clotting system and vascular vessel wall are more frequent in young individuals suffering from ischaemic stroke compared to that observed in healthy subjects. From a clinical point of view this information would be beneficial for studies to investigate the inherited basis of arterial brain thrombophilia.

CO-066

PREVALENCE OF MODERATE HYPERHOMOCYSTEINAEMIA AND OF HOMOZYGOUS C677T MUTATION OF METHYLENTETRAHYDROFOLATE REDUCTASE GENE IN A COHORT OF CONSECUTIVE PATIENTS WITH JUVENILE ISCHAEMIC STROKE

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One hundred and fifteen consecutive patients (58 M, 57 F; mean age 39.23 ± 11.67 , range 4-65 yrs; mean age at first event 35.17 ± 11.06 , range 0-50 yrs) referred to our Centre between January 1998 and April 1999 because of a history of juvenile ischaemic stroke (age at first event <51 yrs) were compared with 198 healthy subjects (91 M, 107 F; mean age 36.19 ± 12.94 , range 5-73 yrs). Genotypes of factor V (FV), prothrombin (PRTH) and methylenetetrahydrofolate reductase (MTHFR) were evaluated in the entire population, while total mean fasting plasma homocysteine (THcy) concentrations were determined in 86 patients and in 88 controls. Frequencies of the genotypes of FV Leiden, and of PRTH G20210A gene mutation were not statistically different between patients and controls (7/115, 6.1%, vs. 11/198; 5.6% for FV Leiden; 10/115, 8.7%, vs. 13/198; 6.6% for PRTH; p always $>.05$), while homozygosity for C677T mutation of the MTHFR gene was slightly more frequent in patients than in controls (30/115, 26% vs 32/198, 16.1%; $p=0.048$, C.I. 1.0-3.3). THcy levels were significantly different between patients ($n=86$, 46M, 40F) and controls ($n=88$, 41M, 47F) (17.26 ± 24.5 mM, vs. 11.95 ± 6.1 mM, $p=0.006$) maximal difference being observed in males (18.4 ± 16.4 mM, vs. 13.96 ± 7.42 mM, $p=0.01$). Moreover, raised levels of homocysteine were twice as common in patients (19/86, 22.1%) than in controls (8/67, 11.9%; $p=0.03$, χ^2 test). We conclude that, in the present cohort of consecutive patients, raised levels of homocysteine and its main genetic determinant (homozygous MTHFR gene mutation) rather than known inherited prothrombotic conditions help identify subjects with juvenile ischaemic stroke.

CO-067**THROMBOGENICITY OF ATHEROSCLEROTIC PLAQUES IN NORMOCHOLESTEROLAEMIC PATIENTS WITH BILATERAL CAROTID STENOSES: EFFECTS OF ATORVASTATIN ON TISSUE FACTOR AND RELATED INHIBITOR: THE ATROCAP STUDY**

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Introduction. Prothrombotic factors (mostly tissue factor (TF)) in core-rich plaques have been suggested to promote the thrombotic complications that cause most acute ischaemic events. Statins seem to exert their overall beneficial effects on prevention of acute ischaemic events both via serum cholesterol lowering, and plaque stabilisation, endothelial cell protection and reduction of prothrombotic plasma activity. Plaque stabilisation probably results from the TF inhibitor (TFPI) effect induced by statins. The *in vivo* testing of this latter hypothesis relies on the possibility of assessing the plaque activity and pathological characteristics before and after treatment with statins. No method allows *in vivo* monitoring of plaque composition and prothrombotic factor contents. **Study aim and design.** To investigate the effects of statins in plaque composition and in TF/TFPI and other thrombogenic factors (t-PA/PAI-1; *Chlamydia p. genoma*) content, we planned a clinical and immunohistochemical study in normocholesterolaemic patients with critical bilateral carotid stenoses undergoing two-step intervention (carotid endarterectomy, CEA). The study is randomised, double-blind, placebo-controlled and multicentre (6 centres). At this time 53 patients (70% males; mean age: 70.7 years) with ischaemic vascular disease (TIAs, angina pectoris, myocardial infarction, and/or intermittent claudication) have undergone the first CEA and been randomly assigned to atorvastatin 20 mg or placebo until the contralateral CEA is performed (within 4-6-months). All patients are given aspirin (100mg). Early results on first CEA samples. Plaques were longitudinally divided to assign adjacent segments for the pathological, biochemical and molecular analyses. All plaques had both fibrous and core components. None was exclusively fibrous. The extent of the core varied from small to an extremely large, dominant component. Plaque rupture was found in 11. Thrombus was seen in 22 plaques, recent in 6 and organising in 16. Severe plaque inflammation was documented in 21, while small clusters of inflammatory cells were seen in 28. Inflammatory cells were constituted of T-lymphocytes and macrophages. Small calcifications were seen in 47 plaques: in 3, they were large and numerous. Neointimal hyperplasia in the superficial layers of the plaques was seen in 36 plaques, and vascular neogenesis in 34. All plaques had measurable TF activity, ranging from 22.9 to 311 U_{TFa}/mg of protein, the mean value being 158.9+24.7. The second CEA sample series will be completed by June 2000. Compar-

ative analyses between the first and the second CEA samples will be performed by November 2000.

CO-068**LOW MOLECULAR WEIGHT HEPARIN ADMINISTRATION IS ASSOCIATED WITH DECREASED TISSUE FACTOR PLASMA LEVELS IN PATIENTS WITH UNSTABLE ANGINA**

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The clinical benefit of using of low molecular weight heparin (LMWH) in the treatment of acute coronary syndromes has been demonstrated by several studies. High tissue factor (TF), tissue factor pathway inhibitor (TFPI) and thrombin-antithrombin (TAT) plasma levels have been documented in patients with unstable angina. The aim of this study was to evaluate whether enoxaparin administration is effective in reducing high TF and TAT plasma levels and whether there is a relationship between TF and TFPI levels after enoxaparin administration (90 IU/Kg bid for 3 days) in patients with unstable angina, according to the ESSENCE protocol. Plasma samples were obtained from 20 patients immediately before, 1 hour and 4 hours after the enoxaparin administration on the 3rd day of treatment. TF, TFPI and F1+2 plasma levels were measured by commercial assays. After the 2nd day of LMWH treatment, on the morning of the 3rd day, immediately previous to the morning injection, TF plasma levels were slightly but not significant lower (195.5, 103-322 pg/mL) than those observed in the base-line blood sample (203.5, 127-322.9 pg/mL). On the same day (3rd day), 1 hour after the morning injection TF plasma levels were significantly reduced (149.9, 77.9-293.3 pg/mL; -25.6 %, p<0.001). A significant reduction (-21.7%) with respect to the base-line levels in TF plasma levels was also observed, 4 hours after the morning injection (156.4, 86.6-310.2 pg/mL; p<0.001). The decrease in TF plasma levels was observed in all but one patients both 1 hour and 4 hours after enoxaparin administration. After 2 days of enoxaparin on the 3rd day in the pre-injection samples changes in TFPI levels (111.7, 69-210 ng/mL) were not statistically significant. Both 1 and 4 hours after the morning injection on the 3rd day TFPI plasma levels increased significantly (p<0.001) (96.4%, 96.9%) with respect to base-line levels (base-line=123.7, 67.6-218 ng/mL; 1 hour=261.8, 135.2-411.1 ng/mL; 4 hours=262.0, 133.2-399.5 ng/mL). The increase was observed in all patients. After enoxaparin administration TAT plasma levels at all observation times (pre-injection: 2.25, 1.2-7.5 µg/L; 1 hour: 2.3, 1.3-7.0 µg/L; 2.0, 1.3-7.0 µg/L) were significantly (p<0.001) lower with respect to base-line levels (4.1, 2.1-8.3 µg/L). In conclusion, enoxaparin treatment was found to be associated with a reduc-

tion in high TF and TAT plasma levels occurring in patients with unstable angina. This inhibitory effect of LMWH may represent a novel mechanism of the antithrombotic properties of this drug.

CO-069

EFFECT OF LIPID-LOWERING TREATMENT ON FACTOR VII PROFILE IN HYPERLIPIDAEMIC PATIENTS

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Clinical trials have demonstrated that inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase greatly reduce cardiovascular events in patients with and without coronary artery disease but few data, at this time, are available on the effects of lipid-lowering treatment on factor VII coagulant activity (FVIIc and FVIIa) and antigen (FVIIAg). Activation of factor VII is indeed the pivotal step in fibrin generation and thrombus formation. Thirty-six consecutive outpatients with primary hyperlipidaemia and without any previous history of ischaemic heart disease (IHD) were selected. All patients were allocated to receive a lipid-lowering diet (American Heart Association Phase I diet) for 4-6 weeks. Patients who after this period did not achieve a reduction in LDL-C below 4 mmol/L, received drug therapy (atorvastatin, 20 mg/daily) for a further 4-6 weeks until the LDL-C achieved the established level. Patients who achieved a reduction of LDL-C below 4 mmol/L after diet, maintained that diet for a further 4-6 weeks. Factor VII was evaluated at baseline and again at the end of treatment, when LDL-C levels had dropped as established. Both atorvastatin and dietary treatment significantly improved lipid profile. After adjustment for the decrease in triglycerides and factor VII levels at the baseline, FVIIc, FVIIAg were significantly decreased by drug therapy (FVIIc: from 145±22 to 122±22%, p<0.05; FVIIAg: from 124±17 to 108±20%, p<0.01); FVIIa levels were not significantly modified (from 69.7±17 to 54.5±21 mU/ml, p=0.30). In contrast, in spite of the improved lipid profile, dietary treatment did not significantly modify FVII profile (FVIIc: from 110±31 to 106±20%, p=0.30; FVIIAg: from 110±28 to 111±29%, p=0.54; FVIIa: from 54±44 to 49±28 mU/ml, p=0.35). Our study suggests a possible non-lipid-related mechanism by which atorvastatin therapy may significantly improve systemic factor VII profile in plasma of hyperlipidaemic patients. These effects may, in part, explain the beneficial effects of statins in primary and secondary prevention of coronary artery disease.

CO-070

PARTIAL REACTIVATION OF THE HAEMOSTATIC MECHANISM AFTER HIRUDIN DISCONTINUATION IN PATIENTS WITH ACUTE CORONARY SYNDROMES

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A transient rebound increase in thrombin generation and activity after cessation of heparin (H) has been observed in patients with acute coronary syndromes (ACS). The possible reactivation of the haemostatic mechanism after hirudin (D) discontinuation has been less investigated. We measured plasma levels of prothrombin fragment 1+2 (F1+2, RIA) and fibrinopeptide A (FPA, ELISA), markers of thrombin generation and activity, respectively, in 35 patients with ACS receiving hirudin or heparin in the GUSTO IIb study. Plasma levels of F1+2 and FPA were measured at baseline, after 3-5 days of drug administration immediately before drug cessation, and 3, 6, and 24 hours after drug termination. Data are expressed as medians (interquartile ranges):

	Pre-suspension	3h after	6h after	24h after
<i>F1+2</i>				
D	0.94 (0.76-1.13)	0.94 (0.71-1.15)	0.95 (0.75-1.10)	1.08* (0.86-1.28)
H	0.98 (0.89-1.22)	1.44** (1.02-1.61)	1.39** (1.04-1.72)	1.35* (1.09-1.67)
<i>FPA</i>				
D	0.90 (0.40-1.00)	1.10* (0.90-1.60)	1.45 (0.90-2.10)	1.60* (1.30-2.30)
H	1.10 (0.90-1.65)	1.40 (1.10-3.58)	2.60* (1.80-5.75)	3.10* (0.90-12.88)

(*p<0.05 ; **p<0.005 vs pre-suspension)

In conclusion, reactivation of the haemostatic mechanism was observed after hirudin and heparin discontinuation. The increase in thrombin generation and activity was significantly less pronounced in hirudin than in heparin-treated patients. However, in GUSTO IIb, the reactivation of procoagulant activity after drug cessation may explain the loss of clinical efficacy of both hirudin and heparin after termination of the treatment.

CO-071

IN ACUTE CORONARY SYNDROMES HOMOCYSTEINE LEVELS ARE HIGHER THAN IN CHRONIC STABLE ANGINA AND AFFECT THE RISK OF RESTENOSIS AFTER PRIMARY CORONARY ANGIOPLASTY

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Few data available in the literature suggest an increase in homocysteine (Hcy) plasma levels in the acute phase after a myocardial infarction (AMI) or a stroke, generating a hypothesis for the association between Hcy, inflammation and tissue damage. In order to investigate this issue, we compared: 106 patients (21 F/ 85 M; age 61 ± 9.7 yrs) with AMI who had undergone a primary coronary angioplasty (PTCA) (group A); 85 patients (20 F/ 65 M; age 62 ± 8 yrs) with effort angina (group B) and 103 healthy subjects (43 F/ 60 M; age 59 ± 14 yrs) as controls (group C). We assayed, on plasma samples collected before PTCA, Hcy plasma levels by HPLC method with fluorimetric detection. Hcy levels were significantly higher in group A (15.5 mmol/L (5.5-46.2)) with respect to group B (8.3 mmol/L (3.0-32.3); $p < 0.001$) and C (7.6 mmol/L (1.0-28.5); $p < 0.0001$). The OR for coronary artery disease (CAD) in the fourth quartile of Hcy with respect to the first quartile at multivariate analysis was higher in group A (OR 36.5; 95% C.I. 4.0-330; $p < 0.001$) than in group B (OR 12.1; 95% C.I. 1.8-81.4; $p < 0.001$). After a median follow up of 6 months, we found 18 angiographically documented restenoses (16.9%) in group A. In these patients Hcy levels within the fourth quartile were associated, at multivariate analysis, with a higher risk of restenosis (OR 10.8; 95% C.I. 1.1-90; $p < 0.05$). In group A we documented fibrinogen (Fg) levels higher than in group B and C (A: 336 ± 92 mg/dL; B: 302 ± 49.2 mg/dl/ $p < 0.0001$; C: 294 ± 43 mg/dL/ $p < 0.001$) and we demonstrated a significant correlation between Hcy and Fg levels ($r = 0.53$; $p < 0.01$). In addition, Fg levels within the third tertile were associated with an increased risk of restenosis at multivariate analysis (OR 11.9; 95% C.I. 1.2-99.4; $p < 0.05$), but this result was not confirmed after adjustment for Hcy levels (OR 6.3; 95% C.I. 0.5-85.6; $p = 0.1$). In conclusion, our findings document a possible relationship between elevated Hcy levels and the acute phase of CAD, and highlight the role of hyperhomocysteinaemia as a risk factor for restenosis after primary PTCA.

CO-072

HIGH CYSTEINE LEVELS IN RENAL TRANSPLANT RECIPIENTS: RELATIONSHIP WITH HYPERHOMOCYSTEINAEMIA. 5-10 METHYLENETETRAHYDROFOLATE REDUCTASE POLYMORPHISM AFFECTS THE RESPONSE TO THE THERAPY

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Cysteine (Cy) is a sulphur amino acid which has great structural similarity to homocysteine. No data are available about Cy levels in renal transplant recipients. The aims of our study were: 1) to determine

levels of Cy and homocysteine (Hcy) in renal transplant recipients; 2) to investigate the association between high levels of Cy, Hcy and the prevalence of 5-10 methylenetetrahydrofolate reductase (MTHFR). We investigated 70 stable renal transplant recipients (40M, 30F/age: 47 ± 8.5 yrs) and 66 age and sex-matched normal subjects as controls. Hcy and Cy levels were determined by HPLC with fluorimetric detection.; 5-10 MTHFR was detected by PCR followed by Hind I digestion. Cy levels were significantly higher in renal transplant recipients with respect to controls (254 μ mol/L (117-466) vs 198 μ mol/L (99-331); $p < 0.001$). Cy levels above the 90th percentile of controls (274 μ mol/L) were detected in 25/70 patients (35.8%). The fasting levels of plasma Hcy were significantly higher in renal transplant recipients than in controls (17.0 μ mol/L (4.0-68) vs 8.1 μ mol/L (2.0-24.0; $p < 0.0001$). Hyperhomocysteinaemia was diagnosed in 53/70 patients with a prevalence of 75.7%. The odds ratios for Cy and Hcy levels within the fourth quartile were markedly increased both in univariate and multivariate analyses, after adjustment for prevalent risk factors and GFR (Cy: 29 (95% CI 7.0-111); Hcy: 29.9 (95% CI: 7.5-118.1). The prevalence of the homozygous C677T genotype did not differ between patients and controls. The mutant allele frequency was 0.48 in patients and 0.47 in controls. After two months of vitamin supplementation based on folic acid (5 mg/die), vitamin B12 (0.5 mg/die) and vitamin B6 (50 mg/die) Hcy levels were significantly decreased ($p < 0.0001$). Patients with homozygous MTHFR polymorphism had the greatest percentage of decrease of Hcy levels with respect to the other genotypes (MTHFR C677T +/- 67.2%; +/- 62.2%; -/- 54.6%). In conclusion, this study demonstrates that in renal transplant recipients not only hyperhomocysteinaemia, but also high Cy levels may represent an independent risk factor. Vitamin supplementation reduces Hcy but not Cy levels and the entity of decrease appears to be influenced by the MTHFR genotype.

POSTERS

Oral anticoagulant therapy

PO-073

THE USE OF ORAL ANTICOAGULANTS IN PATIENTS WITH ATRIAL FIBRILLATION: FROM CLINICAL TRIALS TO CLINICAL PRACTICE

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Background. Atrial fibrillation (AF) is the most common chronic arrhythmia and is the cause of approximately 15% of all ischaemic strokes. Oral anticoagulant treatment (OAT) significantly reduces the risk

of thromboembolic complications and is particularly recommended in patients older than 65 years with concomitant risk factors and in all patients older than 75 years. *Methods.* An observational, cross-sectional study was carried out in one Italian teaching hospital to assess the prevalence of AF and to evaluate the use of antithrombotic drugs. Eight physicians reviewed the charts of all hospitalised patients in a single day, one every month for 5 consecutive months. *Results.* A total of 3121 charts were examined. The prevalence of AF was 7.2% (224 patients), mean age 78.4 years, 7.6% <65 years, 30.3% 65-75 and 62.1% >75. Among the 192 patients with chronic AF, 21.3% were on OAT, 29.7% on antiplatelet drugs, and 49% were not receiving any antithrombotic treatment. Patients on OAT were significantly younger than other patients (72.3 years, 80.6, and 80.7 respectively, $p < 0.0001$). In particular, only 11.3% of patients >75 years were on OAT. Among patients of age <75 yrs. with concomitant risk factors, 43.7% were on OAT and 29.2% were on antiplatelet drugs. Relative or absolute clinical contraindications to OAT were detected in 78 patients, but only 35.9% of the remaining 114 were actually treated. One third of patients had a history of transient ischaemic attacks or ischaemic stroke: secondary prevention with OAT was administered to 14.7% of them, 37.7% were on antiplatelet drugs and 52.4% were not receiving antithrombotic therapy. There were 32 patients with newly diagnosed AF (mean age 75.6), 7 (21.9%) started OAT. *Conclusion.* Despite clear evidence from clinical trials, OAT is significantly underused in patients with AF and the rate decreases with increasing age. In clinical practice the management of OAT is considered problematic, and OAT is often considered more dangerous to patients than AF. Anticoagulant clinics have been shown to reduce OAT-related risks and patients >75 yrs old should be addressed to these clinics in order to evaluate their possibilities of complying with the treatment. An effort to improve the accessibility to the clinics may be cost-effective.

PO-074

HOW MANY ELDERLY PATIENTS WITH CHRONIC ATRIAL FIBRILLATION CAN ACTUALLY BE TREATED WITH ORAL ANTICOAGULANTS?

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Background. Oral anticoagulant treatment (OAT) is clearly effective in preventing patients affected by atrial fibrillation from thromboembolic complications. In an observational, cross-sectional study carried out at the Ospedale di Circolo of Varese, Italy, to assess the prevalence of atrial fibrillation and the use of antithrombotic drugs we have confirmed previous reports of a significant underuse of OAT. Among hospitalised patients affected by atrial fibrillation only 21.3% were receiving oral anticoagulants. In the subgroup of patients older than 75 years, only 11.3%

were treated, often despite a history of ischaemic cerebrovascular events. OAT is commonly felt as unfeasible for patients older than 75. *Objectives and methods.* We prospectively evaluated patients affected by atrial fibrillation who were admitted to the departments of Internal Medicine and Geriatrics of the Ospedale di Circolo of Varese to assess potential contraindications to OAT which could explain its low use. Using a specific questionnaire, we first investigated the presence of clinical contraindications and subsequently the presence of personal problems to complying with the treatment or to attending an anticoagulant clinic. *Results.* One hundred patients with an average age of 80.3 years were included in the study. Of them, 28 (mean age 73.1) were on oral anticoagulants, 39 (mean age 84.4) on antiplatelet agents and 33 (mean age 81.5) were not receiving any treatment. Among the 72 patients (mean age 83.1) who were not receiving oral anticoagulants, we identified clear contraindications to OAT in 22 (30.5%, mean age 84): 15 (mean age 84.8) had clinical contraindications (2 prior bleeding, 1 active peptic ulcer, 5 dementia, 5 prior fall, 2 alcohol abuse) and 7 (mean age 82.2) were considered as non compliant, dependent and inadequately assisted. In 50 patients (69.5%) OAT was considered as potentially feasible despite the patient's age (mean 82.7): 17 (mean age 79.1) were independent, compliant with treatments and assisted by other family members; 33 (mean age 84.5) were dependent, but were closely assisted by family members or nurses in charge of their treatments and able to help them in attending frequent clinical controls. *Conclusions.* Regardless of age, most patients with chronic atrial fibrillation should receive the opportunity to be treated with oral anticoagulants. Anticoagulant clinics have been shown to reduce OAT related risks and a large proportion of patients older than 75 years do have the possibility to attend such clinics. Considering the high risk of thromboembolic complications, an effort to improve the accessibility to these clinics could be highly cost-effective.

PO-075

A SIMPLE PROTOCOL FOR THE MANAGEMENT OF DENTAL EXTRACTIONS IN PATIENTS ON ORAL ANTICOAGULANT TREATMENT

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Background. As assessed in a recent survey of oral and maxillofacial surgeons in North America, there is no consensus on the INR interval at which dental extractions can be safely performed (*MJ Troulis. J Oral Maxillofac Surg 1998; 56: 914*). We report here a simple protocol for reducing warfarin dosage in order to obtain INR values between 1.5 and 2, which can be regarded as safe for procedures having a high risk of bleeding. *Subjects and methods.* Seventy-eight consecutive patients (pts) on treatment with warfarin referred

for dental extraction at high bleeding risk to the Department of Oral and Maxillofacial Surgery of Modena University (46 males, 32 females; mean age 62 years, range 24-92). In each patient we preoperatively reduced warfarin dosage according to the following protocol, aimed at obtaining an INR value between 1.5 and 2:

- 4, 3 and 2 days before the extraction: half the usual dose;
- the day before: dose as usual;
- 6 hours after the extraction: double dose.

In all cases local measures (i.e. tranexamic acid mouth rinsing and sutures) were used to control postoperative bleeding. INR was determined the day of the extraction and one week thereafter; one month later a phone interview was performed in order to assess late complications. *Results.* In 71/78 (91%) of pts. preoperative INR values were within the expected range. Two (3%) pts had an INR higher than 2 (2.10 and 2.12) and 5 (6%) less than 1.5, but always higher than 1.4. After one week 100% of pts returned to the original INR value. Neither postextraction bleeding nor late thromboembolic complications were found. *Discussion.* Despite evidence that "it is time to stop interrupting warfarin therapy for dental surgery" (*MJ Wahl. Arch Intern Med 1998; 158: 1610-1616*), many dental surgeons still withdraw oral anticoagulants before invasive procedures in an empirical way. We demonstrate that our protocol is safe, simple to remember and to use, and obtains only a moderate reduction in INR values. We recommend that it be widely implemented for invasive procedures in patients at high haemorrhagic and/or low thromboembolic risk.

PO-076

DIFFERING SENSITIVITY OF THE PROTHROMBIN TIME TO DIFFERENT THROMBOPLASTINS: A FACTOR VII-DEPENDENT PHENOMENON

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The prothrombin time (PT) explores the extrinsic pathway and is crucial for monitoring oral anticoagulation. Interindividual differences in different commercially available thromboplastins (most of them derived from human placenta, others from rabbit brain), are known to account for the central role of this reagent in the determination of PT. On the other hand, in addition to sec and %, results are expressed according to the International Normalised Ratio (INR) in order to address the issue of the different sensitivities of PT to different thromboplastins. We evaluated a potential factor VII-dependence in the differing sensitivity of the PT to different thromboplastins in citrated blood samples from 157 patients (71 M, 86 F, 36 of whom taking warfarin – INR 2-3), consecutively referred to our Centre. Two

different thromboplastins were employed: a human placenta-derived thromboplastin (Thromborel S[®] Behring Dade) and a rabbit brain-derived thromboplastin (Thromboplastin-S[®] Dasit). They were compared in an automatic Sysmex TOA CA 6000 coagulometer, and the results were expressed in sec, in %, and as INR ratios. In the samples from the 121 non-anticoagulated patients, the levels of factor VII activity were also determined. When human placenta thromboplastin was used, mean prothrombin times were 15.4±7.75 sec; i.e. 76.5±28.42%; the mean INR being 1.36±0.74. Using rabbit brain derived thromboplastin, mean PT was 15.7±6.74 sec; i.e. 82.5±28.8%; the mean INR being 1.27±0.62. The r values (Pearson's) of these values were 0.988, 0.974 and 0.949 respectively, the p value always being <0.01, a finding consistent with an almost complete comparability of the two tests in this setting. When the whole sample was stratified into three groups (group A: FVII coagulant values <70%, group B: FVII 71-120%, and group C:>121%) maximal correlation between the results of the two thromboplastins (in sec, in % and as INR) was found in patients with factor VII levels <70% (r:0.983, p<0.01 for group A), the r and p values being 0.977 and 0.983 for group B and C, respectively, p always <0.01. This finding was confirmed in patients on oral anticoagulants (INR 2-3), the r and the p values (of the results expressed in sec, in % or as INR) in the latter setting always being >0.95 and <0.01, respectively, both before and after stratification for plasma factor VII levels. %. The Arg/Gln genotype of the gene coding for factor VII is known to be involved in the regulation of factor VII plasma levels. Its relevance in the observations reported above has been confirmed in the present setting. These data support the factor VII dependence of these results and the difficulty in detecting PT values with precision in the presence of raised levels of circulating plasma factor VII.

PO-077

PORTABLE MONITOR PROTHROMBIN TIME (CoaguChek) COMPARED WITH TWO DIFFERENT THROMBOPLASTINS: DOES LABORATORY ISI CALIBRATION IMPROVE RELIABILITY AMONG SYSTEMS?

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Background. The use of "near-patient" testing devices has increased in the last few years and many studies have demonstrated the good reliability of standard prothrombin time (PT) tests. However, the evidence that different INR can be obtained in the same patients with different systems remains a critical clinical issue. *Aim of the study.* 1) to evaluate whether reliability between CoaguChek and laboratory INR is comparable to INR performed with two different reagents; 2) to evaluate whether commercial calibration lyophilised plasmas improve reliability among systems. *Material and methods.* We evaluated

one hundred patients on stable oral anticoagulant therapy with different therapeutic INR ranges, depending upon clinical indication. A qualified operator performed CoaguChek PT (Roche Diagnostics, Germany) on all patients, on capillary fingertip blood. Subsequently, venous blood samples obtained from venipuncture, were collected in tubes containing sodium citrate 0.129 M, centrifuged and tested in double series within two hours with two thromboplastin reagents. Portable monitor results were compared with INR performed on an automated coagulometer (STA-R, Roche Diagnostics, Germany) using two different reagents (Neoplastin plus, rabbit brain, Stago and Recombiplastin, recombinant human tissue factor, Ortho Diagnostics). To calculate INR, lyophilized plasma calibrators (Behring) were used. Calibration of Both reagents was performed by testing 20 normal control donors. The mean normal prothrombin time was calculated for each reagent. *Results.* Good correlations were found among systems ranging from $r=0.945$ to $r=0.981$. Agreement between "hear-monitoring" and clinical laboratory results were calculated for the different reagents and after ISI calibration. 93.1% of the paired laboratory and "hear-monitored" INR were within 1.0 INR units with recombiplastin and 98% with neoplastin. After ISI calibration the agreement reached 95.1% with recombiplastin and 99.1% with neoplastin. *Comment.* Differences among systems are independent of the method used for INR determination. As calibration of conventional reagents shows better reliability, portable monitoring calibration could improve reliability when compared with conventional methods.

PO-078

DETERMINATION OF WARFARIN IN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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The effect of warfarin in patients on oral anticoagulants is usually assessed by the prothrombin time (PT) test with results expressed as International Normalised Ratio (INR). However, the measurement of the plasma levels of the drug may be required in some instances to investigate non-compliance of the patient, resistance to anticoagulation, drug metabolism and pharmacokinetics. Methods so far described are based on extraction of warfarin from plasma followed by gas chromatography or reversed-phase high performance liquid chromatography (HPLC). Extraction is the crucial step and may be performed either in liquid- or solid-phase, which requires careful conditions to ensure good recovery of warfarin before sample injection. The solid-phase extraction also requires the preparation of suitable columns, which makes the entire assay procedure time consuming and the sample preparation highly variable. In this report we investigated the suitability of the ready-for-

use, commercially available, miniature-cartridges (Oasis HLB, Waters, Milford, MA) for sample preparation in the measurement of warfarin by HPLC. One mL of test plasma or standards was applied to the cartridge, unbound material was then washed out with 1 mL 5% methanol in water. Bound material was eluted by 1 mL of 2% ammonium hydroxide in 60% methanol. Eluted material was evaporated to dryness under a stream of nitrogen at 40 °C. The residue was eventually dissolved in 100 µL mobile phase and injected into the HPLC system (Waters). Separation was performed on a 3.9x150 mm Symmetry Shield column (RP8, 5 µm, Waters) and peaks were detected with a tunable absorbance UV detector (Waters) at 280 nm. The eluent (25 mM potassium phosphate, pH 7 with 45% methanol) was applied at a flow rate of 1 mL/min (isocratic conditions). The height of peaks for standards and test plasma were measured and the concentration of warfarin was extrapolated from the calibration curve. The within-run reproducibility of the assay was estimated as CV by repeat measurements (n=6) on a plasma sample from a patient on warfarin therapy and was 4.6%. Recovery, estimated by measuring warfarin concentration on a plasma sample pooled from healthy subjects and spiked with known amounts of the drug, was 99%. In conclusion, this method proved to be easy to do, reproducible and specific for warfarin. It does not require sophisticated equipment other than the regular HPLC apparatus that is available in clinical laboratories.

PO-079

ORAL SURGERY IN PATIENTS ON ORAL ANTICOAGULANT: SHOULD ANTICOAGULANTS TREATMENT BE ALTERED?

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The management of patients on oral anticoagulant therapy who need oral surgery is controversial. There has been some debate as to whether, prior to minor oral surgery, anticoagulant treatment should be altered or not. The risk of serious postoperative haemorrhage has to be balanced against the potential for life-threatening thromboembolism. This randomised study was designed to compare two approaches in the management of these patients. A control group of 55 patients had their anticoagulant therapy stopped for 2-3 days prior to having oral surgery, resulting in a reduction in the average pre-operative INR from 2.5 to 1.7. The study group of 56 patients did not have their anticoagulant therapy altered before oral surgery, and had an average pre-operative INR of 2.7. All patients were treated under local anaesthesia on an outpatients basis and local measures consisted of sutures and haemostatic absorbable agents (the patients of the study group were treated with an oral rinse of tranexamic acid). None of the patients had any immediate postoperative bleeding, and only 3 patients from the control group and only 2 from the study group had mild

delayed haemorrhage, which was easily controlled with local measures (Tissucol). *Conclusion.* Despite a theoretical risk of haemorrhage after oral surgery in patients who are at therapeutic levels of anticoagulation, the risk appears to be minimal, the bleeding can usually be easily treated with local measures, and this risk may be greatly outweighed by the risk of thromboembolism after withdrawal of anticoagulation therapy.

PO-080 **DISCREPANT THROMBOPLASTIN SENSITIVITY TO CLOTTING FACTORS IN PATIENTS ON ORAL ANTICOAGULATION: IMPACT ON INR CALIBRATION**

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The prothrombin time (PT) is used to monitor oral anticoagulant treatment because of its sensitivity to vitamin K-dependent factors. To account for the different sensitivity of thromboplastin reagents to factor levels, the International Sensitivity Index (ISI) is assigned to thromboplastin reagents and/or INR calibrator plasmas are used. These approaches imply that the variation in the sensitivity of thromboplastin reagents is the same for all the vitamin K-dependent factors explored by the PT. We measured the PT of 92 patients on stable oral anticoagulant treatment and 23 healthy subjects with 2 reagents (Neoplastin Plus, rabbit brain, NP, and Recombiplastin, recombinant human tissue factor, REC), in random order using the same coagulometer (STA). Factor VII, X, II and V activities were also measured on frozen plasma samples from patients and controls with the two thromboplastin reagents in immunodepleted plasmas (Stago). As expected, PT ratios (sec/MNPT) differed with the two reagents (NP: 2.20 ± 0.62 vs REC: 3.04 ± 1.34 , $p=0.0001$). All clotting factor levels also differed significantly with the two reagents ($p=0.0001$). In a generalised linear model, the reciprocal of factor VII ($p=0.0001$), X ($p=0.0001$), and II levels ($p=0.0001$) were independent predictors of the PT ratio with NP ($r^2=0.857$). Reciprocal of factor II ($p=0.001$) and VII levels ($p=0.0001$) only were independent predictors of the PT ratio with REC ($r^2=0.737$). In addition, the difference in PT ratios with the two reagents was also a function of the difference in factor V ($p=0.001$), X ($p=0.012$), and VII levels ($p=0.03$, $r^2=0.23$). These data point to a discrepant sensitivity of the two thromboplastin reagents to clotting factors measured by the PT. In a Bland-Altman plot (NP-REC) with log-transformed data, the bias in PT ratios (NP-REC) was -24.2% ($p=0.0001$) with limits of agreement $+40\%$ and -59% . After calibration with INR calibrator plasmas (Immuno), the bias in INRs (NP-REC) was -6.5% ($p=0.0001$) with limits of agreement $+16\%$ and -19% . Because clotting factor levels differed with the two reagents in INR calibrator plasmas as they did in the patient plasmas, our results indicate that discrepant

thromboplastin sensitivity to clotting factors is responsible for reagent-dependent variability in calibrated INR values.

PO-081 **ANTICOAGULATION CLINICS: A COST-BENEFIT ANALYSIS**

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Introduction of the INR system and definition of therapeutic ranges have contributed to reduce the complication rate of patients on oral anticoagulant treatment. However, the relatively low incidence of major bleeding and/or thrombotic complications recently reported derives from studies mainly carried out in anticoagulation clinics (AC), services organised to provide patients' education, laboratory monitoring, and to record bleeding and thromboembolic episodes, changes in patients' habits, inter-current diseases and co-medications, with the aim of tailoring the optimal dose of oral anticoagulant drugs. Because of the inherent costs of anticoagulation clinics we performed a meta-analysis of published reports with the aim a) of comparing complication rates recorded in AC vs those of patients managed by routine medical care (RMC), and b) of comparing the economic burden related to the two strategies of patient management (AC vs RMC). We evaluated 13 studies conducted in AC, 4 in RMC, and 7 studies directly comparing the two strategies of management published between 1977 and 1998. Odds ratios (OR) for major bleeding (2,612 patient-years) and for thrombosis (2,246 patient-years) in the 7 comparative studies were 0.49 (95% C.I.: 0.31-0.76) and 0.22 (0.13-0.37). Corresponding OR derived from non-comparative studies were 0.30 (0.25-0.36, 21,114 patient-years) and 0.31 (0.21-0.45, 11,412 patient-years). These data indicate that both major bleeding and thrombotic complications are reduced by 50 to 75% if patient management is carried out in AC. In the ISCOAT studies carried out in 31 Italian AC (2,011 patient-years), the prevalence of major bleeding and thrombotic complications were 1.35 per 100 patient-years and 3.5 per 100 patient-years respectively. By applying Italian DRG rates for any type of complication recorded and adding inherent AC management costs (213 Euro per patient-year), an average economic burden of 366,1 Euro per patient-year was obtained. We assumed that if managed by RMC, patients enrolled in the ISCOAT studies would have suffered a higher rate of complications according to the OR calculated from comparative studies, with the same distribution of outcomes. By this simulation, and including costs related to laboratory monitoring (65.7 Euro per patient-year), an average economic burden of 688.5 Euro per patient-year would be anticipated. Thus, even without taking into account complication-related mortality, permanent disability and social costs, management of patients on oral anticoagulant treatment in anticoagulation clinics leads to an estimated 50% savings in economic resources.

PO-082**ACCURACY OF A PORTABLE PROTHROMBIN TIME MONITOR (COAGUCHECK) IN PATIENTS ON CHRONIC ORAL ANTICOAGULANT THERAPY: A PROSPECTIVE MULTICENTRE STUDY**

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A portable prothrombin time (PT) monitor allows patients on oral anticoagulant (OAC) therapy to measure their PT at home. *Aim of the study.* 1) To evaluate the accuracy and reliability of a portable PT monitor (Coagucheck, Roche Diagnostics, Germany) as compared with laboratory methods in four centres (n.1, n.2, n.3, n.4) of the Italian Federation of Anticoagulation Clinics (FCSA). *Experimental design:* Prospective study. *Study subjects:* seventy-eight subjects on stable OAC therapy (48 men, 30 women, age range: 40-75 years) were selected on a volunteer basis. Dual measurements of INR values were performed in each subject: both from finger capillary blood by the monitor and from venous blood by the anticoagulation clinic laboratory. PT laboratory measurements were performed using the photo-optical MLA Electra 1600 instruments in centres n.1 and 3, MLA Electra 1400 (Hemoliance, Instrumentation Laboratory, Italy) in centre n.4 and STA (Roche Diagnostics, Germany) in centre n.3. The thromboplastin used for PT laboratory measurements were Neoplastin Plus reagent (Roche Diagnostics, Germany, ISI: 1.26) in centre n. 2 and Recombiplastin (Hemoliance, Instrumentation Laboratory, Italy, ISI: 0.9-1) in the other three centres. *Results.* The mean bias and limits of agreement in INR units were 0.07 (+0.51/-0.65), +0.19 (+0.96/-0.67), +0.12 (1.13/-0.89) and -0.40 (+0.72/-1.5) respectively in centres 1,2,3 and 4. The mean bias for INR values lower than 2.0 was -0.07, +0.075, -0.13 and -0.28, respectively, in centres 1,2,3 and 4. The mean bias for INR values between 2.0 and 3.0 was -0.11, +0.17, +0.11 and -0.3, respectively in centres 1,2,3 and 4. The mean bias for INR values greater than 3.0 was -0.021, +0.313, +0.23 and -0.40, respectively, in centres 1,2,3 and 4. In centre 4 both laboratory mean INR values (2.41) and Coagucheck mean INR values (2.09) were significantly lower than in the other Centres because of a high number of patients (18/20) in the low therapeutic range 2.0-3.0 INR (mean laboratory INR values: 2.93, 2.57, 2.86, respectively in centres 1,2,3; mean Coagucheck INR values: 2.72, 2.76, 2.91, respectively in centres 1,2,3). The repeatability coefficients of monitored INR results were 0.23, 0.461, 0.289 and 0.212, respectively in centres 1,2,3 and 4. The variation coefficient was 3.66 %, 8.5%, 4.68% and 4.9%, respectively in centres 1,2,3 and 4. *Conclusions.* Our data confirm that Coagucheck has an acceptable level of accuracy for INR values in the range between 2.0-3.0.

PO-083**ASSESSMENT OF FACTOR IX ACTIVITY IN SUBJECTS ATTENDING AN ANTICOAGULATION CLINIC**

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Markedly reduced factor IX (FIX) levels, leading to aPTT prolongation and associated with severe bleeding complications during oral anticoagulant treatment (OAT) have recently been described in patients (pts) carrying the Ala10Thr mutation in the FIX gene. We aimed at assessing: a) whether a higher incidence of bleeding complications was present in subjects with prolonged aPTT, caused by FIX reduction, and b) whether it may be useful, to reduce the bleeding risk, to identify subjects with markedly prolonged aPTT during OAT. In ten working days 582 pts (mean age 70.0 y, range 18-91 y; M/F 308/274), among those presenting to our out-patient clinic, were included in the study according to the following inclusion/exclusion criteria: 1) OAT lasting > 6 weeks, 2) INR value within the intended therapeutic range on the day of analysis and at least at the previous control, and 3) exclusion of LAC phenomenon. On the day of analysis both PT (INR) and aPTT were performed, and plasma aliquots frozen. The aPTT distribution was assessed in the pts grouped in classes of increasing INR values (each class covering a 0.2 INR difference); FIX activity was determined in samples whose aPTT was distributed in the first and fifth quintiles for each INR class.

	Pts of the 1st quintile in all INR classes (Mean ± DS; n=118)	Pts of the 5th quintile in all INR classes (Mean ± DS; n=116)	p value
INR	2.77 ± 0.52	2.79 ± 0.49	0.81
aPTT (ratio)	1.21 ± 0.11	1.65 ± 0.24	<0.0001
FIX (IU/ml)	0.44 ± 0.14	0.29 ± 0.09	<0.0001

Only one patient, whose INR was in the 3.4-3.6 class and aPTT was 1.63, had a very low FIX (0.03 IU/mL), compatible with the presence of the Ala10Thr FIX gene mutation. By using the clinic's computerised database system, the number of bleeding events during treatment was retrospectively examined; there were 21 (1 major) and 29 (2 major) bleeds in the pts of the first (total follow-up 441 y) and fifth (464 y) aPTT quintiles, respectively (rate: 4.8 vs 6.2% pt/y, n.s.). The subject with very low FIX activity had experienced a minor bleeding event (hematoma) during treatment. In conclusion, FIX during oral anticoagulation seems to vary greatly between subjects in spite of similar achieved anticoagulation intensity (measured by PT-INR). Although the rate of bleeding events was not significantly higher in subjects with lower FIX levels, the procedure adopted in the present study was able to identify the one subject with a very low FIX. It is worth identifying this condition since the case we found had

experienced a bleeding event and his high target could subsequently be reduced.

PO-084

REBOUND HYPERCOAGULABLE STATE AFTER ORAL ANTICOAGULANT THERAPY STEPWISE DISCONTINUATION IS MORE MARKED IN PATIENTS WITH SPONTANEOUS VENOUS THROMBOEMBOLISM

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Previous studies revealed a rebound hypercoagulable state in patients treated with warfarin for venous thromboembolism (VTE) after discontinuation of oral anticoagulant therapy (OAT), with both abrupt and gradual interruption of warfarin. Gradual withdrawal of OAT is followed by less intense and shorter lasting clotting activation. The aims of this study were to evaluate: 1) whether clotting activation is different in patients with spontaneous VTE (sVTE) in comparison to patients with transient risk factors for VTE (trfVTE); 2) whether the rebound hypercoagulable state has a prognostic significance for recurrent VTE. We investigated 59 patients who had suffered from VTE (21 with sVTE and 38 with trfVTE) and who attended the Anticoagulation Clinic of Florence for monitoring OAT. They had clinical indications for warfarin withdrawal after at least 6 months of OAT (therapeutic range INR 2-3). The scheme of anticoagulation withdrawal was 2/3 of the previous week's dose on the first week, 1/3 on the second week. The OAT was completely stopped from the third week. Laboratory tests (prothrombin fragment F1+2, D-dimer) were performed at baseline (T0), once a week for the first three weeks (T1, T2 and T3) and after 6 weeks (T4). All patients were invited to contact the Clinic in the presence of suspected VTE recurrence and were contacted by phone twice a year. Patients were followed-up for a median time of 17 months (range 3-56 months). F1+2 levels increased progressively from T0 to T3 (p at least <0.01) showing the highest levels at T3 (1.2 nmol/L (0.5-10)). At T4 F1+2 levels were not different in comparison to T3 and were still significantly higher with respect to baseline ($p<0.001$). D-dimer levels did not change from T0 to T2. From T2 to T3 D-dimer concentrations increased significantly ($p<0.005$) and remained higher than T0 at T4 ($p<0.005$). Considering diagnosis, we observed that patients who suffered from sVTE showed significantly higher F 1+2 levels at T2 ($p<0.05$) and a similar trend at T3 in comparison to patients with trfVTE. D-dimer levels were significantly higher in patients with sVTE at T3 and T4 (p at least <0.005) with respect to the other patients. During follow-up, 5 patients (2 out of the 21 patients with sVTE and 3 out of the 38 patients with trfVTE) had recurrence of deep vein thrombosis. Recurrences occurred 6-31 months after warfarin withdrawal (median time 9 months). No differences in F1+2 or

D-dimer levels were observed between patients with recurrence and those without. In conclusion, we have confirmed the occurrence of a hypercoagulable state after OAT withdrawal. These preliminary data indicate that this behaviour is more intense in sVTE patients.

PO-085

INCREASING WORKLOAD OF ANTICOAGULATION CLINICS AND COST BENEFITS OF COMPUTER ASSISTED MANAGEMENT: THE P.A.R.M.A. NET

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The number of patients that can benefit from oral anticoagulant therapy (OAT) is rising and proportionally increases the burden on medical, nursing and administrative staff of anticoagulation clinics. Computerised support systems for better patient care therefore became mandatory. The P.A.R.M.A. Program was developed in 1986 by THC of Parma in collaboration with the Instrumentation Laboratory. P.A.R.M.A. was born to help physicians in dosage prescription and data storing and has been gradually improved over the years by implementing several dedicated algorithms such as 'previous dosage confirmation', 'dosage change suggestion' and 'maintenance-dose suggestion' (calculated from the 4th day INR response to the induction dose). From 1996 to 1999 a web connection was introduced to perform all tasks of the OAT centre (patient reception, venous puncture room, laboratory and therapy prescription): the "P.A.R.M.A. system" became "P.A.R.M.A. net" allowing a great reduction of work load and shortening patient waiting time. Consequently a major cut in the management of costs was reached by improving the service efficiency. We are able to carry out a comparison between costs of traditional prescriptions (in which clinical and laboratory data storage, work lists, results transcription and dosage prescription reports are manually executed on paper forms) and the computerised system, including the web link strategy (patient's data storage and prescription print outs with the P.A.R.M.A. system and informatisation of all tasks of thromboses centre with P.A.R.M.A. net). Costs for a single visit were quantified by adding fixed, variable and general costs. Fixed costs, not dependent on the patient management system, include reagents, disposables, instruments pay off and must be considered equal for the three systems. Variable costs depend strictly on the patient management and are considered in relation to the salary of physicians, nurses and technicians involved. General costs depend on heating, cleaning, phone expenses etc. and are calculated on the basis of 20% of previous costs. On this basis we calculated a cost of 11.11 Euro for each traditional visit, 8.38 for a P.A.R.M.A. system visit and 6.77 Euro for a P.A.R.M.A. net one. In this way, for instance, the saving for a centre that monitors 500 patients per year

and therefore carries out about 9,000 visits per year is about 24,570 Euro with P.A.R.M.A. system and 39,060 Euro with P.A.R.M.A. net. Consequently the P.A.R.M.A. system and P.A.R.M.A. net allow substantial financial saving (about 25% to 40% respectively) as compared to traditional management.

PO-086

IMPROVED QUALITY OF ORAL ANTICOAGULANT TREATMENT BY COMPUTERISED DOSING: THE P.A.R.M.A. SYSTEM

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The number of patients receiving oral anticoagulant therapy is constantly increasing world-wide. This has led to the development of computerised support system for a better management of patient care. The aim of our study was to compare the performance of a computerised system (P.A.R.M.A. System) for prescribing anticoagulant therapy (computer group, C) with traditional decisions by experienced medical staff (manual group, M) in achieving the INR therapeutic range. The P.A.R.M.A. system has been developed at the THC of Parma in collaboration with the Instrumentation Laboratory and has been gradually improved over the years by implementing several dedicated algorithms. We retrospectively analysed data from the ISCOAT study (Palareti, Lancet, 1996). This study was not infact designed to evaluate the computer performance and each participant Centre managed patients according to his own system only. Thirty-three centres participated in the ISCOAT study (19 in C and 14 in M); a total of 2619 patients were recruited (1761 in C and 858 in M) with a follow up of about 2891 patient/year (2096 in C, 765 in M) for a total of more than 67000 visits in three years of follow-up. The proportion of time spent within, below or above the therapeutic range and the incidence of haemorrhagic and thromboembolic events were considered in the two groups. Patients monitored with the P.A.R.M.A. system spend more time within therapeutic ranges than those managed manually. These differences were statistically very significant, in fact, we observed that they were 66% vs 58% in the first year; 71% vs 61% in the second year and 72% vs 58% in the third year of time was spent within therapeutic range by patients of C group compared to the M group. We found that this significant improvement was obtained by shortening the time the C group spent at levels below the therapeutic range in comparison with that which occurred in the M group. We also observed that the number of total haemorrhagic events was significantly higher in the C group than in the M group. However the number of major complications was not significantly different and most of the haemorrhagic events in the computer group were due to minor haemorrhages. In contrast, a significantly lower percentage of thrombo-embolic events was observed in the computerised patients probably because they spent less time below the therapeutic

range. In conclusion, from these data derived from a study not specifically designed to compare the benefits of computer in respect to manual traditional dosing, we can say that the P.A.R.M.A. system has been demonstrated to be able to improve the treatment quality in comparison to manual method, by increasing the time spent by patients within the therapeutic range. Moreover the use of the P.A.R.M.A. system has allowed the patients to spend less time below the therapeutic range and therefore to have minor probabilities of thrombo-embolic complications.

POSTERS

Arterial thrombosis: risk factors

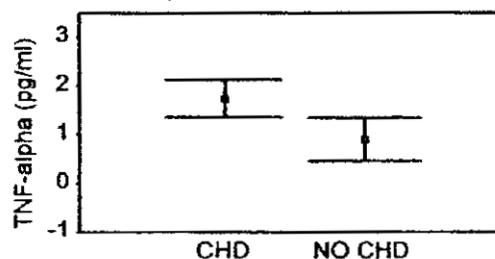
PO-087

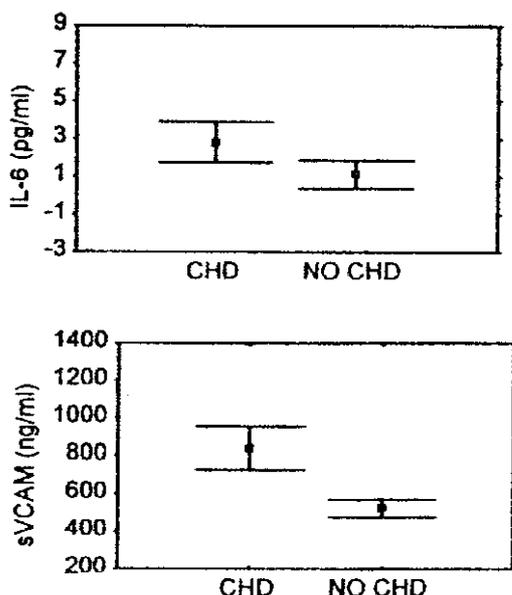
RELATION OF CYTOKINE PRODUCTION AND *HELICOBACTER PYLORI* INFECTION TO CORONARY HEART DISEASE

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Epidemiological data have identified associations between clinically active atherosclerosis and serological evidence of infection with *Helicobacter pylori* (HP). HP is pathogenetic because it triggering leukocyte infiltration of the gastric submucosa, which is mediated by pro-inflammatory cytokines. The role of inflammatory mechanisms in the initiation and progression of atherosclerosis is increasingly acknowledged. This study was, therefore designed to evaluate the behaviour of some cytokines and adhesion molecules [IL-1 β , IL-6, TNF- α , soluble (s)P-selectin, sE-selectin and sVCAM] in 28 dyspeptic patients affected by chronic gastritis [diagnosed by oesophagogastroduodenoscopy (OGDS)] with (n=14) and without (n=14) coronary heart disease (CHD). A correlation with the presence of HP infection was performed. The presence of HP in gastric-biopsies was determined by histological examination and the urea test. CHD patients were treated with beta-blockers or Ca-antagonists; antiplatelet therapy was discontinued 15 days before OGDS. Cytokine and adhesion molecule levels were determined by enzyme-immunoassays (R&D Systems, Inc.).





The results obtained demonstrated the presence of higher of IL-6 ($p<0.03$), TNF- α ($p<0.03$) and sVCAM ($p<0.02$) in CHD patients than in patients without CHD (see Figures). Conversely, sP-selectin, sE-selectin and IL-1 β did not show any difference between the two groups of patients. No difference in the cytokine or adhesion molecule profile was observed between CHD patients with and without HP infection, probably due to the small size of the samples; however, a comparative analysis of the variables analysed in the study demonstrated significant correlations between TNF- α and IL-6 ($\rho=0.721$, $p=0.05$) or sVCAM ($\rho=0.857$, $p=0.01$) only in CHD patients with concomitant HP infection. IL-6 levels correlated directly with sVCAM ($\rho=0.739$, $p=0.05$) in the same group of patients. The findings reported suggest that HP infection might trigger a chronic inflammatory state, as reflected by elevated TNF- α , IL-6 and sVCAM levels as well as their correlations, which may induce endothelial dysfunction and provide a link between HP infection and cardiovascular disease.

PO-088 FACTOR XIII-A GENE MUTATION (Val34Leu) AND ARTERIAL VASCULAR DISEASE

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Factor XIII (FXIII) when activated by thrombin in plasma, forms a stable fibrin clot by covalent crosslinking of the α - and γ -chains of fibrin conferring to crosslinked fibrin a higher resistance to fibrinolysis than non-stabilized clot. FXIII circulates in plasma as a tetramer of two catalytic A-subunits and two accessory B-subunits. Mutations in the FXIII A-sub-

unit gene are classically related to a tendency to spontaneous bleeding. Recently a G to T point mutation in the exon 2, codon 34 of the A-subunit gene has been described. It codes for a valine to leucine (Val34Leu) substitution close to the thrombin activation site. The mutation appears to be protective against myocardial and brain infarction and is also associated with primary intracerebral haemorrhage suggesting a role in both thrombotic and haemorrhagic disorders. We evaluated the prevalence of the Val34Leu mutation in a group of 360 patients with arterial vascular disease (AVD), and in a group of 200 healthy controls. Among the cases with arterial disease, 120 had coronary artery disease (myocardial infarction $n=70$; angina $n=50$), 120 had cerebrovascular disease (cerebral infarction $n=65$; transient ischaemic attack $n=55$) and the remaining 120 patients suffered from peripheral arterial occlusive disease. The frequency of the mutated allele in the populations examined is reported in the table. The significant difference obtained comparing the number of the T alleles of the whole AVD group with the group of controls slightly increased when cases with angina and transient ischaemic attacks were excluded ($p<0.04$ and $p<0.02$ respectively):

	AVD (all)	AVD (excluding angina and TIAs)	Controls
FXIII G	582 (80.8%)	418 (82.0%)	301 (72.25%)
FXIII T	138 (19.2%)	92 (18.0%)	99 (24.25%)
Total alleles	720	510	400
<i>p</i>	<i>p<0.04</i>	<i>p<0.02</i>	--

Although, this rise was not statistically significant, it is to note that a large part of the T alleles were observed in the subgroup of arterial disease with angina or transient ischaemic attacks. This common mutation in the FXIII-A gene is to be considered as a novel factor with a role in the aetiology of atherothrombotic disorders.

PO-089 PREVALENCE OF RISK FACTORS FOR INHERITED THROMBOPHILIA AMONG YOUNG ADULTS WITH MYOCARDIAL INFARCTION

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Both older age and male gender are strongly associated with the incidence of myocardial infarction (MI): myocardial infarction is rare among persons less than 45 years of age and the incidence is particularly low among young women. Among the young, myocardial infarction occurs rarely in the absence of major coronary heart disease (atherosclerotic) risk factors; and, multiple risk factors, including current smoking, obesity, hypercholesterolaemia, hypertension, and diabetes, are typically present. Several studies have examined the association of genetic mutations related to thrombotic markers with myocardial

infarction, but findings of studies that have examined this association are inconsistent. We analysed the rate of 20210 A prothrombin gene variant and 506 Q factor V mutation as risk factors for MI among young adults of age <45 years. Our study included 27 patients (21 male, 6 female) observed over a period of one year. The median age at the time of MI was 44.5 years (range 21-45). The control groups included 35 subjects matched for age and sex. No significant variation was found between the patient group and the controls regarding the prevalence of factor V Leiden. The prothrombin variant was found in 4 patients and 2 controls (14.8% VS 5.7%, $p=0.229$), odds ratio of 2,87 (95% CI, 0.484 to 17.01). Twelve out of 27 patients had one or more metabolic risk factors (hypertension, diabetes mellitus, hyperlipoproteinaemia), fourteen were smokers, and six had a family history of thrombosis. In our experience factor V Leiden and 20210 A prothrombin gene variant are not associated with a significant risk of MI in young patients. The analysis of larger series is necessary in order to clarify the role of the prothrombotic genetic polymorphisms in coronary heart disease.

PO-090

PREVALENCE OF RISK FACTORS FOR INHERITED THROMBOPHILIA AMONG YOUNG ADULTS WITH ISCHAEMIC STROKE

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The prevalence of ischaemic stroke (IS) affecting adults under the age of 45 years ranges from 3 to 12% world-wide and its aetiology remains unknown in approximately 30% of the cases. The purpose of this study was to analyse the role of the following genetic variants as risk factors for IS among young adults: prothrombin gene variant (20210 A) and factor V Leiden (506 Q). Our study included 28 patients (11 male, 17 female), with a median age at first ischaemic event of 39.5 years (range 19 to 45 years) observed over a period of one year at our Neurology Division and 35 controls matched for age and sex. The diagnosis was based on a clinical and neurological examination and at least one objective diagnostic method such as computed tomography or magnetic resonance imaging of the brain in all patients. The patients with a source of cardiac embolism, myeloproliferative diseases or the following metabolic risk factors for arterial disease were excluded: hypertension, diabetes mellitus, hyperlipoproteinaemia and smoking. Factor V Leiden mutation was found in 4 patients and 1 control (14.2% VS 2.8%, $p=0.09$), odds ratio of 5.66 (95% C.I., 0.595 to 53.94). The 20210 A prothrombin variant was found in 5 patients and 2 controls (17.8% VS 5.7%, $p=0.04$), odds ratio of 7.39 (95% C.I., 0.809 to 67.50). All of the patients was identified with combined inherited risk factors

(heterozygous for factor V Leiden and heterozygous for prothrombin 20210 A variant). A high prevalence of prothrombin mutation in the control group probably was overlooked due the small size of the sample. In our experience the prothrombin gene variant is a significant risk factor for IS in young adults. Molecular analysis of FV and FII should be included in a screening program me these of patients in the setting.

PO-091

WHOLE BLOOD VISCOSITY DOES NOT INCREASE WITH AGING

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In the last decade several prospective epidemiological studies showed that increased whole blood viscosity, primarily dependent upon haematocrit value and fibrinogen concentration, is a major risk factor for ischaemic heart disease and stroke. At the same time many investigations found that plasma fibrinogen concentration rises with advancing age. A few studies explored the relationship between rising age and whole blood viscosity. Nevertheless, some reports based on the results of investigations carried out on very small population samples, tend to state that elderly healthy subjects display increased whole blood viscosity. The aim of this study was to evaluate the relationship of whole blood viscosity and its major determinants to rising age in both sexes, considering the role of hemorheological abnormalities in the prevalence of ischaemic heart disease and stroke, that are the major causes of death in an aging population. *Subjects.* A total of 249 subjects (mean age 49.9 ± 21.5 ; range 19-102 years) were included in the study. They were divided into three groups: [(A) <30 years of age, n° 58; (B) 30-60 years, n° 107; (C) >60 years, n° 84]. *Methods.* Whole blood viscosity at two different rate of shear (450 and 45 sec^{-1}) was evaluated using a cone-plate digital viscosimeter. The haematological parameters (haematocrit, haemoglobin and blood cell's count) were evaluated using an automatic Coulter Counter. Plasma fibrinogen concentration was measured by a clotting method. *Results.* When both sexes are considered together, whole blood viscosity shows no significant difference among age groups. Plasma fibrinogen concentration significantly increases with age, haemoglobin, red blood cell count and platelet count, on contrary, are significantly lower in aged group. Only in the male sex blood viscosity at higher shear rate (450 sec^{-1}) significantly correlates in a negative manner with the rising age. *Conclusion.* Whole blood viscosity doesn't significantly change with rising age in female sex. Whole blood viscosity at higher shear rate significantly decreases with ageing in male sex. The age-related decrease of haematocrit value in male sex accounts for this event.

PO-092**THE G20210A PROTHROMBIN GENE MUTATION IS INCREASED IN ACUTE CORONARY SYNDROMES WITHOUT METABOLIC OR ACQUIRED RISK FACTORS OR WITH LIMITED EXTENT OF DISEASE**

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Background. The G20210A prothrombin gene mutation is an established risk factor for venous thrombosis, but its role in ischaemic heart disease is matter of debate. The prevalence of this mutation in patients with acute coronary syndromes without major risk factors has not been investigated. The aim of our study was to evaluate the prevalence of G20210A allele among patients with ischaemic heart disease. **Patients and methods.** The polymorphisms G/A at locus 20210 of the prothrombin gene and at locus 1691 of factor V were investigated in 247 patients <65 years of age with myocardial infarction (n=190) or unstable angina (n=57) and in 247 healthy age- and sex-matched controls. Coronary angiography in 156 patients revealed not-significant or single-vessel disease in 81 and multivessel disease in 75. **Results.** The prevalence of the 1691A factor V allele was similar in cases and controls (odds ratio OR 1.1, 95%CI 0.4-3.2). The prevalence of the 20210A prothrombin mutation was 2.8% in controls and 6.5% in patients (OR 2.4, 95%CI 1.0-5.9), increasing to 8.7% in those with a family history of myocardial infarction (OR 3.3, 95%CI 1.2-9.1), to 9.8% in those with <1 vessel disease (OR 3.8, 95% CI 1.3-10.8), and to 13.0% in the normocholesterolaemic, non-diabetic, normotensive and non-smoking group (OR 5.1, 95%CI 1.2-21.4). No homozygous individuals were found. **Conclusions.** The 20210A prothrombin genotype appears to contribute significantly to the genesis of acute coronary syndromes, especially in patients with a family history of myocardial infarction, without other major risk factors or with limited coronary disease. Inherited hypercoagulability may thus predispose to acute ischaemia in the absence of extensive atherosclerosis or in the absence of major metabolic and acquired risk factors.

PO-093**ANGIOPLASTY INCREASES INTRACORONARY F₂-ISOPROSTANE FORMATION. EVIDENCE FOR *IN VIVO* OXIDATIVE STRESS DURING ANGIOPLASTY**

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Isoprostanes, stable end-products of oxygen free radical mediated-lipid peroxidation, were measured

in the coronary vessels during percutaneous transluminal coronary angioplasty (PTCA) to provide direct evidence for enhanced oxidative stress in a local milieu *in vivo*. PTCA is associated with complications such as myocardial stunning and accelerated restenosis, which at least in part are mediated by oxygen free radicals. Because isoprostanes are markers of oxidant stress and potent vasoactive compounds, the formation of which is not inhibited by aspirin treatment *in vivo*, it is possible that these mediators are increased locally during PTCA. In 12 coronary artery disease patients who were given aspirin and ticlopidine, blood samples from the coronary sinus were taken immediately before and immediately upon balloon deflation during PTCA. Isoprostane F_{2α}-III, isoprostane F_{2α}-VI, and TxB₂ were quantified after extraction and chromatography using a stable dilution isotope gas chromatography/mass spectrometry assay. Intracoronary blood levels of iPF_{2α}-III and iPF_{2α}-VI at baseline were (mean±SEM) 40±9 pg/mL and 115±10 pg/mL, respectively. TxB₂ levels were undetectable. After PTCA isoprostane levels markedly increased (mean±SEM): iPF_{2α}-III, 125±12 pg/mL (p<0.001); iPF_{2α}-VI, 295±20 pg/mL (p<0.001), whereas TxB₂ levels remained undetectable. These results indicate that PTCA induces coronary sinus increase in F₂-isoprostane formation and provide direct evidence for enhanced oxidative stress in a local milieu *in vivo*. Thus, increased F₂-isoprostane formation could play a role in the pathogenesis of some untoward PTCA-associated events.

PO-094**HAEMOSTATIC ABNORMALITIES IN END-STAGE HEART FAILURE**

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Background. Patients with heart failure are at increased risk of thromboembolic events. Both blood stasis and endothelial dysfunction have been recognised to be involved in haemostatic imbalance. In patients with end-stage heart failure the haemostatic alterations could become particularly severe and could preclude a successful cardiac transplantation. **Methods.** In the present study we evaluated coagulation, fibrinolysis, the contact system and endothelial parameters in 30 male patients with end-stage heart failure (NYHA class IV resistant to intravenous inotropic treatment, aged from 22 to 65 years; mean 54±10) compared with 30 healthy controls matched for age and gender with patients. Plasma levels of thrombin-antithrombin (TAT) complexes, prothrombin fragment F1+2, plasmin-antiplasmin (PAP) complexes, fibrin fragment D-Dimer, tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor 1 (PAI-1), von Willebrand factor (vWF), factor VII antigen (FVII:Ag) and activated factor XII (FXIIa) were measured by immunoenzymatic methods,

fibrinogen by a coagulometric method and cleaved high molecular weight kininogen (HK) by SDS-PAGE and immunoblotting analysis. **Results.** Plasma levels of TAT, PAP, t-PA, D-dimer, fibrinogen and vWF were significantly higher in patients than in normal controls ($p=0.0006$ - $p=0.0001$). Plasma levels of FVIIAg and PAI-1 were significantly lower in patients than in controls ($p=0.0009$ - $p=0.03$). F1+2, XIIa and cleaved HK were not significantly different in patients compared with controls. Patients receiving oral anticoagulant treatment (OAT) ($n=12$) had lower levels of FVII:Ag and F1+2 than patients not receiving OAT ($n=18$) ($p<0.001$); patients not receiving OAT had F1+2 levels higher than normal controls ($p=0.01$). FVII:Ag and F1+2 plasma levels were inversely correlated with INR values ($r=-0.753$ and $r=-0.606$ respectively). **Conclusions.** Our results indicate that patients with end-stage heart failure have increased levels of markers of coagulation and fibrinolysis activation and of endothelial dysfunction. The clinical relevance of the reduction of F1+2 induced by OAT in patients with end-stage heart failure deserves further investigation.

PO-095

ENDOTHELIAL PERTURBATION IN DEMENTIA

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Introduction. Vascular endothelium is known to play an aetiopathological role not only in vascular dementia (VD), but also in Alzheimer's disease (AD). Histochemical and *in vivo* studies have shown the presence of proteins involved in the haemostatic process such as factor XII, within the neuritic plaques. Thrombomodulin (TM) is a high affinity receptor for thrombin and it is also considered a molecular marker of endothelial damage. In a previous study, we have shown a state of hypercoagulability both in VD and in AD. **Aim of the study.** To investigate a state of endothelial perturbation measuring plasma levels of TM, vWF and the role of the conat phase by activated factor XII (FXIIa). **Subjects.** Twenty patients fulfilled NINCS-ADRDA criteria for probable AD (M=8 F=12; age 77 ± 8 yrs). Twenty patients with VD according to NINDS-AIREN criteria (M=9 F=11; age 84 ± 6 yrs). Thirty controls subjects, judged to be physically and mentally healthy (M=13 F=17; age 81 ± 6 yrs). **Methods.** FVIIa was measured by a functional method; vWF, FVIIAg, FXIIa and TM were measured using an ELISA. **Results.**

	Controls (n=30)	AD (n=20)	VD (n=20)
FVIIa, ng/mL	2.9±0.9	3.8±1.0**	3.6±0.9*
FVIIAg, U/dL	87±18	84±22	89±23
FXIIa, ng/mL	2.3±1.0	1.7±0.9*	2.5±0.8
vWF, U/dL	157±51	216±130*	282±84**
TM, ng/dL	3.4±0.6	2.8±0.8*	4.0±1.2**

Discussion. Normal levels of FVIIAg correspond to significantly high levels of FVIIa, suggesting that an activation of coagulative process is present *in vivo* in both forms of dementia. FXIIa seems to be reduced in AD; this could result from a process of consumption due to a long-lasting inflammatory response within the senile plaques. vWF and TM are positively correlated ($r=0.4$; $p=0.0004$). TM is considered a marker of endothelial dysfunction both when its plasma concentration decreases and when it increases. This could be explained by the up and down-regulation of TM plasma levels modulated by inflammatory cytokines secreted when an endothelial perturbation occurs.

PO-096

OXIDATIVE STRESS AND THIOL REDOX STATE IN PATIENTS WITH CORONARY OR CAROTID ARTERY ATHEROSCLEROSIS

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Clinical and experimental studies suggest that high concentrations of total plasma homocysteine (tHcy) are associated with an increased risk of arterial atherosclerosis and free radical-induced endothelium damage. The aim of the study was to evaluate changes in thiol redox status, namely tHcy, cysteine, cysteinylglycine, reduced (GSH) and oxidised (GSSG) glutathione, and the antioxidant vitamins (α -tocopherol, ascorbic acid) during methionine loading (PML) in patients with abnormal tHcy and coronary or carotid atherosclerosis. In 26 patients (7 females and 19 males, 47 ± 14 years), with documented risk factors and/or atherosclerotic vessel disease, blood samples were drawn fasting and PML. Blood was collected at different intervals (2, 3, 4 h PML). On the basis of plasma free malondialdehyde (MDA), a marker of lipid peroxidation and α -tocopherol (Vit. E) changes PML, patient behaviour was classified as antioxidant, when MDA decreased compared with Vit. E and pro-oxidant when the increase of MDA was associated with a decrease of Vit. E. Data are expressed as mean±SD. Concentrations are in μ M

	Basal	Antioxidant Group (n=15)	
		PML, 4h	P
GSH	428±201	530±193	ns
GSSG/GSH	2.8±1.3	1.8±0.8	0.03
tHcy	32±25	78±50	0.0003
Reduced Hcy	0.6±0.6	2.9±3.6	0.0003
Ascorbic acid	68±36	71±39	ns
Vit. E	34±12	34±10	ns
Free MDA	1.4±0.8	1.1±0.6	ns

	Prooxidant Group (n=11)		P
	Basal	PML, 4h	
GSH	574±428	574±435	ns
GSSG/GSH	2.1±1.1	2.4±1.2	ns
tHcv	42±37	86±51	0.02
Reduced Hcv	0.9±1.1	3.2±3.0	0.01
Ascorbic acid	59±27	61±29	ns
Vit. E	34±12	30±12	ns
Free MDA	0.79±0.14	0.98±0.20	0.01

Data show that PML did not always cause a pro-oxidant response despite high tHcy fasting levels. Among the thiol components evaluated in plasma and blood PML, GSSG/GSH distinguishes had the different pattern between the antioxidant and pro-oxidant group. In fact, in the antioxidant group (n=15) the decrease in GSSG/GSH PML was associated with a stable of Vit. E and a decrease in MDA production. Conversely, in the pro-oxidant group (n=11), the decrease in GSSG/GSH remained stable or increased PML, and was associated with a decrease in Vit. E and an increase in MDA concentration. This finding suggests a consumption of the antioxidant system and an activation of lipid peroxidation in the latter group. In conclusion, PML appears to be an effective test not only for evaluating hyperhomocysteinaemia, but also for identifying different patterns of dynamic redox state in subjects at risk of cardiovascular diseases.

PO-097 DISTRIBUTION AND ALLELE FREQUENCY OF P-SELECTIN POLYMORPHISM THR715PRO IN ITALIAN CONTROL SUBJECTS AND ISCHAEMIC HEART DISEASE PATIENTS

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The new polymorphism Thr715Pro of the P-selectin gene displays a variable genotype distribution, as shown in the ECTIM study, in which the mutated allele Pro715, not homogeneously distributed in Europe, was found to be more frequent in Northern Ireland than in France. In the same study, the mutated Pro715 allele was found to protect against myocardial infarction in patients from Belfast, but not from France. The aim of the present study was to evaluate the genotype distribution of Thr715Pro P-selectin polymorphism in 163 patients with ischaemic heart disease and 291 control subjects from Italy. Controls subjects were recruited randomly from the same geographical areas as the patients and stratification by age was employed to match approximately the age distribution of the control subjects (median age 55, range 34-78 years) with that of patients (median age 57, range 35-79). The fragment containing the A->C substitution at

nucleotide 2304 in exon 13 of the P-selectin gene, which causes the amino acid substitution at codon position 715 Thr->Pro was amplified and digested by the restriction enzyme Hinc II. The P-selectin genotype distributions for the ischaemic heart disease patients and for control subject groups were in Hardy-Weinberg equilibrium. In ischaemic heart disease patients the frequency of the 715Pro allele (0.10±0.005) was similar to that found in control subjects (0.09±0.011). The genotype Pro715 Pro and Thr715Pro occurred in 18.4% of the ischaemic heart disease patients, and in 16.8% the control group. There was no statistical difference in the genotype distribution of Thr715Pro polymorphism in patients and control subjects. The allele frequency observed in Italian controls was lower than that found in Belfast, but quite similar to that found in France; whereas in Italian patients the allele frequency was similar to that observed in ECTIM study in Northern Ireland and in France. In conclusion, our findings failed to demonstrate a significantly different distribution between ischaemic heart disease patients and control subjects. This result stresses the different genetic background in European populations which have to be taken in account in evaluating the "protective" role of P-selectin polymorphism in acute coronary syndromes.

PO-098 LOW FREQUENCY OF THE PRO715 ALLELE OF P-SELECTIN POLYMORPHISM IN PATIENTS WITH ISCHAEMIC STROKE

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Cerebral infarction is a condition influenced not only by environmental but also by genetic factors. However, few haemostasis-related genetic factors have been shown to be associated with ischaemic stroke. A large number of studies reported platelet activation in patients with cerebrovascular disease. P-selectin is an adhesive molecule involved in the interaction of activated endothelial cells or platelets with leukocytes. Recently, new P-selectin polymorphism, Thr715Pro, has been identified which has been found to be associated with a reduced risk of myocardial infarction. In the present study, we investigated the prevalence of the P-selectin polymorphism in 74 ischaemic stroke patients (mean age 66.5, range 50-83 years) and in 145 unrelated control subjects (mean age 67.5, range 49-83 years). The Thr715Pro polymorphism was identified by PCR amplification of a fragment of exon 13 followed by digestion with the restriction enzyme Hinc II. The P-selectin genotype distributions for the ischaemic stroke patients and control subjects were in Hardy-Weinberg equilibrium. The allele frequency of 715Pro

of P-selectin polymorphism in the ischaemic stroke patients (0.061 ± 0.019) was significantly lower ($p < 0.01$) than that found in the control subjects (0.15 ± 0.021). The genotype Pro715 Pro and Thr715Pro occurred in 10.8% of the ischaemic stroke patients, whereas they occurred in the control subjects in 24.1%. The distribution of genotype frequencies of patients was different in comparison to those of the control subjects ($p < 0.05$). Our results show that the 715Pro allele frequency differs between stroke patients and control subjects, suggesting a role for this gene in cerebrovascular disease.

PO-099

PERSISTENT BLOOD CLOTting AND FIBRINOLYSIS ACTIVATION IN THREE PATIENTS WHO UNDERWENT PERCUTANEOUS TRANSLUMINAL MYOCARDIAL REVASCLARIZATION

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Percutaneous transluminal myocardial revascularization (PTMR) is a new procedure for the treatment of angina pectoris that is untreatable by surgery or conventional catheter-based intervention. PTMR allows the creation of myocardial channels through the controlled delivery of holmium laser energy from the ventricular chamber. In the common clinical application PTMR-treated patients undergo anticoagulation of intensity and duration comparable to that used for invasive procedures such as percutaneous transluminal coronary angioplasty. In the literature, to the best of our knowledge, no data are available on whether the anticoagulation used is sufficient for blunting blood clotting activation during the procedure and whether a state of hypercoagulability occurs during the hours following the PTMR. At the Catheterisation Laboratory (University of Florence) 3 patients with refractory angina (2 males and 1 female) underwent the PTMR procedure by a 9F directional catheter carrying flexible fibre optics used with a holmium laser (Eclipse system) and placed across the aortic valve into the left ventricle cavity to create channels with a depth of 5 mm from the endocardial surface into the myocardial tissue. Blood clotting and fibrinolysis activation were investigated by prothrombin fragment 1+2 (F1+2, ELISA), thrombin-antithrombin complexes (TAT, ELISA), D-dimer (D-D, ELISA) and plasmin-antiplasmin complexes (PAP, ELISA) before (T0), at the end (T1) of the procedure, and after 12 (T2) and 24 hours (T3). All patients received a 5000 IU Heparin bolus before the procedure. The behaviour of F1+2 and D-dimer plasma levels is reported in table, TAT and PAP showed a parallel pattern.

	F1+2 (nmol/L)				D-D (ng/mL)			
	T0	T1	T2	T3	T0	T1	T2	T3
pt.1 (50 yrs)	3.1	2	3.6	12.6	72	92	118	347
pt.2 (73 yrs)	8.1	6.7	12.2	17.8	46	61	92	114
pt.3 (74 yrs)	4.4	4	7.3	16.7	85	108	110	146

Our data suggest that 1) clotting activation does not take place during the PTMR, but it occurs after the procedure, possibly in relation to the heparin suspension, 2) if this observation is confirmed as the study continues, future clinical research should evaluate prevention with prolonged administration of antithrombotic drugs.

PO-100

TISSUE FACTOR INCREASE AND TISSUE FACTOR PATHWAY INHIBITOR DECREASE IN SEVERE OVARIAN HYPERSTIMULATION SYNDROME: A MECHANISM RESPONSIBLE FOR BLOOD CLOTting ACTIVATION

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Ovarian stimulation for ovulation induction or *in vitro* fertilization (IVF) is currently used in infertile couples. The most commonly used method for IVF involves the controlled pharmacologic stimulation of ovarian follicles using GnRH analogues (GnRH-a) and exogenous gonadotropins. This process results in a marked increase in the number of developing ovarian follicles and in ovarian enlargement. During the procedure, endogenous oestradiol plasma concentrations exceed physiological levels and a clinical severe ovarian hyperstimulation syndrome (OHSS) may occur. This syndrome is characterised by ovarian enlargement and the production of a protein rich exudate, resulting in severe hypoalbuminaemia and the development of pleural effusions and ascites. In this context, fluid- electrolyte disturbance is commonly associated with a hypercoagulable state. The aim of the present study was to evaluate the effects of the increase of endogenous oestrogens on blood coagulation and fibrinolysis. Twenty-five patients (age range 23-43 yrs) who were hospitalised because of a severe form of OHSS and 25 healthy, non pregnant and age-matched women were investigated. On the day of admission, we examined a number of haemostatic markers, including prothrombin fragment 1+2 (F1+2), thrombin- antithrombin complexes (TAT), D-dimers (D-D), plasmin-antiplasmin complexes (PAP), tissue factor (TF), tissue factor pathway inhibitor (TFPI) and vonWillebrand factor:Ag (vW). Patients showed significantly higher levels of F1+2 ($p < 0.0001$), TAT ($p < 0.0001$), D-D ($p < 0.0001$), PAP ($p < 0.0001$), TF ($p < 0.005$), vW ($p < 0.0001$) and significantly lower levels of TFPI ($p < 0.05$) vs controls. In conclusion, our data suggest that high TF plasma lev-

els, associated with reduced TFPI concentrations may play a role in inducing the marked hypercoagulability observed in this condition.

PO-101

ACE I/D AND AT1R A1166C POLYMORPHISMS IN ANGIOGRAPHICALLY DOCUMENTED RESTENOSIS AFTER PRIMARY CORONARY PERCUTANEOUS TRANSLUMINAL ANGIOPLASTY

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Percutaneous transluminal coronary angioplasty (PTCA) is a widely used technique for myocardial revascularisation in patients with ischaemic heart disease, but the efficacy of PTCA is limited by restenosis. Only one study has investigated the influence of both angiotensin converting enzyme (ACE) I/D and angiotensin II type 1 receptor (AT1R) A1166C polymorphisms on restenosis after PTCA. The aim of this study was to analyse the effect of ACE and AT1R genes on the occurrence of restenosis after primary PTCA. Eighty-six patients, who had undergone successful primary PTCA, were genotyped for ACE I/D and AT1R A1166C polymorphisms by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. A higher prevalence of ACE D allele was observed in patients in comparison to reference values. In 18 of 86 patients restenosis was angiographically documented (>50% stenosis at follow-up). In the whole sample ACE D and AT1R C allele frequencies were 0.59 and 0.31, respectively. A higher, but not statistically significant, rate of restenosis was found in ACE D allele carriers, suggesting a codominant expression of ACE D allele for restenosis. As far as AT1R A1166C polymorphism is concerned, no difference was observed between the two groups. No association was found between restenosis and traditional risk factors (dyslipidaemia, diabetes, hypertension, smoking habit, BMI>25). Genotype distribution and allele frequency were in agreement with the Hardy-Weinberg equilibrium. Further studies are needed to evaluate the influence of genetic polymorphisms.

PO-102

LIPOPROTEIN(a) AND MIGRAINE

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The evidence that migraine is a risk factor for cerebrovascular disorders is growing, and lipid and haemostasis-related markers of thrombotic risk have been investigated. In the present study, lipoprotein(a) (Lp(a)) was investigated by ELISA in 93 patients (mean age 36±13 years [ys], range 16-62 ys) affected by migraine. The patients were subdivided into 2 subgroups with (20) and without aura (73)+, according to IHS classification criteria. A control group was composed of 50 healthy subjects matched for age and sex. A higher prevalence of high (>300 mg/L) lipoprotein(a) plasma levels was found in migraine affected patients 23/93 (24.7%) than in controls 3/50 (6%), without any significant difference between the two subgroups. Taking into account the prothrombotic effects of Lp(a), our results suggest that high Lp(a) plasma levels may represent a possible mechanism responsible for the increased risk of stroke observed in migraine affected patients.

PO-103

ANALYSIS OF ASSOCIATION OF FIVE NEWLY IDENTIFIED POLYMORPHISMS IN α AND γ FIBRINOGEN GENES WITH PLASMA FIBRINOGEN LEVELS

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It has been demonstrated that elevated plasma fibrinogen levels increase the risk of developing cardiovascular disease. Smoking, age and gender have a role in influencing fibrinogen circulating levels, and the same holds true for genetic variations. Up to now sixteen polymorphisms located on the three fibrinogen chain genes have been described and 12 of them were found in the β -chain gene. In particular two polymorphisms, β -455 G/A and β -854 G/A, were associated with significantly increased plasma fibrinogen levels and therefore can be considered as thrombotic risk predictors. In this frame, we searched for new polymorphisms in the fibrinogen cluster possibly associated with low or high plasma fibrinogen levels. We identified five new polymorphisms, three in the α -chain gene and two in the γ -chain gene. Allelic and genotype frequencies were analysed in a population from Northern Italy, composed of 200 unrelated healthy individuals. A correlation study between each polymorphism and plasma fibrinogen levels revealed a statistically significant association ($p=0.023$) between one polymorphism (α -128 C/G), located in the promoter region of the α -chain gene, and higher fibrinogen levels. Haplotype analysis allowed us to identify associations between some haplotypes and the tendency of fibrinogen levels toward lower or higher values.

PO-104**PLATELET GLYCOPROTEIN RECEPTOR IIIA POLYMORPHISM PL^{A1}/PL^{A2} AND THE RISK OF FAMILIAL MYOCARDIAL INFARCTION**

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Platelet membrane glycoprotein (GP) IIa/IIIb may contribute to the pathogenesis of myocardial infarction (MI) by promoting thrombus formation due to its role in platelet aggregation. A common variant (PL^{A2}) of the GP IIIa gene has been associated with vascular disease in several studies, but other authors failed to confirm such an association. The aim of this study was to investigate whether the PL^{A2} allele is associated with MI, in a homogeneous sample of Italian MI patients with a high likelihood of inherited risk, defined by the presence of a family history of thrombosis. To determine associations (odds ratio >2.0) of PL^{A2} carriers with MI, we conducted a case-control study of 135 patients with familial MI and 216 controls without a personal or family history of cardiovascular disease. The frequency of the PL^{A2} allele was very similar in cases (0.15) and in controls (0.16; p=0.80). In multivariate analysis, the odds ratio for carriers of the PL^{A2} allele was 1.07 (95% CI: 0.55 to 2.06). After stratification for age >60 or age <60, smoking habits, fibrinogen levels, ACE I/D, PAI 4G/5G and fibrinogen Bcl1 polymorphisms, no significant interactions were found with PLA1/PLA2 polymorphism. However, carriers of fibrinogen B2 and PL^{A2} alleles showed an increased risk of familial MI as compared with B1B1/ PL^{A2} PL^{A2} homozygotes. Our data suggest that, in this cohort, the effect of the PL^{A2} allele of GPIIIa on MI, if any, is weak and can be increased in carriers of the B2 allele of Bcl I polymorphism of fibrinogen gene.

PO-105**GENE-MODULATED INTERACTIONS BETWEEN PAI-1 AND BODY MASS INDEX ARE DIFFERENT IN MYOCARDIAL INFARCTION PATIENTS AND HEALTHY SUBJECTS**

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The 4G/5G PAI-1 gene polymorphism modulates plasma PAI-1 levels and is a putative risk factor for

myocardial infarction (MI). Previous studies have suggested that this genetic variant could affect the interaction between PAI-1 and lipids or inflammatory mediators. Aim of our study was to assess gene-environment interactions between PAI-1 4G/5G genotype, PAI-1 blood levels, metabolic variables and inflammatory markers in patients with MI at young age compared to healthy controls. 143 patients who had experienced myocardial infarction at young age (males ≤45 yrs, females ≤50 yrs) and 137 healthy age- and sex-matched subjects were enrolled. In each subject, PAI-1 antigen, total cholesterol, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL), C-reactive protein (CRP) levels, body mass index (BMI), white blood cell count (WBC) levels and PAI-1 genotype at 4G/5G locus were determined. The 4G/5G genotype was found to be associated with plasma PAI-1 antigen levels in healthy controls but not in MI patients suggesting that different gene-environment interactions may occur in the two groups. In the MI group the 4G/5G genotype, was found to modulate the correlation between PAI-1 antigen and BMI (r 4G/4G vs r 5G/5G: p=0.04). In the healthy subjects group 4G/5G genotype was found to modulate the correlation between PAI-1 antigen and BMI (r 4G/4G vs r 5G/5G: p=0.04), total cholesterol (r 4G/4G vs r 5G-carriers: p=0.04), LDL-cholesterol (r 4G/4G vs r 5G/5G, p=0.01). The present study suggests that gene-environment interactions between PAI-1 and metabolic variables are different in healthy subjects and in MI patients.

PO-106**POLYMORPHISMS OF THE ANGIOTENSIN-CONVERTING ENZYME (I/D) AND OF PLASMINOGEN ACTIVATOR INHIBITOR-1 (4G/5G) GENE IN END STAGE RENAL FAILURE PATIENTS**

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An association between progressive renal disease, raised cardiovascular risk and angiotensin-converting enzyme (ACE) plasma levels has been shown. Likewise, an association between plasminogen activator inhibitor-1 (PAI-1) levels and cardiovascular ischaemic diseases has been shown. Plasma levels of the ACE and of PAI-1 are modulated by insertion (I)/deletion (D) and by deletion (4G)/insertion (5G) polymorphisms, respectively. To evaluate the genotype frequencies of the I/D polymorphism in terminal renal failure, we enrolled 341 pts. (321 on haemodialysis and 20 on peritoneal dialysis) ongoing dialysis treatment in a district of Southern Italy (Foggia). As controls, 1307 subjects from the same area were

enrolled. Genomic DNA was obtained from leukocytes and ACE I/D and PAI-1 4G/5G polymorphisms were determined as described. Among uraemics, 151 subjects (44.3 %) carried the DD genotype, 149 (43.7%) the ID, and 41 (12.0%) the II genotype. In controls, 560 subjects (42.8%) had the DD genotype, 577 (44.1%) the ID, and 170 (13.1%) the II genotype ($p=n.s.$). The PAI-1 4G/4G genotype was observed in 94 uraemics (27.6%), whereas 163 carried the 4G/5G genotype (47.8%) and 82 (24.6%) were homozygous 5G/5G. Among patients, the frequency of DD subjects was higher in men (48.3%) than in women (39.7%, $p<0.01$). In controls, 355 subjects (27.2%) had the 4G/4G genotype, 621 (47.6%) the 4G/5G, and 329 (25.2%) the 5G/5G genotype ($p=n.s.$). A slightly different frequency of the DD genotype was found according to the duration of dialysis treatment: 47.5% in patients on dialysis up to 60 months, 41.7% and 40.6% in those with dialysis of 60-120 and >120 months, respectively (p for trend: $n.s.$). Patients with or without cardiovascular diseases, such as hypertension, left ventricular hypertrophy, coronary artery disease and chronic cardiac failure, did not exhibit any difference in ACE I/D or PAI-1 4G/5G allele and genotype frequencies (p always >0.05). In conclusion, frequencies of the ACE DD and of the PAI-1 4G/4G genotype were similar in uraemics and in controls, and did not differ between patients with and without cardiovascular diseases.

PO-107

MUTATIONS IN HEREDITARY HAEMOCHROMATOSIS GENES AND THE RISK OF MYOCARDIAL INFARCTION

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Atherosclerosis is a multifactorial disease in which iron may play a role by promoting oxygen radical formation and lipid peroxidation. Increased body iron stores have been suggested to be an independent risk factor for the occurrence of acute myocardial infarction. Hereditary haemochromatosis (HH) consists in an excess iron accumulation and a mutation in the haemochromatosis-associated gene HFE (Cys282Tyr) is the major cause of the disease. The His63Asp variant of the HFE gene accounts for a small fraction of HH. In men, carriership of this common gene variation has been associated with a higher risk of acute myocardial infarction, whereas a significant relationship with cardiovascular death has been found in postmenopausal women. We have investigated the association between the occurrence of acute myocardial infarction and the Cys282Tyr and the His63Asp mutations of the HFE gene in 132

patients from the GISS-2 trial population over 45 years of age with myocardial infarction and at least one first-degree relative who had had a myocardial infarction before the age of 65 years and in 214 controls. The Tyr282 allele was found in 5 cases (3.8%) and in 7 controls (3.3%). The Asp63 allele was found in 42 cases (31.8%) and in 65 controls (30.4%). The associated risk estimates (95% confidence intervals) of having myocardial infarction were 1.2 (0.4-3.7) and 1.1 (0.7-1.7), respectively. No difference was found after stratification for sex, hypertension, diabetes mellitus, cigarette smoking, hyperlipidaemia. The estimated risk of having myocardial infarction according to different mutations of the HFE gene was further evaluated in a logistic model after stratification for age, sex and additional risk factors (hypertension, diabetes mellitus, cigarette smoking, hyperlipidaemia). No significant relationship was observed between a history of myocardial infarction and the Tyr282 allele or the Asp63 allele. In contrast, a significant relationship was observed with the presence of hypertension, diabetes mellitus, cigarette smoking, hyperlipidaemia and with the increasing age. In conclusion, in a setting of patients with a family history of arterial thrombosis, in which the contribution of genetic factors is emphasised, the results obtained cannot support the hypothesis that the Cys282Tyr and the His63Asp mutations of the HFE gene enhance the risk of myocardial infarction.

PO-108

HYPERCOAGULABILITY AND THROMBOTIC RISK IN UNRELATED BONE MARROW TRANSPLANTATION. STUDY OF TWO CASES

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Introduction. Hypercoagulability and thromboembolic events constitute the most frequent complications in cancer patients. Venocclusive disease is a serious complication of bone marrow transplantation (BMT). Chemotherapy and growth factor in BMT improves the risk of thrombosis. In many patients with haematological malignancies allogeneic transplantation is the only hope of cure. The use of unrelated donors has expanded the applicability of allogeneic BMT. Aim of the study is evaluate the thrombotic risk in patients who received unrelated bone marrow transplantation. **Patients.** Two patients with malignancies (Patient 1: CP 1 CML. Patient 2: CR 1 ALL). Mayor characteristic are as follow: conditioning regimen with Bucy 2 (Patient 1) Thiotepa-Cy-TBI (Patient 2). Total nucleated cells infused: 450x10⁶/kg (Patient 1) and 405x10⁶/kg (Patient 2). GVHD prophylaxis ATG-MTX-CSA (Patient 1) and MTX-CSA (Patient 2). **Methods.** We evaluated the hypercoagulability, the thrombotic risk and the vascular damage by study of: antithrombin III (AT III), protein C (PC),

protein S (PS), activated protein C resistance (APCR), plasminogen, thrombin antithrombin complex (TAT), prothrombin fragment 1+2 (F1+2), plasmat-ic homocysteine (OHO), D-dimers, plasmat-ic tissue factor (TF), von Willebrand factor (vWF) and Plas- matic Thrombomodulin (TM). These parameters were evaluated at many time intervals: basal after chemotherapy and transplantation. *Results.* In patient with LAL we observed a reduction of AT III, PC and Plasminogen in almost all controls; markers of hyper- coagulation (TAT and F1+2) were increased after chemotherapy and in day +13 from trasplantation; endothelial damage markers (TM and vWF) TF and OHO increased only after chemoterapy. Patient with LMC showed a basal situation of reduction of AT III and activation of coagulation (TAT and F1+2 higher than normal), low levels of PC at time +3 to +11 from BMT, high level of OHO present at all times and activated endothelial markers at time +24, +31, TF was increased after chemotherapy. *Conclusions.* Our data confirm that chemotherapy constitutes a great stim- ulus who determines hypercoagulability and endothe- lial damage. Unrelated BMT not appear to be a more severe condition than related BMT.

PO-109

PLASMA FIBRINOGEN AS AN INDEPENDENT RISK FACTOR FOR INCIDENT MYOCARDIAL INFARCTION IN THE ITALIAN POPULATION: FIRST RESULTS OF THE VITA PROJECT

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Background. Several studies have suggested that increased levels of plasma fibrinogen are associated with subsequent development of acute myocardial infarction (AMI). Presently, there are no data from populations of Southern Europe, which may be notably different as regards exposure to other estab- lished cardiovascular risk factors. *Aim of the study.* To evaluate the association between hyperfibrino- genaemia and incident myocardial infarction in a population-based prospective study in an Italian population. *Methods.* From 1993 to 1997, 15 109 subjects aged 18 - 65 years were randomly enrolled in the Vicenza Thrombophilia and Atherosclerosis (VITA) Project. Fibrinogen was measured at baseline examination with the PT-derived method, using an internal standard calibrated with a gravimetric method to improve method accuracy (Palareti, 1991). Incident cases in the VITA cohort were ascer- tained by evaluating all the discharge diagnoses in the Veneto Region from 1993 to 1999. Relative risk was computed from the coefficient of a Cox propor- tional hazards model. *Results.* We recorded 93 new cases of IMA in 65,990 observation years. After age adjustment, there was a clear trend indicating an independent, excess risk for fibrinogen, which was statistically significant for the fourth percentile, cor- responding to fibrinogen above 321 mg/dL.

Relative Risk 95% Confidence Intervals

Fibrinogen			
2 nd quartile (230-275 mg/dL)	0.93	0.40	2.16
3 rd quartile (276-321 mg/dL)	1.54	0.72	3.27
4 th quartile (>321 mg/dL)	2.39	1.18	4.84
Male gender	3.67	2.29	5.87
Cholesterol >240 mg/dL	1.94	1.28	2.92
Smoking	1.74	1.13	2.67

Conclusions. These preliminary prospective data pro- vide evidence that a fibrinogen level above 320 mg/dL is a risk factor for myocardial infarction also in a population from Southern Europe, with a magnitude of risk (2.39) comparable to that of hypercholes- terolaemia.

PO-110

IDIOPATHIC CENTRAL NERVOUS SYSTEM ISCHAEMIA IN CHILDHOOD: THROMBOPHILIA SCREENING PROTOCOL

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Stroke in childhood is certainly less frequent than in adulthood, nevertheless it is socially relevant because of the severity of the outcome. The aim of the present study was to assess the role of throm- bophilia risk factors, both acquired and hereditary, in a cohort of children with idiopathic central nervous system (CNS) ischaemia. The thrombophilia profile includes: measurement of factors VIIIc, VIIIIR: Ag, VII, XII, AT, protein C, protein S, APCR, plasminogen, prothrombin G20210 mutation, MTHFR mutation, LAC, ACA, homocysteinaemia and lipid profile. Since October 1998 we have studied 14 patients (10 females and 4 males), aged 7 months to 13 years, showing idiopathic CNS ischaemia (brain ischaemia in 11 cases, spinal cord ischaemia in 1, TIA in 2), occurring during pregnancy to the age 13. 7 years patients have a family history of stroke. They have been submitted to the protocol for thrombophilia and a sample of blood has been stored for further genetic analysis. *Results.* A thrombotic risk factor was found in 13 cases (93%): 6 patients were positive for LAC (associated with ANA in 1 and with hypercho- lesterolaemia in 2), 3 were heterozygotes for MTHFR, 1 for the prothrombin polymorphism, 3 had dyslipi- daemia and a persistently high level of VIIIc factor. Neuroradiologic evidence of ischaemia (focal alter- ations at MRI with perfusion technique in the first hours, then a precise delimitation of the lesion) was found in all. Four patients are receiving rehabilitation treatment for neurologic outcomes and 1 for motor disorders; 5 take antiepileptic drugs. Ten children are currently receiving secondary prophylactic treatment with ASA (8 patients) and warfarin (2 patient, both with LAC). We underline the importance of a com-

plete thrombophilia screening protocol in children with CNS ischaemia, since we demonstrate a high incidence of risk factors, both genetic (30%) and acquired. We further underline the need for careful neurologic diagnosis and extensive neuropsychiatric follow-up for better knowledge of the disease in this age and prevention of further episodes.

POSTERS

Vascular and cellular haemostasis

PO-111

IN VITRO INHIBITION OF XA/VA PROCOAGULANT ACTIVITY AND IN VIVO INHIBITION OF NEOINTIMAL THICKENING AFTER BALLOON INJURY IN THE RABBIT AORTA BY N-ACETYL-L-CYSTEINE

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N-acetyl-L-cysteine (NAC) regulates activity of cellular membranes and intracellular redox potential, inhibits *in vitro* proliferation of cultured smooth muscle cells and promotes vasorelaxation in aortae of animals. In this study, we compared the extent to which NAC and unfractionated heparin (UHF) inhibited *in vitro* procoagulant activity promoted by Xa/Va complex and regulated *in vivo* intimal thickening in abdominal aortae of rabbits after balloon injury. For the *in vitro* study we used platelet rich plasma from different donors, that was rapidly clotted after adjusting platelet count to 200,000/cc. Clots were washed, incubated at 37°C for 30 min in HEPES saline buffer alone, or additioned with increasing concentration of UHF (final: 0.1-0.5-1 IU/mL) or I-NAC (NAC- final 10-50-100 mM). Clots were again washed, and factor Xa/Va activity was detected by measuring clot-dependent activation of added 0.5 mM human prothrombin with S-2238, a thrombin specific chromogenic substrate. Procoagulant activity in the treated clots was expressed as mean % of inhibition of Xa/Va activity of clots incubated with HEPES saline buffer alone.

% inhib. at	UHF 0.1	UHF-0.5	UHF-1	NAC-10	NAC-50	NAC-100
5 minutes	36%	63%	75%	63%	66%	72%

For the animal study, NZW rabbits were injured with an overinflated 4F-balloon catheter, and harvested 4 weeks later. The animals received twice daily sc. injections of either saline (control), 215 IU of UHF, or daily oral supplementation with 250 mg/kg NAC, or both, started 2 weeks before injury. After the

sacrifice, Verhoff's van Geison stained aortic sections were digitally analysed and the ratios of intimal to medial (I:M) thickening calculated (mean ± S.E.).

% inhib. at	Control	UHF	NAC	NAC + UHF
I/M ratio	.64±.02	.45±.04*	.35±.04*	.34±.06*

(* p: <.05 vs control)

The efficacy of NAC in attenuating neointimal thickening may reflect a decreased platelet activation or deposition after injury, as suggested by the attenuation of *in vitro* thrombus-dependent Xa/Va activity.

PO-112

PLATELET ADHESION TO PG-M/VERSICANS OF THE VASCULATURE UNDER FLOW: COOPERATIVE EFFECT WITH COLLAGEN TYPE I

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Vascular-specific isoforms were purified from bovine adult aorta by GuHCl-extraction followed by alternated gel permeation and ion-exchange chromatographies and a final Zn²⁺-chelating chromatography step to remove contaminating smaller glycoproteins/proteoglycans. Isolated proteoglycans were characterised by SDS-PAGE, SDS-agarose gel electrophoresis and TEM involving rotary shadowing/negative staining, and by ELISA/Western blotting utilising a number of monoclonal antibodies against glycosaminoglycans and core protein-associated epitopes raised against crude preparations. This combined ultrastructural and immunochemical analysis demonstrated that purified vascular PG-M/versicans were composed of isoform V1/2 carrying unusual dermatan/chondroitin sulphate chains partly resistant to chondroitinase ABC. The potential involvement of vascular PG-M/versicans in haemostatic and thrombotic phenomena was explored by examining the ability of human platelets to adhere and aggregate on PG-M/versican substrates under high and low shear rates. These experiments were performed by utilising a computer-aided microscopy-system *in vitro*. Vascular PG-M/versicans supported vWF- and fibrinogen-independent platelet adhesion at low, but not high shear rates. Platelet adhesion was not inhibited by the addition of anti-GPIIb and anti- α IIb β 3 monoclonal antibodies, while EGTA completely blocked it. This binding activity could not be reproduced by other members of the PG-M/versican proteoglycan subfamily, such as various aggrecans. Furthermore, a putative V1 PG-M/versican isoform from ovarian follicular fluid was similarly incapable of pro-

moting platelet adhesion, suggesting that the effect was tissue isoform-specific. Combined enzymatic and competition experiments revealed that platelet binding to the PG-M/versicans occurred through the side chains of the proteoglycans. Chondroitin sulphate B appears to be responsible for this phenomenon. The pattern of platelet adhesion was different from that observed with other substrates like collagens and vWF: platelets are tethered to PG-M/versicans for a few seconds but no platelet aggregation or platelet traslocation was observed. In addition platelet adhesion and aggregate formation to collagen type I was enhanced in a dose-dependent manner, by the addition of vascular PG-M/versicans, suggesting that it may act in the vascular bed of venules and smaller capillaries as potentiators of the physiological ECM-mediated haemostatic processes.

PO-113

EFFECT OF RETINOIDS ON APOPTOSIS AND PROCOAGULANT ACTIVITY OF ESTROGEN RECEPTOR POSITIVE (ER+) AND NEGATIVE (ER-) HUMAN BREAST CANCER CELL LINES

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All-trans-retinoic acid (RA) is a cyto-differentiating drug used in treatments for solid tumours and leukaemias. In leukaemic cells, RA downregulates cellular procoagulant activities (PCA), i.e. tissue factor (TF) and cancer procoagulant (CP); further, RA-induced CP loss is associated with cell differentiation. In order to evaluate whether TF and CP modulation by RA occurs in breast cancer cells and is associated with cell maturation, we studied human breast cancer cell lines ER+ (MCF-7 and ZR-75.1) and ER- (MD-MB-231). We characterised: 1. constitutive TF and CP expression; 2. the effects on them of RA and of two selective RA receptor (RAR) agonists, Am580 (RAR α agonist) and CD2019 (RAR β agonist); and 3. the relation of PCA modulation to retinoid-induced cell apoptosis and differentiation. CP and TF were identified by specific functional chromogenic and immunological assays, cell proliferation by growth curves analysis, apoptosis by annexin V-propidium iodide (PI) staining and Bcl-2 protein expression, cell differentiation by ER analysis. The results show that the three cell lines possessed both TF and CP. Of the thres lines, MD-MB-231 had levels of CP and TF significantly greater than the other two. Further, 96 h RA treatment significantly inhibited CP and TF of MCF-7, ZR-75.1 and MDA-MB-231 cells compared to untreated controls. Am580 reduced CP and TF expression in a dose-dependent manner, while the RAR β agonist, CD2019, affected TF and CP of the cell lines only at the highest concentrations. All the retinoids also induced a dose-dependent inhibition of cell proliferation in MCF-7 and ZR-75.1. Maximum

effect was obtained at 10⁻⁵M retinoid concentration, while anti-proliferative activity was almost undetectable at or below 10⁻⁸M. In MDA-MB-231 only the 10⁻⁵M retinoid dose slightly inhibited cell proliferation. Apoptosis evaluation showed that RA and Am580, but not CD2019, increased apoptosis in a dose- and time-dependent fashion in ER+ cells. In the ER- cells, MDA-MB-231, none of the retinoids significantly induced apoptosis. Bcl-2 analysis showed that RA and Am580 significantly decreased the levels of Bcl-2 protein in MCF-7 cells. ER mRNA was not present in MDA-MB-231 cells, even after RA treatment. ER mRNA was not modulated by treatment in MCF-7 cells, while a downregulation was observed in ZR-75.1 cells after RA treatment. In conclusion, this study demonstrates that retinoids modulate TF and CP expression in breast cancer cells. It also suggests that retinoid-induced CP and TF reductions may involve both RAR α and RAR β . Moreover, in MDA-MB-231 the retinoid-induced reduction of PCA is independent of apoptosis, while in MCF-7 cells PCA loss appears associated to apoptosis and in ZR-75.1 cells to cellular differentiation. Therefore, modification of PCA could act as a marker of these phenomena in these cell lines.

PO-114

ENHANCED RELEASE OF PLATELETS SUPEROXIDE ANION IN PATIENTS WITH SYSTEMIC HYPERTENSION: A ROLE FOR RECEPTORS OF ANGIOTENSIN II?

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Patients with hypertension have a reduced vasodilatation response to acetylcholine, suggesting an involvement of nitric oxide (NO) in such vascular dysfunction. Among the putative mechanisms leading to the reduced NO activity, an early inactivation by superoxide anion (O₂⁻) has been postulated. To test this hypothesis we evaluated platelet O₂⁻ release, detected by lucigenin chemiluminescence, in 20 patients with mild systemic hypertension (sitting systolic blood pressure- Sit SBP-, 160-179 mmHg and sitting diastolic blood pressure -Sit DBP-, 100-109 mmHg) and in 20 healthy subjects, matched for sex and age. Patients with systemic hypertension showed a significantly higher platelet O₂⁻ release compared to control subjects (1.99 vs 1 nmoles/L; p<0.05). In order to evaluate whether angiotensin II-receptor antagonists could decrease platelet O₂⁻ release, 10 patients with systemic hypertension were given irbesartan 150 mg/daily for four weeks. Compared to baseline values platelet O₂⁻ release in patients treated with irbesartan (2.18 vs 1.53 nmoles/L; p<0.05) was significantly decreased. The study shows the existence of an enhanced oxidative stress in platelets taken from hypertensive patients and suggests a potential role of angiotensin II in this phenomenon.

PO-115**OXIDANT STRESS AND CLOTTING ACTIVATION IN PATIENTS WITH POLYGENIC HYPERCHOLESTEROLEMIA**

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The mechanism accounting for enhanced expression of monocyte tissue factor (TF) in hypercholesterolaemia has never been investigated. We speculated that oxidant stress, that is elevated in hypercholesterolaemic patients, could have a role because oxygen free radicals are implicated in the expression of monocyte TF. Therefore we investigated whether vitamin E, a known antioxidant agent, influences circulating levels of prothrombin fragment F1+2, a marker of thrombin generation, and monocyte TF in patients with hypercholesterolaemia. Sixteen patients (9 males, 7 females, age 52 +/- 10 years) with polygenic hypercholesterolaemia were randomly assigned to diet (total fat intake <30% of total calories, cholesterol <300 mg/day, polyunsaturated/saturated fatty acids=1.0) treatment alone (n=8) or diet plus 600 mg/day vitamin E (n=8) for four weeks. At baseline, no differences in sex, age, lipid profile, smoking habit, plasma levels of vitamin E and F1+2 and monocyte TF were observed between the two groups. In patients treated with diet alone no changes in serum cholesterol, vitamin E, F1+2 and monocyte TF were detected after the treatment period. In patients given vitamin E, serum cholesterol did not change (mean ± SD 320±75 vs 307±61 mg/dL, p>0.05) and plasma vitamin E rose from 12.9±3.2 to 29±11.6 mM, p<0.05; conversely a significant decrease (Wilcoxon matched pair test) of F1+2 (-39%; p<0.02) and monocyte TF (-70%; p<0.005) was observed. Comparing the diet and vitamin E groups at the end of the treatment period (Mann-Whitney U test), the vitamin E group showed lower values of F1+2 (p<0.04) and monocyte TF (p<0.007) and higher values of plasma vitamin E (p<0.05). The study shows that in patients with polygenic hypercholesterolaemia, supplemental vitamin E *in vivo* decrease thrombin generation and monocyte expression of tissue factor.

PO-116**VITAMIN E ADMINISTRATION INHIBITS OXYGEN FREE RADICAL RELEASE BY HUMAN MONOCYTES IN PATIENTS WITH POLYGENIC HYPERCHOLESTEROLAEMIA**

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Background. Patients with hypercholesterolaemia have accelerated atherosclerosis and enhanced risk of thrombotic disease. Oxidant stress, which is elevated in hypercholesterolaemic patients, could be involved

in this process. It is therefore interesting to study the relationship between vitamin E, an antioxidant agent, and oxygen free radicals (OFR) in this setting. **Aims.** To demonstrate the role of vitamin E in monocyte release of oxygen free radical from hypercholesterolaemic patients. **Materials and methods.** We measured OFR release from monocytes in 7 patients (5 males, 2 females, 59 to 72 years of age) with polygenic hypercholesterolaemia (cholesterol 277.6±15.38 mg/dL, mean±SD). Exclusion criteria were: diabetes mellitus, previous cardiovascular diseases, concomitant inflammatory disease and drugs modifying lipid metabolism. We measured O₂⁻ release from monocytes before and after 1 month of therapy with vitamin E (300 mg/day). Peripheral blood mononuclear cells (PBMCs) were isolated from heparinised venous blood. Monocytes (adherent cells) were stimulated with 4 ng/mL of lipopolysaccharide (LPS) for 60 minutes at 37° C. After incubation, O₂⁻ release from monocytes was measured using lucigenin as a chemoluminescent detector. **Results.** No changes of serum cholesterol were observed at the end of interventional study; conversely compared to baseline values vitamin E administration reduced O₂⁻ release from monocytes; the percentage decrease was 36.3% (p<0.05). **Conclusions.** This study shows that monocyte production of OFR may be reduced by vitamin E administration in patients with polygenic hypercholesterolaemia, suggesting that vitamin E plays an important role in modulating release of OFR in conditions of enhanced oxidative stress.

PO-117**NEUTROPHIL ACTIVATING PROTEIN OF HELICOBACTER PYLORI STIMULATES THE SYNTHESIS OF TISSUE FACTOR AND PAI-2 BY HUMAN BLOOD MONONUCLEAR CELLS**

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Infection of the human stomach mucosa by the Gram- bacterium *H. pylori* causes gastritis and peptic ulcer, diseases marked by chronic inflammation, tissue damage and altered tissue repair. Recent clinical evidence also suggests that *H. pylori* infection is a risk factor for ischaemic heart and cerebrovascular diseases. Mononuclear cells (MNC) are key participants in these processes. In this study we show that *H. pylori* neutrophil activating protein (HP-NAP), a major antigen of *H. pylori* known to activate the respiratory burst in phagocytic cells, strongly enhances the procoagulant activity of MNC by inducing a dose- and time-dependent increase of tissue factor (TF) activity, antigen and mRNA (>30-fold and >15-fold increase in TF activity and antigen, respectively, at 4 h). This effect is abolished by heating HP-NAP at 100° C for 30 min and is not influenced by anti-CD14 antibodies, thus excluding a contamination with endotoxin. MNC fibrinolysis is also hampered by HP-NAP through the

promotion of the synthesis and extracellular secretion of PAI-2, with no production of u-PA (PAI-2 antigen in control cells vs cells treated with 5 mM HP-NAP: 11.4 ± 3 vs 153 ± 23 ng/ 10^5 monocytes). MNC infection with a nap+ *H. pylori* strain and with the corresponding isogenic nap- mutant demonstrates that HP-NAP clearly increases (about 4-fold) the potency of the whole bacterium to induce TF and PAI-2 production. TF and PAI-2 expression due to HP-NAP are fully prevented by the PKC inhibitor H7, and by CAPE and HNE, two drugs interfering with the activation of the transcription factor NFkB, while partially decreased (60-68% inhibition) by the antioxidant drug PDTC, believed to inhibit reactive oxygen intermediates (ROI)-dependent activation of NFkB. TF induction is also fully blocked by the PTK inhibitor genistein, while that of PAI-2 is only partially affected. These data suggest that HP-NAP induces the synthesis of TF and PAI-2 by activating parallel, slightly differentiated signalling pathways involving PTK, PKC and NFkB. Finally, by using chronic granulomatous disease (CGD) monocytes, we proved that cell stimulation by HP-NAP, although favoured by ROI, can proceed independently of the concomitant activation of the NADPH-oxidase. The reported changes in monocyte coagulation-fibrinolysis, induced by HP-NAP, might contribute to the inflammatory reaction of gastric mucosa and, possibly, to the increased risk of thrombotic vascular diseases.

PO-118

KIDNEY FAILURE AND ENDOTHELIAL DYSFUNCTION: EFFECTS OF HAEMODIALYSIS

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Patients with kidney failure, on chronic haemodialysis, have a high incidence of ischaemic cardiovascular disease. Endothelial dysfunction, with the loss of flow-mediated, nitric oxide (NO)-related arterial dilatation (FMD), is a precocious marker of vascular damage in various categories of patients at high risk of ischaemic events and it has been shown to be reversible with the correction of several risk factors. The accumulation of toxic substances, e.g. homocysteine or advanced glycation end-products (AGEs), may contribute to endothelial dysfunction in patients with kidney failure. It is conceivable that haemodialysis, by removing toxic factors, may improve endothelial function and that an optimal haemodialysis regimen may, in the long term, reduce the cardiovascular risk. We have carried out a study to test whether patients on chronic haemodialysis present a defective endothelium-dependent, flow-mediated vasodilatation and whether the haemodialysis procedure ameliorates it. Fourteen patients on chronic, periodic haemodialysis for over 6 months were tested before and at the end of a standard dialysis for FMD (diam-

eter of the brachial artery by B-mode ultrasonography), plasma homocysteine, plasma nitrites and nitrates and AGEs. **Results.**

	Controls	Kidney failure Pre-dialysis	Kidney failure Post dialysis	Pre dialysis vs control (p)	Pre dialysis vs Post dialysis (p)
FMD (%)	9.8±1.7	2.9±1.4	2.4±1.6	<0.01	n.s.
Homocysteine (µM)	10.1±0.4	20.9±2.4	15.9±2.5	<0.05	<0.005
AGEs (AU/mL)	30±3	1257±330.9	613±134.4	<0.05	<0.05
NO2 (µmol/L)	0.7±0.3	0.9±0.2	0.8±0.1	n.s.	n.s.
NO3 (µmol/L)	67.8±8.4	104.4±12.1	91.1±9.1	<0.05	n.s.

In conclusion, patients on chronic haemodialysis present an impairment of endothelium-dependent vasodilatation. Haemodialysis does not ameliorate endothelial function, despite a significant reduction of homocysteine and of AGEs, though not to the levels of normal controls. Moreover, haemodialysis does not affect endogenous NO synthesis which remains, on the average, somewhat higher than that of normal controls. The endothelial dysfunction of patients on haemodialysis appears to be the consequence of a decreased response of endothelium to NO rather than to a reduced synthesis of it.

PO-119

THE POLYPHENOLIC COMPOUNDS RESVERATROL AND QUERCETIN DOWNREGULATE TISSUE FACTOR EXPRESSION BY HUMAN ENDOTHELIAL AND MONONUCLEAR CELLS

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Many epidemiological studies have shown that consumption of wine can reduce the risk of coronary heart disease (CHD). Resveratrol and quercetin, polyphenolic compounds found in red wine, have been shown to prevent low-density lipoprotein oxidation. Resveratrol and quercetin inhibit human platelet aggregation *in vitro* and modulate eicosanoid synthesis. Tissue factor (TF) is the cellular receptor that initiates blood coagulation. TF gene is constitutively expressed in various extravascular cells and inducibly expressed in vascular cell types, such as endothelial cells and monocytes. TF-dependent blood coagulation plays a primary role in haemostasis following tissue injury and in the pathogenesis of many thrombotic events. In order to understand further the mechanisms of the association between red wine consumption and CHD, we investigated the role of resveratrol and quercetin on TF expression by endothelial and mononuclear cells. Confluent human umbilical vein endothelial cells (HUVEC) stimulated with bacterial lipopolysaccharide (LPS) (10 µg/mL) for 4h were treated with increasing concentrations of resveratrol (0 to 50 µM) or quercetin (0 to 200 µM). Mononuclear cells, collected from healthy donors,

were coincubated with LPS (0.1 µg/mL) and the same concentrations of resveratrol or quercetin for 6h. The LPS-induced TF activity in both cell types was significantly reduced in a dose-dependent manner, as revealed by one stage clotting assay. This inhibition was not associated with cytotoxic effects of these compounds, since no LDH release from treated-samples could be detected. Resveratrol and quercetin were also able to reduce cytokine-stimulated TF activity in HUVEC. Since TF expression is mainly regulated at transcriptional level, we evaluated the effects of polyphenolic compounds on TF mRNA levels. Northern blot analysis indicates that resveratrol strongly reduces TF mRNA in HUVEC. Resveratrol and quercetin are also able to reduce the translocation/binding of NF-κB to the TF-κB oligonucleotides, as revealed by EMSA (electromobility shift assay). These results provide a molecular basis which could help to explain the protective effect of red wine consumption against cardiovascular disease.

POSTERS

Hyperhomocysteinaemia

PO-120

HOMOCYSTEINE AND MTHFR GENOTYPE IN YOUNG ADULTS WITH RETINAL VEIN OCCLUSION

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Retinal vein occlusion (RVO) is one of the most common retinal vascular diseases. It is frequently seen in older patients and often associated with many ocular and systemic conditions. In young adults these factors are uncommon and the cause is unknown in the majority of cases. In some studies abnormalities of congenital risk factors for thrombosis have been found in young subjects with RVO. Few data are known about hyperhomocysteinaemia, which is an independent risk factor for arterial and venous thrombosis. In 22 young adults (aged between 33 and 50 years) with RVO (13 with central RVO and 9 with branch RVO) and in 20 matched controls we evaluated fasting and post-methionine load homocysteine concentrations and C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) gene. Plasma total homocysteine was measured while subjects were fasting and 4 hours after a standardised methionine-loading test, which involves the administration of 100 mg of methionine per kilogram. Homocysteine was determined by HPLC with fluorescent detection. HindI restriction enzyme analysis

of polymerase chain reaction (PCR) amplified C677T MTHFR gene was performed. A slight but not significant increase in homocysteine fasting concentrations was shown in patients (8.0±3.8 mmol/L) compared with controls (7.2±4 mmol/L); no difference was shown in post-methionine load homocysteine concentrations and in folate and B12 levels. A significantly different distribution of the C677T mutation of MTHFR was shown: 6% of patients and 13% of controls had no mutation, 54% of patients and 25% of controls were heterozygous and 18% of patients and 10% of controls were homozygous for the mutation (p<0.05). Our data suggest that the MTHFR genotype could be considered as risk factor for RVO, whereas the role of homocysteine is still unclear.

PO-121

ROLE OF METHIONINE LOAD IN DETECTION OF MILD HYPERHOMOCYSTEINEMIA

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Hyperhomocysteinaemia is accepted as an independent risk factor for cardiovascular disease and venous thrombosis. Methionine load is generally used to detect mild hyperhomocysteinaemia which is inadequately reflected by basal homocysteine blood levels. The role of methionine load is, however, still controversial. Our study included 53 patients with premature arterial and venous thrombosis (20 patients with ischaemic stroke, 22 patients with retinal vein occlusion and 11 patients with deep venous thrombosis, all under 50 years of age) and 20 matched control subjects. Plasma total homocysteine was measured while subjects were fasting and 4 hours after a standardised methionine-loading test, which involves the administration of 100 mg of methionine per kilogram. Homocysteine was determined by HPLC with fluorescent detection. Hyperhomocysteinaemia was defined as fasting homocysteine concentration and/or an increase in homocysteine concentration after methionine loading (concentration after methionine loading minus fasting concentration) exceeding the normal range derived from 50 healthy subjects (mean fasting homocysteine concentration 12.4 mmol/L, mean increase in homocysteine concentration after loading 15.8 mmol/L). In 15% of controls and 11% of patients, defined as having hyperhomocysteinaemia, the condition was detected by an elevated fasting and after loading homocysteine concentration. Fifty-five percent of controls and 53% of patients were diagnosed as having hyperhomocysteinaemia only after the methionine loading test. Our results show that the methionine loading test may identify more than 50% of subjects with hyperhomocysteinaemia, confirming that this test is a more sensitive method for detecting mild hyperhomocysteinaemia.

PO-122**PLASMA AND SERUM TOTAL HOMOCYSTEINE CONCENTRATIONS IN PAEDIATRIC PATIENTS**Minniti G,* Cerone R,* Piana A,^o Armani U,^o Campanella D,* Lorini R*

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Numerous studies have indicated that a mild degree of hyperhomocysteinaemia is associated with an increased risk of developing occlusive vascular diseases and that homocysteine is a potent inducer of atherosclerosis. Genetic factors, such as heterozygosity for homocystinuria and the thermolabile variant of methylenetetrahydrofolate reductase, or nutritional factors such as folate or cobalamin deficiency partially seem to explain the aetiology of the hyperhomocysteinaemia. Few studies have investigated total homocysteine (tHcy) concentrations in children. This study presents the first reference information on tHcy concentrations in a sample of the Italian childhood population. Children with a severe illness (renal, heart, respiratory, endocrine, or neurologic disease) or requiring chronic treatment were not included in the study. Total homocysteine was determined in plasma and serum of 96 healthy children. Blood samples for the measurement of plasma homocysteine and serum folate and vitamin B12 were taken after subjects had fasted overnight. Homocysteine was determined by HPLC with fluorescent detection. The mean \pm SD concentration of plasma total homocysteine in 50 males (mean age 11.6 years, range 8.5-12.5) and 46 females (mean age 10.5 years, range 7.5-11.5) was 7.4 \pm 2.15 mmol/L (range 4. -10.7) and 6.1 \pm 1.18 mmol/L (range 4.12-9.5) respectively. Serum concentrations were 7.8 \pm 2.6 mmol/L in male and 6.6 \pm 1.5 mmol/L in female children. The relative simplicity and stability of this assay should facilitate the screening of large populations who may be at risk of vascular diseases.

PO-123**HYPERHOMOCYSTEINAEMIA AS A RISK FACTOR FOR CEREBRAL VEIN THROMBOSIS**Marietta M, Cobelli M,* Ventura P,^o Castelli I, Bertesi M, Torelli G

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Background. Many inherited or acquired predisposing factors for cerebral vein thrombosis (CVT) have been described. Hyperhomocysteinaemia (HHcy) is a well-known risk factor for venous and arterial thrombosis, but data about its role in the pathogenesis of CVT are lacking. The aim of this study was to investigate whether HHcy represents a risk factor for CVT. **Subjects and methods.** Fourteen consecutive, unrelated patients (pts) with idiopathic CVT referred to the Dept. of Neurology of Modena University (5 males,

9 females; mean age 30.6 years, range 19-49). CVT was diagnosed by MR and angio-MR. A control group was formed for 28 healthy volunteers (10 males, 18 females, mean age 29.4 years, range 24-40). In pts and controls we determined factor V(FV) G1691A and prothrombin (PT) G20210A gene mutations, lupus anticoagulant and anticardiolipin antibodies, protein C and S, antithrombin III and fasting total homocysteine (tHcy) levels by a HPLC method. HHcy was defined as tHcy levels >12 nmol/L. The use of oral contraceptives (OC) was also assessed. **Results.** We found heterozygous FV G1691A in only in 1 pt; neither PT G20210A nor other thrombophilic defects were found in either pts or controls.

subjects	tHcy levels (nmol/l, \pm SE)		p
	controls	TVC patients	
all	8.97 \pm 0.71	15.31 \pm 2.22*	0.004
males	8.36 \pm 0.65	24.54 \pm 3.05*	0.005
females	9.22 \pm 1.03	10.18 \pm 0.81	ns

*p<0.005 vs. controls

HHcy was found in 50% of patients and 14% of controls; the difference was statistically significant ($X^2=3.31$, p=0.03). Seventy seven percent of pts and 33% of controls were OC users; this difference too, was significant ($X^2=3.00$, p=0.04). **Discussion.** We found FV G1691A in 7.1% of patients and 0% of controls, and PT G20210A in 0% of pts and controls. Both these figures disagree with previously published data, but we think that the difference could be due to a different sample size. On the other hand, our data confirm that OC use is an important risk factor for CVT, carrying an odds ratio of 3.5. Moreover we demonstrate that HHcy is a strong risk factor for CVT, with an odds ratio of 2.4. The lower prevalence of HHcy in females suggests that this condition could be a stronger risk factor for male subjects. We recommend that tHcy assessment be included in the initial evaluation of CVT pts.

PO-124**THROMBOEMBOLIC DISEASE AND THROMBOTIC RISK FACTORS: THE EFFECT OF TREATING HYPERHOMOCYSTEINAEMIA ON PHYSIOLOGICAL ANTICOAGULATION**

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In subjects with thromboembolic complications it is common to find alterations in more than one thrombotic risk factor. It is not clear whether these alterations are due to an excessive consumption or to a persistent defect of biological activity. **Aim of the study.** To examine the behaviour of physiological anticoagulants after therapy to normalise homocysteine (Hcy) levels. Out of 52 patients with hyperHcy (17 females,

35 males, aged 27 to 88 years) 7 were affected by protein C (PC) deficiency, 2 protein S (PS) deficiency, 3 antitrombin (AT) deficiency, 4 PC and PS deficiencies, 1 PC and AT deficiencies, 1 PS and AT deficiencies, 5 PC and PS and AT deficiencies. Fifteen patients (6 females, 9 males, aged 27 to 88 years) underwent therapy for 60 days with pyridoxine (600 mg daily), calcium folinate (30 mg daily) and once/60 days vitamin B12 (1000g). At the end of the therapy the Hcy levels, PC, PS and AT were measured again. *Results.* 1) after therapy Hcy levels returned to within the normal range (before therapy: range: 15.3-54.7 mM/L; $M \pm 1SD$: 23.19 ± 9.84 mM/L; median value: 22 mM/L; post therapy: range: 4.3-13.4 mM/L; $M \pm 1SD$: 9.13 ± 2.56 mM/L; median value: 8.5 mM/L. $p < 0.01$); 2) the table shows the behaviour of the deficiencies anticoagulant after therapy;

PC	PS	AT
1 improved	4 improved	-
3 normalized	3 normalized	3 normalized

3) no modification occurred in subjects with normal values. *Conclusions.* The correction of hyperHcy produces an improvement in the biological activity of physiological anticoagulants in patients with hereditary defects. It is possible to hypothesize that hyperHcy produces a chronic consumption of physiological anticoagulants reducing the efficiency of haemostasis. The therapy to normalise Hcy levels is recommended in order to reduce the additional thromboembolic risk.

PO-125 THE ROLE OF HOMOCYSTEINE IN REGULAR HAEMODIALYSIS PATIENTS

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Epidemiological studies showed that hyperhomocysteinaemia is an independent atherosclerotic risk factor in the coronary, cerebral and peripheral circulation. Plasma homocysteine is elevated in renal failure and is associated with the severity of renal insufficiency. Although homocysteine levels are positively correlated to creatinine concentrations, the cause of homocysteine increase in chronic renal failure has not been definitively clarified. In this study we measured the plasma homocysteine levels to evaluate the impact of another risk factor on premature atherosclerosis observed in patients receiving regular

haemodialysis (RH). We studied 74 uraemic patients on RH (44 M and 30 F, aged 61 ± 14.3 years). One hundred healthy subjects matched for sex and age acted as a control group. Total homocysteine levels were assayed by high performance capillary electrophoresis. The levels of tHcy in RD patients were significantly higher in male patients (54.8 ± 40 mM/L) than in control males (12.6 ± 4.3 mM/L) and in female patients (38 ± 37 mM/L) compared to female controls (7.8 ± 3.2 mM/L). Elevated tHcy (>95th percentile) as 19.3 mM/L in males and 13.5 mM/L in females was detected in 38 out of 44 male patients ($X^2=22.2$; $p < .001$) and in 28 out of 30 female patients ($X^2=14.0$; $p < .001$). Hyperhomocysteinaemia detected in the patients' group was not associated with a higher prevalence of +/+ MTHFR mutation compared to healthy subjects (16% vs. 14%; $p=ns$). Hyperhomocysteinaemia in chronic renal failure increases the risk of the atherosclerosis to a greater extent in RH patients since long term regular haemodialysis treatment already produces vascular damage which triggers coagulation with a consequent hypercoagulability.

PO-126 PLASMA HOMOCYSTEINE MEASUREMENT: COMPARISON OF THREE DIFFERENT ASSAY METHODS IN PLASMA FROM VASCULAR PATIENTS

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Background. The relevance of plasma homocyst(e)ine level (Hcy) as a risk factor for the development of arterial and venous thrombosis has increased continuously over the last years. The reference method for the determination of Hcy is HPLC with fluorometric detection. New assay methodologies have been recently developed to make Hcy determination applicable in most clinical laboratories. *Aim of the study.* To compare three different methods for Hcy determination in acute myocardial infarction, stroke and venous thromboembolism patients undergoing a standard methionine load (100 mg/kg). *Materials and Methods.* Three assay methodologies for Hcy determination were compared: HPLC (Homocysteine by HPLC, BioRad Laboratories GmbH, Munchen, Germany), EIA (Axis" Homocysteine, Axis Biochemicals ASA, Oslo, Norway) and FPIA (Axis Biochemicals for the IMX System, Abbott Laboratories, Abbott Park, IL, US). One hundred plasma samples (62 baseline and 38 post-methionine load, PML) were assayed. Statistical analysis: Mean intra- and inter-assay CV were calculated for each method on patient plasma samples with Hcy concentrations of 5 (baseline), 20 and 40 (PML) mmol/L. The methods were compared by conventional regression analysis and by the paired difference approach proposed by Bland and Altman (Lancet 1986,i,307-10). *Results.* Main results are shown in the following table:

CV: % (range)	HPLC	EIA	FPIA
Intra-assay	2.87 (2.02 - 4.32)	9.47 (8.26 - 11.30)	6.85 (4.72 - 8.18)
Inter-assay	5.88 (4.09 - 8.11)	12.94 (11.53 - 15.75)	7.52 (6.32 - 8.64)

	All samples	Baseline samples	PML samples
Correlation coefficient at regression analysis vs HPLC(R2)			
EIA	0.9509	0.9723	0.8850
FPIA	0.9514	0.9808	0.8836
Mean difference* vs HPLC (95%CI), mmol/L			
EIA	0.48 (-0.25 - 1.21)	0.10 (-0.48 - 0.68)	1.01 (-0.56 - 2.58)
FPIA	-2.97 (-3.85 - -2.09)	-1.69 (-2.17 - -1.20)	-4.77 (-6.70 - -2.85)
Limits of agreement vs HPLC (% of the measured concentration)			
EIA	-27 to +39	-27 to +37	-27 to +42
FPIA	-34 to +12	-30 to +8	-38 to +18

*A positive difference means a higher HPLC result

Conclusions. Both EIA and FPIA methods resulted to be suitable for clinical use. A slightly higher variability was observed for PML versus baseline samples. When HPLC is not feasible, EIA could constitute an interesting option for laboratories with automated EIA facilities and high workload. The FPIA assay is likely to be preferable for correlability to HPLC and flexibility of use.

PO-127

HYPERHOMOCYSTEINAEMIA AND RETINAL VEIN OCCLUSION

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Retinal vein occlusion (RVO) is a disease of older age. Established risk factors are hypertension, diabetes mellitus, glaucoma and history of cardiovascular disease. Pathogenetic mechanism(s) are poorly understood. The role of thrombophilias (inherited/acquired) is still matter of debate. Clinical studies focusing on factor V Leiden and antiphospholipid syndrome (lupus anticoagulant and anticardiolipin antibodies) generated controversial results. Antithrombin-III (AT-III), protein C, and protein S deficiency have been described in isolated casereports. G20210A prothrombin allele was not associated with RVO in one study. Very few data regarding hyperhomocysteinaemia (hHCY) are available. De Brujine reported high fasting/post-methionine loading (PML) homocysteine (HCY) levels in 22 patients. However there was no control group, the patients mean age was high (65±11), and finally, risk factors for RVO were frequently present. We studied six consecutive patients (mean age 38±10yrs) with RVO in the absence of hypertension, diabetes mellitus, glaucoma and history of cardiovascular disease. Fasting/PML

HCY levels, serum total folic acid/cyanocobalamin were determined after exclusion of other causes of thrombophilia (factor V Leiden, G20210A prothrombin allele, AT-III, protein C, protein S deficiency, lupus anticoagulant activity and anticardiolipin antibodies). In addition search for C677T point mutation of methylenetetrahydrofolatereductase was performed (30% of each genotype in both groups). Each case-patient was matched for age, gender and geographical area with healthy control subjects. **Results.** We found statistically significantly higher PML HCY levels in patients with RVO than in controls. (Table), while just a trend was present for fasting HCY levels. No difference was detected for C677T genotype, serum total folic acid and cyanocobalamin.

	Patients Mean/SD	Controls Mean/SD	P value
Fasting HCY	11,55 / 5,90	6,62 / 2,56	0,089
PML HCY	40,98 / 8,04	22,67 / 10,93	0,007
Serum folic acid	5,60 / 2,50	7,40 / 3,88	0,361
Serum cyanocobalamin	407,50 / 101,82	476,00 / 180,35	0,436

Conclusion. hHCY is an established risk factor for arterial/venous thrombosis. Whether this is the case in patients with RVO in the absence of risk factors remains to be demonstrated. A larger prospective case-control study to test this hypothesis is ongoing.

PO-128

PREVALENCE OF METHIONINE SYNTHASE A2756G POLYMORPHISM AND OF MODERATE HYPERHOMOCYSTEINAEMIA IN A COHORT OF CONSECUTIVE PATIENTS WITH JUVENILE VENOUS THROMBOSIS

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A raised plasma level of homocysteine is an independent predisposing factor to venous thrombosis. The vitamin B12-dependent methionine synthase (MS) catalyses remethylation of homocysteine to methionine. We evaluated the influence of the A2756G polymorphism of MS on total fasting plasma homocysteine (THcy) levels in patients with juvenile thrombophilia. THcy levels and the A2756G mutation of the MS gene were evaluated in 65 consecutive patients (33 M, 32 F; mean age 40.7±12.9 yrs, mean age at first event 33.6±12.7 yrs) with documented early-onset venous thrombosis and in 129 age and sex-matched healthy controls (69 M, 60 F;

mean age 41.4±11.9 yrs). THcy levels were significantly different between patients and controls (15.22±13.67mM, vs. 12.56±7.36 µM; p=0.044, t-test). Homozygous A2756G MS genotype (+/+) was observed in 1/65 (1.6%) patients and in 5/129 (3.9%) controls, while (+/-) heterozygous genotype occurred in 13 (20%) vs 42 (32.5%) and (-/-) homozygous genotype in 51 (78.5%) vs 82 (63.6%) patients and controls, respectively; these differences were not statistically significant (p>0.05, Fisher's exact test). Moreover, THcy levels of the whole population were not significantly different according to the MS genotypes (14.2±11.2 µM for -/- homozygotes; 12.1±6.4 µM for +/- heterozygotes; 10.3±5 µM for +/+ homozygotes; p>0.05, Scheffé *post hoc* test); the same result was obtained by dividing the population into patients and controls (p always >0.05, Scheffé *post hoc* test). In the same population we also evaluated the prevalence of homozygous C677T mutation (+/+) of methylentetrahydrofolate reductase gene (MTHFR): homozygotes were significantly associated with raised THcy levels when compared with heterozygotes (+/-) and homozygotes (-/-) (21.28±11.2 µM for +/+ homozygotes; 11.41±6.4 µM for +/- heterozygotes; 10.97±5 µM for +/+ homozygotes; +/+ vs +/-, +/+ vs -/- p<0.05, Scheffé *post hoc* test). Thus the present data confirm and extend the concept that the A2756G methionine synthase mutation has no apparent influence on THcy levels.

PO-129

POST-METHIONINE LOADING LEVELS OF TOTAL PLASMA HOMOCYSTEINE IN A COHORT OF APPARENTLY HEALTHY SUBJECTS IN NORTHERN ITALY: RELATION TO AGE, SEX AND NUTRITIONAL STATUS

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Methionine-loading test (ML-test) is a measure of total plasma homocysteine (tHCy) concentration after ingestion of a single oral dose of methionine. A positive ML-test might not represent a heterozygous cystathionine β-synthase defect, but rather impairment of the "transulphuration pathway" as a result of vitamin B6 as pyridoxal-5'-phosphate (PLP) deficiency. Fasting tHCy can be increased because of impairment of the "remethylation pathway" as a result of vit. B12 and folate deficiency. Little is known, though, about the response to the ML-test in relation to vitamin status. The aims of our study were the following: 1) to measure fasting tHCy and 2 hour post-load tHCy (PML), and absolute increments after ML (PML minus fasting tHCy= PML-tHCy); 2) to determine the correlation with age, sex and levels of folate, vitamin B12 (vit. B12) and PLP in a cohort of free-living apparently healthy subjects in Northern Italy (Bologna); 2) to define reference values for 2 hour PML-tHCy in the population in the same geographic area. *Methods.* Apparently healthy subjects (n=97;

53 men, 44 women, age range: 14-94 y) were selected from the general population in the area of Bologna, Italy. ML-test: L-methionine at the dose of 100 mg /Kg b.w. was administered orally in fruit juice. High performance liquid chromatography (HPLC) assays were used to measure plasma levels of the following: 1) tHCy, according to Araki and Sako method (1987). 2) PLP according to the method of Sassi *et al.* (1997). Folate and vit. B12 serum levels were measured by automated chemiluminescence assay (Chiron Diagnostics, East Walpole, MA, USA). *Results.* The geometric mean (GM) of plasma PML levels for men and women was 23.4 and 20.75 mmol/L, respectively. The GM of plasma Delta tHCy levels for men and women was 12.43 and 11.95 µmol/L, respectively. PML and Delta tHCy are influenced by sex. PML and Delta tHCy are negatively correlated with folates. No variation was observed in PML and Delta tHCy above the 60th percentile of folates. *Conclusions.* On the basis of these results, we calculated reference values of PML and of Delta tHCy for a subgroup of subjects with folates above the 60th percentile. The 90th percentiles of PML for men and women were 24.92 and 26.77 µmol/L, respectively. The 90th percentiles of Delta tHCy for men and women were 14.87 and 18.81 µmol/L, respectively.

PO-130

PLASMA HOMOCYSTEINE LEVELS IN A COHORT OF APPARENTLY HEALTHY SUBJECTS IN NORTHERN ITALY: RELATION TO AGE, SEX AND NUTRITIONAL STATUS

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Several cross-sectional and prospective studies have demonstrated that increased levels of total plasma homocysteine (tHCy) are an independent risk factor for cardiovascular disease. Genetic disorders and vitamin deficiencies can lead to moderately elevated levels of tHCy. The definition of normal fasting tHCy is still a matter of debate. The aims of our study were the following: 1) to measure fasting tHCy and determine the correlation with age, body mass index (BMI), sex and levels of folate, vit. B12 and pyridoxal-5'-phosphate (PLP) in a cohort of free-living apparently healthy subjects in Northern Italy (Bologna); 2) to define reference values for fasting tHCy for the population in the same geographic area. *Methods.* Apparently healthy subjects (n=147; 82 men, 65 women, age range: 14-94 y) were selected from the general population in the area of Bologna, Italy. High performance liquid chromatography (HPLC) assays were used to measure plasma levels of the following: 1) tHCy, according to the Araki and Sako method (1987); 2) PLP according to the method of Sassi *et al.* (1997). Folate and vit. B12 serum levels were measured by automated chemiluminescence assay (Chiron Diagnostics, East Walpole, MA, USA). *Results.* tHCy was significantly higher in men than

women (GM: 9.9 and 8.0 mmol/L, respectively), but no difference was observed for folate, PLP and vit.B12 levels between men and women. tHCy was positively correlated with age and negatively correlated with folate, vit.B12 and PLP. Folate, vit.B12 and PLP concentrations were negatively correlated with age. In a stepwise multivariable regression analysis, age and folate levels explained 16.6% and 16.9% of the tHCy variance in men, respectively, while no significant effect was due to BMI, vit.B12 and PLP. In women, age and vit.B12 explained 18.3% and 18.6% of tHCy variance respectively, while no significant effect was due to BMI, folate and PLP. Reference ranges were calculated in the subgroup of patients with both serum folates concentrations above the 60th percentile and vitamin-B12 concentrations above the 40th percentile (n=42, 28.6% of subjects). This cut-off value was considered to indicate optimal levels of folates and vitamin-B12. The upper reference values tHCy were 9.2 mmol/L for men (90th percentile) and 8.9 mmol/L for women (90th percentile). **Conclusions.** 1) tHCy concentrations are influenced by age, sex and vitamin status; 2) serum folates and vitamin-B12 concentrations are suboptimal in a cohort of healthy subjects in Northern Italy; 3) the reference values for tHCy should be calculated taking into account vitamin levels and sex.

PO-131

STROKE MECHANISMS IN PATIENTS WITH C677T/MTHFR MUTATION

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Background. Homozygotes (TT genotype) for the C677T mutation in the gene of methylene tetrahydrofolate-reductase (C677T/MTHFR mutation) constitute about 12% of the Caucasian population. They may have mild hyperhomocysteinaemia which is an established risk factor for cardiovascular disease. In patients with MTHFR mutation-associated stroke, the mechanisms of the stroke and the anatomical distribution of lesions caused by the various stroke mechanisms have not been well determined. **Patients and Methods.** We studied stroke mechanism, infarct distribution, and clinical findings among 29 patients (16 M and 13 F, mean age 42 yr ± 9) from the Cardarelli Hospital Stroke Registry in whom genetic analysis showed a mutation of the MTHFR gene. On the basis of TOAST criteria, we classified the patients into the following five groups: 1) large-artery atherosclerosis (ATR), 2) small vessel occlusion or lacunae (LAC), 3) cardioembolism (CEMB), 4) other determined causes (OTH), 5) undetermined causes (UND). **Results.** Two patients had a transient ischaemic attack. According to the TOAST classification, the remaining 27 patients were distributed as follows: group 1 (ATR) 2 cases (7.5%); group 2 (LAC) 18 cases (66.5%); group 3 (CEMB) 0 cases (0%);

group 4 (OTH) 2 cases (7.5%); group 5 (UND) 5 cases (18.5%). **Conclusions.** The great majority of MTHFR mutation-associated strokes are caused by lacunar (small vessel) disease. Large-artery atherosclerosis, cardiac or intra-arterial embolism are less common.

PO-132

HUMAN LONGEVITY: ANY ROLE FOR A1298C METHYLENETETRAHYDROFOLATE REDUCTASE MUTATION AND 844INS68 CYSTATHIONINE BETA-SYNTASE INSERTION VARIANT

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Increased homocysteine (Hcy) plasma levels has been documented with age, but contrasting results have been reported about the prevalence of the C677T 5,10-methylenetetrahydrofolate reductase (MTHFR) gene mutation. Recently a second mutation (A1298C), influencing the metabolism of Hcy, in the gene codifying for MTHFR has been reported in neural tube defects as well as a "gene-gene" interaction between C677T MTHFR mutation and the 844ins68 insertion variant in the cystathionine-beta-synthase (CBS) gene. The aims of our study were to evaluate: 1- the allele and genotype frequencies of the A1298C MTHFR and 844ins68 CBS polymorphisms in a cohort of Italian healthy ultranonenarians, 2- whether some genotypes are associated with successful aging and 3-whether polymorphisms of MTHFR and CBS genes have a role in influencing Hcy plasma levels in healthy Italian young and ultranonenarians. We investigated 75 healthy Italians over the age of ninety (range 90-106) and 54 unrelated young healthy controls (range 20-55) from the same geographical area. In the multiple logistic regression model, 1298CC (p=0.018) and 1298AC (p=0.048) MTHFR genotypes were found to be factors hindering longevity. The 677TT (p=0.062) MTHFR genotype as well as the compound heterozygote 677CT/1298AC (p=0.09) display a tendency toward an association with a decreased chance of subjects becoming ultranonenarians. No association between CBS and longevity was found. Hcy plasma levels in ultranonenarians (12.2±3.9 µmol/L) were significantly higher (p<0.00001) than in young controls (8.4±3.8 µmol/L). The ordinal logistic regression analysis performed in the young controls showed a significant association between 677TT MTHFR genotype and Hcy plasma levels (p=0.036). The 1298CC MTHFR genotype was also associated with higher Hcy plasma levels but this did not reach statistical significance (p=0.068). The ordinal logistic regression analysis in the ultranonenarians showed a similar trend to that in young subjects although less

evident because of the confounding variable as well as altered diet and lower efficiency in biological functions. Our results on the allele frequency and genotype distribution of the A1298C MTHFR mutation strongly suggest the need to design studies in order to understand the role of this mutation in cardiovascular disease.

PO-133

PHENOTYPIC VARIABILITY OF CARDIOVASCULAR MANIFESTATIONS IN MARFAN'S SYNDROME: POSSIBLE ROLE OF HYPERHOMOCYSTEINAEMIA AS A RISK FACTOR

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Marfan's syndrome is an inherited connective tissue disease, with a dominant transmission due to mutations in fibrillin 1 gene. It is a pleiotropic disorder with major manifestations in the cardiovascular, ocular, skeletal and nervous (dural ectasia) systems. Thoracic aortic aneurysm (TAA) is the major clinical manifestation of the cardiovascular system. Recently, the association between abdominal aortic aneurysm and high homocysteine (Hcy) plasma levels has been reported. Hyperhomocysteinaemia is associated with the presence of the mutation C677T in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene codifying for an enzyme of the homocysteine metabolism. The aim of this study was to evaluate homocysteinaemia and the prevalence of the C677T MTHFR gene mutation in Marfan's patients. We studied 52 Marfan's patients (according to revised diagnostic criteria, De Paepe *et al.*, 1996) and 64 healthy control subjects comparable for sex and age. Hcy plasma levels were determined by HPLC with fluorescence detection. For mutation detection, DNA was amplified by PCR using specific oligonucleotides and the C677T mutation was detected by restriction enzyme analysis. The C677T MTHFR genotype distribution was in Hardy-Weinberg equilibrium. Plasma homocysteine levels were significantly higher in patients with Marfan's disease than in control subjects (11.3 ± 6.1 micromol/L vs 8.4 ± 3.5 micromol/L, $p=0.014$). The prevalence of homozygotes for the C677T MTHFR mutation in Marfan patients was higher (30.8%) than in control subjects (15%), but the difference did not reach statistical significance ($\chi^2=4.9$, $p=0.086$). In conclusion, our results suggest that mild hyperhomocysteinaemia might represent a synergic risk factor for TAA in Marfan patients and explain, at least in part, the phenotypic variability of cardiovascular manifestations. If these data are confirmed in a larger number of Marfan patients, possible pharmacological therapy could be considered.

PO-134

HIGH PREVALENCE OF ELEVATED PLASMA LEVELS OF HOMOCYSTEINE AND LIPOPROTEIN (a) IN PATIENTS WITH CAROTID ARTERY ATHEROSCLEROSIS

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A number of studies have investigated metabolic and haemostasis-related risk factors in patients with carotid artery atherosclerosis, but only few data are available about the plasma levels of both homocysteine and lipoprotein (a) – Lp (a) and of endothelial cell activation in patients with carotid atherosclerosis. In this study 86 patients (62 M and 24 F; mean age 70.3 ± 7.9 years; range 33-86 years) with carotid artery stenosis $>85\%$ and 82 healthy subjects matched for age and sex were included. Fifty-two patients had hypertension and 16 suffered from NIDDM, 65/86 patients were current or former smokers. Plasma homocysteine concentrations were evaluated by HPLC and fluorimetric detection and Lp (a), thrombomodulin (TM) and von Willebrand antigen (vW) plasma levels by ELISA. In patients with carotid stenosis homocysteine plasma levels were significantly higher than in controls both in males (12.7 ± 5.8 $\mu\text{mol/L}$ vs 9.5 ± 3.8 $\mu\text{mol/L}$; $p<0.001$) and females (11.1 ± 4.2 $\mu\text{mol/L}$ vs 6.8 ± 2.3 $\mu\text{mol/L}$; $p<0.0001$). Hyperhomocysteinaemia defined as homocysteine plasma levels above the 95th percentile of the control subjects (15 $\mu\text{mol/L}$ in males and 10.5 $\mu\text{mol/L}$ in females) was detected in 35/86 (40.7%) patients. Lp (a) plasma levels were significantly higher in patients than in controls (median 203 mg/L; range 1-1388 mg/L vs median 143 mg/L; range 10-651 mg/L) ($p=0.023$). Of the 86 patients 36 (41.9%) (27 males and 9 females) had elevated Lp (a) (>300 mg/L) plasma levels. Hyperhomocysteinaemia and high levels of Lp (a) were simultaneously found in 13/86 (15.1%) patients. Plasma levels of TM in patients with carotid stenosis (median 33.5 ng/mL; range 5 – 102 ng/mL) were significantly higher than those of the control subjects (median 19 ng/mL; range 13-44 ng/mL; $p<0.0001$). TM levels above 40 ng/mL were observed in 23/86 (26.7%) patients. vW plasma levels were significantly higher in patients (median 145%; range 60-289%) than in controls (median 110%; range 60-182%) ($p<0.0001$). No significant correlation was found between homocysteine and/or Lp (a) plasma levels and endothelial cell activation (TM and vW levels). In conclusion this study shows a high prevalence of both hyperhomocysteinaemia and elevated Lp (a) in patients with carotid artery atherosclerosis. In these patients an activation of endothelium is detectable. This activation is not strictly dependent on the presence of high plasma levels of homocysteine and/or Lp (a).

POSTERS

Antiphospholipid antibody syndrome**PO-135****LUPUS ANTICOAGULANT, ANTICARDIOLIPIN ANTIBODIES, FACTOR V Q506 MUTATION AND RECURRENT FOETAL LOSS**Lo Pinto G, Galletti A,* Barone E,* De Cassan P,^o Perroni L^o*A.S.L. 4 "Chiavarese", Medical Department, Santa Margherita Ligure, Genoa, Italy; *Laboratory; ^oLaboratory of Human Genetics, Galliera Hospital, Genoa, Italy*

In the last 5 years we treated 27 women aged 25 to 39 years, affected by recurrent foetal loss (1-4 events). Most of foetal losses occurred between the 6th and 13th week of pregnancy. Two patients suffered from late foetal loss due to severe pre-eclampsia and *abruptio placentae* with disseminated intravascular coagulation. When possible, placental examination was performed and thrombotic vascular lesions searched for. We detected lupus anticoagulant (LAC) in 15 patients, anticardiolipin antibodies (aCL IgG and IgM) in 4 patients and factor V Q506 Leiden mutation in 2 patients; 6 patients did not show any genetic or acquired thrombophilia. In 24 women prophylactic therapy with a low dose of aspirin (100 mg once a day) was given, if possible before conception, to prevent foetal loss. In three pregnant women it was not possible to perform this prophylactic protocol: one case with proximal deep venous thrombosis of a leg was treated with subcutaneous non fractionated heparin; a case of evident disseminated lupus erythematosus was treated with corticosteroids; a case with high levels of aCL antibodies was treated with corticosteroids plus aspirin. We obtained the following results: 13 women treated with aspirin before conception had a regular pregnancy, foetal growth and delivery (including one twin birth); one woman treated with aspirin before conception had foetal loss at the 13th week of pregnancy; 4 women treated with aspirin post-conception had foetal loss within the 13th week; one woman treated with aspirin post-conception had a regular pregnancy, foetal growth and delivery; one woman with factor V Leiden mutation treated with aspirin before conception had a regular pregnancy, foetal growth and delivery; one woman with factor V Leiden mutation has not yet had another pregnancy nor have the remaining 6 women.

PO-136**ANTIPHOSPHOLIPID ANTIBODIES ASSOCIATED WITH MIGRAINE IN PATIENTS SUFFERING FROM TRANSIENT ISCHAEMIC ATTACKS**De Lucia D,* d'Alessio D,^o Del Giudice V,* Lauretano M,* Maisto G,* Marotta R,* Mosavat M,* Marra M,# Quarantiello M,# Carbone R,# Caizzo P,# Papa ML^o**Institute of General Pathology and Oncology, II University of Naples, Naples; ^oG. Moscati Hospital, Avellino; #Stroke Unit, General Hospital, Caserta, Italy; *Laboratory of Thrombosis and Haemophilia, New Pellegrini Hospital, Naples, Italy*

Antiphospholipid antibodies (aPL) have been associated with a variety of neurological disorders, mostly linked to focal ischaemia or infarction. Many studies have reported an association between coagulation derangements and patients with a history of transient ischaemic attacks (TIA). It is acknowledged that the status of "migraine-related TIA" is still a debated and unresolved issue. Multiple factors (such as clotting inhibitors, APC-resistance and aPL) may be in play and it is not at all clear whether their interaction is very relevant in the pathogenesis of TIA. We aimed at investigating the role of natural clotting inhibitors and aPL in TIA patients with a history of migraine for at least 10 years. Fifty patients (18 M and 32 F) who satisfied the WHO definition for the diagnosis of TIA history in anterior or posterior brain districts were enrolled into the study. Among the 32 females, 6 subjects were under hormonal treatment with contraceptive pills. All had had at least one episode of TIA following migraine attacks. Fifty healthy subjects were chosen as a control group. Extensive screening for coagulation abnormalities (AT III, PC, PS both -total and -free, APC-resistance and FV gene R506Q mutation) yielded negative results. Only 3 patients (1 M and 2 F) had APC-resistance with FV Q506 allele. Eighteen subjects out of fifty (36%) had high titres of aPL (above cut-off point of 10.5 U/mL for IgG and of 7.5 U/mL for IgM), without positivity for the kaolin clotting time (KCT) test (>1.20 ratio). In the cases presented herein, the TIA episode occurred in the setting of a long history of migraine. Our findings suggest that in a sample of the Southern Italy population, aPL are independently associated with the risk of TIA episodes, and represent a strong predictor of ischaemic events.

PO-137**ANTIPHOSPHOLIPID SYNDROME, ADRENAL FAILURE, DILATED CARDIOMYOPATHY AND CHRONIC HEPATITIS: AN UNUSUAL ASSOCIATION OF MULTIORGAN AUTOIMMUNE INJURY?**Coppola A, Tauchmanovà L,* Albisinni R, Madonna P, Guiotto G, Rossi R,* Di Minno G^o*Centro di Riferimento Regionale per le Emocoagulopatie and *Dipartimento di Endocrinologia e Oncologia Molecolare e Clinica, Università di Napoli "Federico II", Napoli; ^oCattedra di Gerontologia e Geriatria, Istituto di Medicina Interna e Geriatria, Università degli Studi di Palermo, Italy*

The association of antiphospholipid syndrome with the development of adrenal failure has been reported in more than 40 patients, mostly due to bilateral cortical haemorrhage secondary to multiple thromboses of adrenal vessels. The presence of antibodies against adrenal cortex (ACA) was never documented in these cases. A 27-year old man was referred to our Institution because of a history of recurrent thrombophlebitis of the lower limbs since the age of 20 years. Six months earlier he had developed acute adrenal insufficiency and cortisone acetate therapy had been started. ACA were positive and abdominal ultrasonography showed morpho-

logically normal adrenals. There was no family history of endocrine, autoimmune or thromboembolic disease. Laboratory evaluation showed positive VDRL, a slight increase in alanine and aspartate-aminotransferases and prolonged APTT, not corrected by the addition of normal plasma; KCT and DRVVT were similarly abnormal; IgG and IgM anticardiolipin (ACL) were significantly positive; these data were confirmed after three and six months, suggesting an antiphospholipid syndrome. Oral anticoagulation was started (INR 2.5-3.5) and no further thrombophlebitis was subsequently reported. One year later, after an arrhythmic episode, dilated cardiomyopathy was diagnosed; coronarographic study did not document any occlusive coronary disease. As liver enzymes were persistently increased, the patient underwent a liver biopsy that showed mononuclear portal infiltrate and mild fibrosis; these findings, together with the presence of antibodies to mitochondria and to liver/kidney microsomes, suggested an autoimmune hepatitis. Two years later, titres of ACA and ACL were increased and an abdominal CT scan showed adrenal dysmorphisms with findings suggestive of previous haematomas. In this young man we report the unusual coexistence of antiphospholipid syndrome and autoimmune adrenalitis; in addition there was clinical evidence of multiorgan autoimmune injury. In this respect, a pathogenic role for micro-vascular injury related to the presence of ACL, inducing the release of intracellular antigens and activation of the immune system, may be hypothesised.

PO-138
CLINICAL SIGNIFICANCE OF ANTI- β 2-GLYCOPROTEIN I AND ANTI-PROTHROMBIN ANTIBODIES TOWARDS ARTERIAL AND VENOUS THROMBOSIS

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Arterial and venous thrombosis are the most frequent and clinically relevant complications of patients with antiphospholipid antibodies (aPL). The role of anti- β 2-glycoprotein I (a β 2-GPI) and anti-prothrombin (aPT) antibodies - 2 among the most studied aPL - as risk factors of thrombosis has not yet been clearly established. Therefore, we reviewed the literature, to perform an analysis of the association between thrombosis and these antibodies. Sixteen studies analyzed a β 2-GPI antibodies in 2481 patients: they were 1 "nested" case-control study and 15 retrospective studies. 56% of cases (n=1381) suffered from systemic lupus erythematosus (SLE); the presence of aPL was the enrollment criterium of other 476 patients (19%), whereas the other cases had been studied because of other autoimmune diseases (n=79), myocardial infarction (n=265), or other conditions (n=280). The G isotype was investigated by 7 studies, the G+M isotypes by 8 studies, the A+G+M isotypes by 1. "Home-made" assays were used in all

cases: ELISA (n=14), and Western or dot-blot (n=2). The sensitivity towards thrombosis ranged from 24 to 100%, and the specificity from 36 to 93%. In univariate analysis most studies demonstrated a significant association with thrombosis (p<0.0001-0.0189; RR: 1.47-14.1); 3 studies also performed the multivariate analysis: 2 of them confirmed the statistically significant association. Eleven (1 prospective, and 10 retrospective) studies analyzed aPT in 1707 patients: 820 suffered from SLE, the presence of aPL was the enrollment criterium of other 314, whereas the other cases have been studied because of deep vein thrombosis/pulmonary embolism (n=265), myocardial infarction (n=106), or other disease (n=202). The G isotype was investigated by 4 studies, the G+M isotypes by 6. "Home made" ELISA assays were used in all cases: their sensitivity for thrombosis ranged from 33 to 76%, their specificity from 55 to 100%. By univariate analysis, the association between aPT and thrombosis was significant in 7 studies. In conclusion, even though these studies suggest the association between a β 2-GPI and aPT and thrombosis, the clinical relevance of these antibodies needs to be confirmed by further investigations, which must take into account a better methodological standardization.

PO-139
PREVALENCE OF THROMBOPHILIC GENOTYPES IN PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME

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Background. The antiphospholipid syndrome (APS) is the first cause of acquired thrombophilia. It is characterised by arterial and venous thromboses associated with a persistent presence of lupus anticoagulant and/or anticardiolipin antibodies. Recently, new inherited coagulation disorders associated with an increased risk of thrombosis have been identified: the factor V Leiden (FVL), the A20210 mutation of the prothrombin gene and the mutation CT677T of the methylene tetrahydrofolate reductase (MTHFR) which, when homozygous, (TT677) predisposes to hyperhomocysteinemia. The presence of these genotypes may further increase the thrombotic risk in patients with APS. *Aims.* We looked for FVL, the A20210 mutation of prothrombin and the TT677 genotype of MTHFR in 90 patients with APS: 47 (16 males, 31 females, median age 32 years, range 14-68) with a previous thrombotic episode (venous 30, arterial 11, arterial and venous 5, endocardiac 1) and 43 (7 males, 36 females, median age 34 years, range 18-77) without history of thrombosis. *Results.* Five patients (5.5%) were heterozygous for FVL: all of them had had a previous venous thrombosis. The prevalence of FVL was significantly different between the groups of patients with and without venous thrombosis (p=0.02). The A20210 mutation of pro-

thrombin was found in 10 patients (11%), 6 with thrombosis (2 venous, 3 arterial and 1 endocardiac) and 4 without thrombosis; the difference between groups with or without thrombosis was not significant either for arterial or venous thrombosis. The TT677 genotype of MTHFR was present in 18 patients (20%), 12 with thrombosis (venous 11, arterial 1) and 6 without thrombosis. In this group, increased levels of homocysteine were found in 9/12 of patients with thrombosis and only 1/6 of patients without thrombosis. Combined thrombophilic genotypes resulted in 4 (4.5%) patients with thrombosis (FVL+ TT677 MTHFR 3, A20210+ TT677 MTHFR 1). **Conclusions.** The presence of FVL is an additional risk factor for venous thrombosis in patients with APS. The role of hyperhomocysteinaemia associated with the TT677 genotype of MTHFR needs to be investigated in a larger group of patients.

PO-140

THE EFFICACY OF AN INDEX IN THE DETECTION OF FALSE POSITIVE RESULTS IN THE DIAGNOSIS OF LUPUS ANTICOAGULANTS

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Lupus anticoagulants (LA) have long been recognised. They are immunoglobulins reacting with phospholipids and prolonging clotting in phospholipid-dependent coagulation assays. LA may be detected in plasma of subjects suffering from several clinical manifestations, including thrombosis, recurrent foetal loss, thrombocytopenia and cerebro-vascular disease. At present LA screening includes the following tests: prothrombin time (PT), partial thromboplastin time (PTT), kaolin clotting time (KCT), tissue thromboplastin inhibition test (TTIT) and dilute Russell's viper venom time (dRVVT). LA diagnosis, however, remains quite difficult due to the differences in sensitivity and specificity of the tests, and to the heterogeneity of reagents and to the relatively good condition of samples. The TTIT is a particularly sensitive test for LA. It is commonly employed in the clinical laboratory to confirm the presence of LA. This test is a modified PT. A decreased phospholipid concentration in the test system accentuates sensitivity to LA. Unfortunately, TTIT is not specific for LA; consequently false-positive results may be seen with specific factor inhibitors, factor deficiencies and with anticoagulant therapy. The purpose of this study was to increase test specificity to LA. This is accomplished by an Index as the ratio of TTIT to PT. This index reveals the false-positive results. TTIT times, using the Manchester Comparative Reagent in dilutions 1:100, were determined on platelet poor plasmas (PPP) from 100 normal individuals and 278 patients with prolonged times. Test results were interpreted from the ratio patient seconds to normal control seconds. After determining the PT results, the index was expressed. The following table shows the results obtained.

Samples	N.	PT>1.15	TTIT>1.30	INDEX>1.50	PNP +
OAT	225	225	220	0	0
Extr path def	35	35	30	0	0
Heparin	70	7	3	1	0
Intr path def	30	8	4	1	0
LA +	40	3	22	38	39
Control	100	0	3	0	0

From our study we can conclude that the index allows us to optimise TTIT results.

PO-141

A COMPARATIVE EVALUATION OF A NEW, HIGHLY SENSITIVE REAGENT SYSTEM IN THE DIAGNOSIS OF LUPUS ANTICOAGULANT

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Lupus anticoagulants (LA) are immunoglobulins which interfere with *in vitro* phospholipid-dependent tests of coagulation. They have long been recognised as markers for a high risk of developing a peculiar triad of clinical manifestations: thromboembolic diseases, thrombocytopenia and recurrent foetal losses. Laboratory identification of LA is, therefore, of increasing clinical importance. A variety of tests have been introduced such as: prothrombin time (PT), partial thromboplastin time (PTT), kaolin clotting time (KCT), tissue thromboplastin inhibition test (TTIT), dilute Russell's viper venom time (dRVVT), platelet neutralization procedure (PNP) and PTT-LA (Stago). However, due to the marked heterogeneity of these antibodies and to the differences in sensitivity of the reagents and tests, the diagnosis is difficult. Consequently, the detection and characterisation of LA is a laborious procedure which requires a number of associated tests. The aims of the present study were: 1) to compare the LA sensitivity of a group of routine coagulation tests with the modified APTT performed by using an activator source of Immuno-Baxter; 2) to validate the results of this new laboratory method for LA. We evaluated plasma samples from 24 patients with a well-defined phospholipid antibody syndrome. Twenty plasmas were found to be LA positive according to both screening and confirmatory tests (in these pts the dRVVT was positive in 86%, the PTT in 94%, the KCT in 82%, the PNP in 97% and PTT-LA in 88%). Plasma samples from 20 healthy donors (mean age 30 years), all with negative LA tests results, served as controls. The new test was performed on patients' plasma, pooled normal plasma and a 1:1 mixture of both using the APTT reagent (SiO₂/Al₂O₃) and two different concentrations of phospholipids on a Thrombothrack-4. Our criterium on LA positivity was to determine the LCA index, whose basic formula is $b-c/a \cdot 100$ where a is the clotting time of patients' plasma, b is the clotting time of patient + normal plasma and c is the clotting time of normal plasma. The normal value of the index was 15.

The presence of an inhibitor was documented by a value >15. Twenty pts fulfilled the criteria for positive LA status. Our results show that the new test by Immuno-Baxter is high sensitive for LA and can be a useful test in the diagnosis of LA.

PO-142

CLINICAL SURVEY ON ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS SUFFERING FROM LUNG DISEASES

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Lupus anticoagulant (LA) and anticardiolipin antibodies (ACA) are closely related phospholipid binding autoantibodies which interfere in a number of laboratory tests. They have long been detected in plasma and in serum of subjects suffering from different clinical manifestations. However, to date, the likely strong correlation between the naturally occurring prevalence of LA and ACA and the onset of micro-macroangiopathic disorders has not been extensively studied. To clarify and evaluate the accuracy of such an interaction we performed several laboratory investigations. Forty-five patients (30 men, range 20 - 68 years, 15 women, range 36 - 72 years) suffering from lung diseases (hypertension or embolism) were tested. The clinical features of these patients were as follow: 22 pts had pulmonary embolism (EP) and 23 pts had pulmonary hypertension. The diagnostic investigations included on clinical findings (history and physical examinations) and pulmonary scintigraphy and echocardiography. A control - group consisting of 50 healthy volunteers (25 males and 25 females, aged 18 to 60 years) served to establish the reference values for all tests. Several assay systems were used in order to detect the presence of LA (screening tests) and to confirm the antiphospholipid nature of the inhibitor (confirmatory tests). Screening tests comprised the: prothrombin time (PT), partial thromboplastin time (PTT), kaolin clotting time (KCT), tissue thromboplastin inhibition test (TTIT) and dilute Russell's viper venom time (dRVVT). The platelet neutralization procedure (PNP) was carried out as a confirmatory test. ACA (IgG and IgM isotypes) were quantified by a standardised MELISA. The results are shown below,

	N.	LA+	ACA+	LA+/ACA+
I.P.	23	3	7	5
E.P.	22	5	6	3

Our investigations provide evidence of a precise clinical statistical incidence of antiphospholipidies in group of the patients with pulmonary pathologies.

PO-143

ANTIPHOSPHOLIPID ANTIBODIES IN MIGRANEOUS PATIENTS

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Antiphospholipid antibodies (aPL) including lupus anticoagulant and anticardiolipin antibodies (aCL) are recognised markers for an increased risk of stroke. Even though some studies reported the presence of aPL in migraneous patients the association between these autoantibodies and migraine is still controversial. The aim of this study was to evaluate the prevalence of aPL (LA and aCL) and the presence of blood clotting activation (F1+2 plasma levels) in migraneous patients. LA (KCT, dRVVT, diluted aPTT and TITT) and IgG and IgM aCL (ELISA) were detected in 93 patients (mean age 36±13 years; range 16-62 years) affected by migraine subdivided in 2 subgroups with (20) and without (73) aura according to IHS criteria and in 50 healthy subjects without migraine matched for age and sex. F1+2 plasma levels were evaluated by ELISA. aPL was positive in 23/93 (24.7%) patients and in 2/50 (4%) control subjects (p<0.004). The prevalence of aPL was similar in patients with (25%) or without (24.6%) aura. Among aPL positive patients 17/23 were positive for LA and 8/23 for aCL (6 IgG and 2 IgM). Only 2 patients were positive for both LA and aCL. F1+2 levels were significantly higher in patients than in controls (p<0.0002), whereas the difference in F1+2 plasma levels between aPL positive (median 1.2; range 0.6-8) and aPL negative patients (median 1.0; range 0.3-5.1) was not statistically significant (P=0.07). The presence of F1+2 levels above the normal range was observed in 9/23 (39.1%) aPL positive and in 19/70 (27.1%) aPL negative patients. In conclusion in migraneous patients a pathogenetic involvement of "antiphospholipid autoimmunity" may occur. A prothrombotic state is detectable, to which mechanisms other than aPL contribute.

PO-144

DETERMINANTS OF ENHANCED THROMBOXANE BIOSYNTHESIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Objective. To evaluate the rate of thromboxane biosynthesis in systemic lupus erythematosus (SLE) patients, exploring the interplay between antiphos-

pholipid antibodies (aPL), and markers for endothelial perturbation, thrombin generation and platelet activation. *Methods.* A comparison of 11-dehydrothromboxane (TX) B excretion, a marker for *in vivo* platelet activation, aPL, von Willebrand factor (vWF) and tissue-plasminogen activator (t-PA), two circulating markers for endothelial perturbation, and plasma levels of the prothrombin fragment F1+2, marker for thrombin generation, was performed in 40 SLE patients and 40 healthy subjects. Thromboxane metabolite excretion was also measured in 8 SLE patients before and after low-dose aspirin. *Results.* SLE patients had significantly higher 11-dehydro-TXB, excretion, plasma F1+2, vWF and t-PA levels than controls. A statistically significant correlation was found between plasma vWF and t-PA and thromboxane metabolite excretion. Moreover, significantly higher 11-dehydro-TXB was found in patients with aPL positivity and endothelial perturbation. Low-dose aspirin suppressed 11-dehydro-TXB by 80 percent suggesting a predominant platelet source of enhanced thromboxane biosynthesis. After a median follow-up of 48 months, all SLE patients who experienced major cardiovascular events had TX metabolite excretion, aPL positivity and signs of endothelial perturbation. *Conclusion.* We have characterised a sensitive marker of platelet activation, which is abnormal in SLE patients showing aPL positivity and endothelial perturbation. This analytical approach may help in identifying those patients at increased thrombotic risk as potential candidates for antiplatelet therapy.

POSTERS

Diagnosis and treatment of arterial thrombosis

PO-145

HAEMOSTATIC EFFECTS OF CHOLESTEROL-LOWERING THERAPY WITH ATORVASTATIN

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Increased serum cholesterol is regarded as an important cause of atherothrombotic events. Haemostatic abnormalities have been described in hypercholesterolaemic patients and a possible interrelationship between hypercholesterolaemia and haemostatic changes may play a role in increased thrombotic risk of these subjects. Statins, HMG-CoA (3-hydroxymethyl-glutaryl Co-enzyme A) reductase inhibitors, are widely used in the treatment of hyperlipidaemia. It has been shown that long-term treat-

ment with statins is safe and improves survival in patients with coronary heart disease. These effects may be correlated with other beneficial properties besides their cholesterol lowering effect. This study assessed the effects of atorvastatin on some haemostatic variables in 32 patients, of both sexes, with hypercholesterolaemia, without endocrine-metabolic disorders or clinical signs of arteriosclerotic vasculopathy. In the patients and in 25 control subjects, matched for sex, age and body weight, plasma levels of tissue-type plasminogen activator (t-PA), plasminogen activator-inhibitor (PAI-1), D-dimer (DD) and prothrombin fragment 1+2 (F1+2) had previously been measured. Only the levels of PAI-1 and F1+2 were found to be higher than in controls. All these haemostatic evaluations were carried out in hypercholesterolaemic patients after 6 months of treatment with atorvastatin, 20 mg/day. This treatment significantly lowered the mean cholesterol level in the whole group of patients. Moreover in the patients' group, six months of atorvastatin treatment not only lowered serum cholesterol level, but also significantly reduced PAI-1 and F1+2, which were both increased at baseline. Other parameters did not change with therapy. The present results show that a reduction of haemostatic abnormalities, existing in hypercholesterolaemia, may be another, important, effect of atorvastatin therapy.

PO-146

HIGH CIRCULATING LEVELS OF PAI-1 IN PATIENTS WITH ISCHAEMIC STROKE

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The nature of haematologic disorders in different stroke subtypes remains uncertain. PAI-1 plasma levels and 4G/4G polymorphism within the promoter region of the PAI-1 gene have been consistently associated with ischaemic complications of atherosclerosis such as acute myocardial infarction and ischaemic stroke. We performed measurements of antigenic PAI-1 plasma levels and investigated the association with PAI-1 4G/5G gene variants in 30 patients with acute cardioembolic stroke, 164 patients with atherothrombotic stroke, 50 patients with lacunar stroke and 164 age-matched healthy controls. A marked elevation of plasma concentrations of PAI-1 was observed in patients (75 ± 45 ng/mL) when compared to controls (20.5 ± 11.5 ng/mL, $p < .001$; Fisher exact test). However, the highest levels of PAI-1 were found in cardioembolic stroke (97.5 ± 11.7 ng/mL) and in atherothrombotic stroke (68.5 ± 27.5 ng/mL). No significant changes of this marker were observed in lacunar stroke (27.5 ± 10.5 ng/mL). The PAI-1 plasma levels were

significantly elevated on day 7 ($p < .001$) after the ischaemic event and declined by day 30 both in cardioembolic stroke and in atherothrombotic stroke. PAI-1 4G allele was detected in 58% of patients with cardioembolic stroke ($X^2=0.6$; $p=0.4$), and 55% of those with atherothrombotic strokes ($X^2=0.1$; $p=0.6$) and only in 51 % of lacunar infarcts ($X^2=0.005$; $p=0.9$) compared to healthy subjects (50%; baseline). Thus, polymorphisms of the PAI-1 gene had the same frequency in individuals suffering from ischaemic stroke compared to healthy subjects in this setting. However, in a multivariate analysis PAI-1 plasma levels consistently identified subjects with a history of cardioembolic and atherothrombotic episodes and PAI-1 was the strongest discriminator. The difference in the time-course of this marker during the acute phase suggests that the nature of the altered fibrinolytic state appears to be different depending on stroke pathophysiology. In conclusion, it is very difficult to assess whether PAI-1 plays an aetiological role or is affected secondarily, especially as PAI-1 behaves in the plasma as an acute-phase reactant which increases rapidly in many clinical conditions including myocardial infarction and stroke.

PO-147 HYPERHOMOCYSTEINAEMIA AND STROKE: INCIDENCE IN YOUNG PATIENTS AND PROSPECTIVE THERAPEUTIC IMPLICATIONS. PRELIMINARY DATA

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The mechanisms by which hyperhomocysteinaemia contributes to thrombogenesis are incompletely understood, but data from epidemiological studies suggest that this condition represents an independent risk factor for thromboembolic disease. The effect of homocysteine-lowering treatment by folic acid and vitamin B6 and B12 administration in decreasing clinical vascular events is not proved by prospective studies. The main objectives of our study in young patients with ischaemic stroke were 1) to examine the percentage of fasting and post-methionine load hyperhomocysteinaemia in these patients 2) to establish the incidence of associated risk factors 3) to provide long-term care with vitamins in the context of a prospective evaluation. In the last year, 63 patients aged 27-58 years (mean 41.2) admitted to the Neurology Dept. because of ischaemic cerebrovascular disease were tested for fasting and post-methionine load homocysteinaemia. The table shows that 44% of patients were positive, and that 50% of them had a normal fasting test.

Patients	Homocysteine Fasting Test	Homocysteine Post-Methionine Load
63	14	28

Thirty-fives percent of patients were smokers and 30% had arterial hypertension. We do not know whether hypertension is an associated risk factor or the result of endothelial oxidative stress. In fact hyperhomocysteinaemia induces impairment of endothelial function and oxidation of low density lipoproteins, leading to a prothrombotic environment in areas of endothelial injury. Our data confirm epidemiologic evidence that implicates hyperhomocysteinaemia as a risk factor for atherothrombotic disease in young people. We think it useful to evaluate how known genetic polymorphism are related to phenotypic expression in young patients with stroke.

PO-148 EVALUATION OF HAEMORHEOLOGY AND COAGULATION AND D-DIMER IN PATIENTS SUFFERING FROM DILATED CARDIOMYOPATHY IN THE PRESENCE OF SPONTANEOUS ECHO CONTRAST

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The aim of this study was to evaluate the behaviour of blood viscosity, fibrinogen, PT, aPTT, red cells morphology and D-dimer in patients with dilated cardiomyopathy, some of them with chronic atrial fibrillation (AF), with or without spontaneous echo contrast (SEC). Patients with major valve disease were excluded. We studied 45 patients, 35 males and 10 females, (mean age 72.15 ± 9.28 yrs). We measured whole blood viscosity, plasma fibrinogen, PT, aPTT, D-dimer, haemochrome and red cell morphology with Zipursky-Forconi's method. Transthoracic and transoesophageal echocardiography was performed in all patients to evaluate the presence of SEC in the left atrium. We divided all the patients into two groups: the 1st group of 20 patients, 16 males and 4 females (mean age 76.38 ± 6.9) with SEC and AF in 80% of cases, and the 2nd group of 25 patients, 19 males and 6 females (mean age 69.23 ± 9.69) without SEC and AF in 31%. Our results show that in patients with SEC there is a statistically significant increase of whole blood viscosity and plasma fibrinogen in comparison with patients without SEC: 8.56 ± 1.61 , 7.27 ± 1.59 cPs at 10 s⁻¹ ($p < 0.1$); 438.31 ± 96.04 , 373.86 ± 89.66 mg % ($p > 0.5$) respectively. The hemochrome, PT, aPTT have not shown significant variations in both groups. Red cells morphology evidence in all patients an increase of discocytes, with a reversed EMI (erythrocyte morphological index), without substantial differences in these two groups. D-dimer, which indicates fibrinolytic activation, was out of the range of normality in about 1/3 of the patients, equally distributed in the 1st and 2nd group. An analysis of our results points out that in patients with SEC and AF, who are those with a major risk factor for cardioembolic stroke, alterations of haemorheology are present, with an increase of whole blood viscosity and fibrinogen that seems to be

caused by an increase of red cell aggregability favoured by fibrinogen. Other values do not indicate any significance difference between the groups. In patients with dilatated cardiomyopathy and AF, haemorheological study is an important marker for cardioembolic risk stroke evaluation.

PO-149

CARDIOGENIC CEREBRAL ISCHEMIA IN PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME: SOURCE OF THROMBI EUROPEAN PROJECT (STEP)

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The pathogenesis of the cerebral ischemia in patients affected by antiphospholipid antibodies syndrome (APS) is uncertain and has been the subject of our study. Numerous recent studies have stressed a link between these antibodies and cardiac valvular lesions, and have also suggested the possibility that antiphospholipid antibodies (aPL) can provoke a cerebral ischemia through cardiac emboli. In order to evaluate this hypothesis, we have studied 15 patients with primary APS and cerebral ischemic events. These patients have been selected on the basis of their positivity to lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and anti β 2GP1 antibodies, from a wider group of patients. Neuroimaging studies have shown different cerebral lesions (11 cortical, 4 subcortical and 4 both cortical and subcortical). Cardiac abnormalities detected by transesophageal echocardiography have resulted very frequent (80%) and in line with literature. In particular, the aortic and mitralic valvular insufficiencies were the most common lesions, found in 8 patients (53.3%). The nodular thickenings of aortic and mitralic valvular flaps also resulted very frequent (6 patients, 40%). From literature it emerged that in this sort of patients these thickenings are made of overlapped layers of thrombi. This condition might represent a potential cardiac source of cerebral embolism. Other relevant abnormalities, detected in 2 patients, have been thickenings of the aortic arch, rare in young people. Given the infrequent finding of patent foramen ovalis, the hypothesis of the paradoxical thromboembolism in these patients, does not seem to be very probable. This study seems to support the cardiac-embolic hypothesis of the cerebral ischemia that is also suggested *ex adiuvantibus* by the efficacy of oral anticoagulant drugs in the prevention of new ischaemic events in these patients.

PO-150

FLUVASTATIN ALONE AND IN COMBINATION TREATMENT (FACT STUDY). EFFECTS ON PLASMA LEVELS OF FIBRINOGEN, PAI-1 AND C REACTIVE PROTEIN IN CORONARY ARTERY DISEASE PATIENTS WITH COMBINED HYPERLIPIDAEMIA

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Evaluation of lipid-modifying agents in clinical trials has focused primarily on anti-lipidaemic effects. A potentially favourable haemostasis-modulating activity has been reported, but no definitive data are available on the effects of fibrates and statins on coagulation parameters. We studied the effects of fluvastatin (F) and bezafibrate (B) in monotherapy and in combination on fibrinogen, t-plasminogen activator (tPA) and tPA inhibitor (PAI-1) in patients with coronary artery disease (CAD) and mixed hyperlipidaemia. This is a randomised, double blind, multicentre trial. Treatments: 1) F 40mg; 2) B 400mg; 3) F 20mg + B 400mg; 4) F 40mg + B 400mg. Treatment duration was 24 weeks. Three hundred and thirty-three patients (254 males, 79 females), aged 56+8 years, affected by stable angina pectoris or previous MI or coronary revascularisation and combined hyperlipidaemia (LDL-cholesterol 135-250mg/dL and triglycerides (TG) 180-400mg/dL after a 7-week placebo/dietary run-in) were randomised. There were 80, 86, 85 and 82 patients per group, respectively. *Methods.* Fibrinogen (functional Clauss assay), tPA (ELISA), PAI-1 (chromogenic), baseline (bas) fibrinogen, LDL and HDL-cholesterol, and triglycerides values (mean+SD) and mean % changes after 24 weeks of treatment are reported :

Treatment	Fibrinogen mg/dL		LDL-chol mg/dL		HDL-chol mg/dL		Triglycerides mg/dL	
	Bas	% ch	Bas	% ch	Bas	% ch	Bas	% ch
F40	352±83	-4.0	189±35	-22.5 [^]	40±8	0.3	263±64	-6.0
B400	322±51	-9.2 [#]	179±32	-9.6	43±10	17.4 ^o	257±65	25.1 ^o
F20+B400	339±69	-14.1 [#]	186±34	-23.3 [^]	41±8	20.9 ^o	253±57	-35.7 ^o
F40+B400	322±57	-16.2 ^{#§}	192±36	-23.6 [^]	42±9	21.9 ^o	259±71	-37.7 ^o

[#] p < 0.001 vs F40; [§] p = 0.009 vs B400; [^] p < 0.001 vs B400; ^o p < 0.001 vs F40

No significant changes were observed in PAI-1 and c-reactive protein (CRP) plasma levels. At baseline fibrinogen was positively correlated with CRP (r=0.493). The combined effects on fibrinogen and plasma lipids achieved by fluvastatin and bezafibrate combination treatment might be more useful than the simple reduction of cholesterol in preventing ischaemic cardiovascular disease.

PO-151**EFFECT OF ETHINYLOESTRADIOL-CYPROTERONE ACETATE ON TISSUE FACTOR PATHWAY INHIBITOR**

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Introduction. Several epidemiological studies note an association between oral contraceptive (OC) use and both venous thrombosis and arterial diseases. Moreover is known that OC therapy induces several changes in haemostatic plasma proteins. Aim of the study to evaluate the effect of ethinyloestradiol 35 mg combined with cyproterone acetate 2mg therapy on components of the tissue factor pathway and its main inhibitor in young women before and 4 months after OC therapy. We also assessed other haemostatic variables in the same groups of subjects. **Materials and methods.** We enrolled 35 female patients (aged 15-35 yrs.) affected by resistant acne after a careful history taking anamnesis to exclude inherited thrombotic diseases, obesity and smoking. Plasma factor VII zymogen was measured by ELISA using monoclonal antibodies (FVII:Ag), factor VII coagulant activity (FVII:C) by the one stage method, activated FVII (FVIIa) according to Wildgoose, tissue factor pathway inhibitor (TFPI) by a modified functional chromogenic assay according to Sandset. The levels of activated factor XII (FXIIa) were measured by ELISA (Shield Diagnostics); the levels of factor VIII activity (FVIII:C) were measured by one stage method and those of von Willebrand factor (vWF) by ELISA.

Results.

		time 0	4 months	p
FVII:Ag	(U/dl)	94±20	137±40	0.0001
FVII:C	(U/dl)	88±16	136±40	0.0001
FVIIa	(ng/ml)	3.0±1.3	5.3±3.0	0.0001
TFPI	(%)	100±21	81±14	0.0001
FXIIa	(ng/ml)	1.0±0.3	1.6±0.5	0.0001
FVIII:C	(U/dl)	103±15	118±24	0.0035
VWF	(U/dl)	92±19	111±30	0.0031

Conclusions. Plasma levels of VWF, FVIII:C, FVII and FXIIa were significantly higher after OC in accordance with previous reports, confirming the role of hormonal changes on the contact phase and the tissue factor pathway. The significant decrease of TFPI levels after OC therapy provides support for a pro-coagulant imbalance of the tissue factor pathway. The latter finding may be relevant to the increase risk of thrombosis in OC using women. The decrease in TFPI could be an early predictor of thrombotic events.

PO-152**PERIPHERAL ARTERIOPATHY: EFFECTS OF CLORICROMENE ON CLAUDICATION AND QUALITY OF LIFE. A RANDOMISED, DOUBLE BLIND, PLACEBO CONTROLLED TRIAL IN PATIENTS TREATED WITH ASPIRIN**Gresele P,* Migliacci R,^o Di Sante G,[#] Nenci GG,* Avruscio GP,^e Bicchi M,^e Buzzigoli A,^e Coccheri S,^e Curzi T,^e D'Addato M,^e de Regis F,^e Falcinelli F,^e Di Giacomo E,^e Filippini M,^e Flamini F,^e Forconi S,^e Lattanzi S,^e Massi Benedetti M,^e Mollaioli M,^e Montagnani M,^e Ribaldi E,^e Scloverano F^e**Institute of Internal and Vascular Medicine, University of Perugia, Perugia, Italy; ^oDivision of General Medicine, Cortona Hospital, AR, Cortona, Italy; [#]Fidia S.p.A., Abano Terme; ^eCRAMPS Investigators, Italy*

The main aim of medical treatment of peripheral arteriopathy is the reduction of the mortality and morbidity from ischaemic cardiovascular disease. However, symptomatic treatment of intermittent claudication (IC), with the aim of improving exercise performance and the overall quality of life, may also be an important target of the clinical management of these patients. Cloricromene, a drug provided with antithrombotic and anti-ischaemic activities, has previously shown some promising results in patients with claudication. We carried out a clinical trial to assess the effect of cloricromene on the claudication distance and on the quality of life of patients with IC chronically treated with aspirin. One hundred and fifty-nine patients with IC, Stage II (Fontaine), were enrolled in a double-blind, randomised, prospective, multicentre study comparing cloricromene (100 mg orally BID) or an identical placebo for six months. All patients received aspirin 160 mg/day. The primary end point was the improvement of initial claudication distance (ICD) at six months as measured by a treadmill test. Secondary end points were absolute claudication distance (ACD) at six months, percentage of responders to treatment (improvement of ICD of at least 40%), changes in the ischaemic window (IW), quality of life as assessed by the SF36 questionnaire, and occurrence of major cardiovascular events. Initial claudication distance increased significantly in both treatment groups (plac. +44m, clor. +57m, both $p < 0.01$), with a not-significant difference at 6 months in favour of cloricromene of +12.3 meters. ACD, percentage of responders, and IW also improved in both groups with a slight, not-significant trend in favour of cloricromene. Pretreatment quality of life scores showed only a slight worsening as compared with an age-matched, healthy population and did not change upon treatment. A *post hoc*, subgroup analysis showed a significant benefit from cloricromene in patients with an ICD at enrolment higher than the median of the patient population. In conclusion, treatment with cloricromene for 6 months does not significantly improve claudication symptoms in patients with stage II Fontaine peripheral arteriopathy chronically treated with aspirin. An improvement of 40-60 meters in the ICD on a standardised treadmill test does not translate into a self-perceived improvement in the quality of life as assessed by the SF36 questionnaire.

PO-153**LOW MOLECULAR WEIGHT HEPARIN ADMINISTRATION FOR 2 DAYS IN PATIENTS WITH UNSTABLE ANGINA AFFECTS THE HYPERCOAGULABILITY BUT NOT THE INFLAMMATORY STATE**

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Blood clotting activation and consequent thrombus formation play a relevant role in acute coronary syndromes. A large number of experimental and clinical studies have demonstrated that inflammatory reactions participate in the pathogenesis of acute coronary syndromes. Elevated plasma levels of both interleukin-6 (IL-6) and C-reactive protein (CRP) have been found in patients with unstable angina and their elevation is associated with an unfavourable outcome. Several studies comparing unfractionated heparin (UFH) with low molecular weight heparin (LMWH) have been conducted and most of them have demonstrated an equal benefit or a better outcome with LMWH in respect to UFH in patients with unstable angina. The aim of this study was to evaluate whether enoxaparin administration (90 IU/Kg bid for 3 days), according to the ESSENCE study, is effective in reducing the high prothrombin fragment (F1+2) plasma levels in unstable angina patients and the possible alterations of the inflammatory state during LMWH administration. Plasma samples were obtained from 20 unstable angina patients immediately before, 1 hour and 4 hours after the enoxaparin administration on the 3rd day of treatment. F1+2, IL-6 and CRP plasma levels were measured by commercial assay. We observed that after enoxaparin administration F1+2 plasma levels at all observation times (pre-injection=1.45, 0.9-2.2 nmol/L; 1 hour=1.2, 0.6-1.8 nmol/L; 4 hours: 1.3, 0.7-2 nmol/L) were significantly ($p < 0.001$) lower than base-line levels (2.15, 0.9-3.2 nmol/L). At all observation times, after base-line withdrawal, both IL-6 and CRP levels markedly increased in 5/20 patients and decreased in 5/20; in the other patients the levels remained unchanged. In conclusion, enoxaparin treatment is effective in reducing increased F1+2 plasma levels in patients with unstable angina. No alterations of inflammatory markers during short-term LMWH treatment were observed.

POSTERS**Laboratory tests and diagnosis****PO-154****ABNORMALLY HIGH TpP PLASMA LEVELS IN PATIENTS WITH CARDIOEMBOLIC STROKE**

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One of the key events in the formation of thrombus (blood clot) is conversion of the circulating soluble plasma protein fibrinogen to the insoluble cross-linked fibrin polymer. Thrombin cleaves and removes a set of peptides from the fibrinogen molecule resulting in the formation of a species known as des AABF fibrin. These soluble polymers are the immediate precursor to insoluble fibrin and are thus referred to as thrombus precursor protein (TpP). The aim of our study was to investigate the role of TpP in ischaemic stroke as a new marker of thrombophilia. We recruited 88 adult patients (aged 59 ± 17 years). Sixty-eight patients had a documented diagnosis of atherothrombotic stroke (lacunar and large vessel disease) and 20 a diagnosis of cardioembolic stroke. Eighty-eight healthy subjects with the same ethnic background acted as a control group. TpP plasma levels were assayed from citrated platelet poor plasma by the EIA method. Normal values in our general population ranged from 0.2 to 3.6 mg/mL. An effective cut-off was determined by examination of TpP values in our control population tested at two sites ($n=400$). The best estimate of an effective cut-off value was determined to be 3.65 mg/mL by employing a percentile evaluation (97.5 percentile in a box and whisker plot). Thus normal values from healthy volunteers should produce results < 3.65 mg/mL. In patients with an atherothrombotic episode we observed values significantly higher compared to those of controls (6.15 ± 0.85 mg/mL, $p < .001$ Mann Withney U-test). Notably, the highest levels of TpP were found in patients with a cardioembolic stroke (20.5 ± 5.7 mg/mL, $p < .0001$). Our data suggest a hypercoagulable state in stroke patients by means of TpP plasma levels considered indicative of enhanced fibrin formation. Thus, the early diagnosis of cerebral thrombosis, with respect to the identification of subjects at major risk, should be significantly reduce the mortality and morbidity in patients with an ischaemic episode and could be helpful in identifying subgroups of patients at higher risk of thrombi formation. It is also suggested that TpP may be used to monitor the outcome both in atherothrombotic and cardioembolic stroke. The authors feel that TpP plasma levels may represent an additional tool for risk factor assessment of thrombophilia in ischemic stroke in this population.

PO-155 IMPROVING SENSITIVITY OF THE APC RESISTANCE TEST

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Activated protein C acts a naturally occurring anticoagulant mechanism that inactivates procoagulant factors VIIIa and Va. Resistance to activated protein (APCr), an important coagulation defect leading to venous thrombosis, is mostly due to the factor V Leiden (Arg506Glu) mutation. However other inherited and acquired conditions have been described that lead to APCr. On the other hand, evidence is accumulating that APCr is unable to identify all subjects with factor V Leiden mutation. We evaluated APCr in citrated blood samples obtained from 81 consecutive subjects referred to our Centre. In these patients, we investigated the sensitivity and specificity of the PCA-Ratio test (Dasit® S.p.A) in an automatic Sysmex TOA CA 6000 coagulometer. The results were expressed in ratios. In addition to the common screening test, this kit also provides a confirmatory test based on dilution with factor V depleted plasma. The cut-off value for APCr was experimentally established to be >2.2. Of the 44 subjects with APCr >2.2, 22 (50%) were heterozygous for factor V Leiden mutation; of the 17 subjects with APCr <2.2, 14 were heterozygotes and 2 homozygotes for the mutation (94%). In 20 subjects (2 heterozygous for FV Leiden, 10%) the APCr could not be determined because of insufficient clotting after adding activated protein C. Positivity of the confirmatory test was obtained in 43 subjects with APCr >2.2 (3 were heterozygous for factor V Leiden mutation, 7%), in 38 subjects with APCr <2.2 (35 heterozygous and 2 homozygous for mutation, 97%) and in no subject with indeterminable APCr. We conclude that the present assay is a fairly sensitive and specific test to evaluate APCr, being also very predictive of factor V Leiden mutation. Not all subjects with the Leiden mutation are, however, identified by this test.

PO-156 A NEW TEST FOR OVERALL ASSESSMENT OF THE ACTIVATED FACTOR II -ANTITHROMBINS SYSTEM

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The venom from *Echis carinatus* selectively activates factor II to thrombin, forming an intermediate product with thrombin-like enzymatic activity, known as meizothrombin. The new test measures the clotting

time of plasma after addition of this venom, with (Ta) or without (To) heparin. Time To is directly correlated with the coagulant activity of factor II and the conversion of fibrinogen into fibrin, while the prolongation of clotting time (Ta-To) is directly correlated with the functional status of the anticoagulant system based on substances with antithrombin activity. We investigated how several substances affected the activation of factor II: the venom concentration, the ionic strength (Na⁺, K⁺ concentration) and the concentrations of the ions required as cofactors in the normal biochemical reactions involved in the coagulation cascade (Ca⁺⁺, Mg⁺⁺). We then optimised the anticoagulant activity, using different heparin concentrations and types (non-fractionated and LMW products, dermatan sulphate). The new test can be easily automated and the reagents need no particular precautions in handling and storage. The intra- and inter-assay repeatability is good, giving a coefficient of variation of respectively <2% and <4%. The test's diagnostic ability lies in the fact that it gives an overall assessment of the coagulant activity of activated factor II against fibrinogen, and the anticoagulant activity of antithrombin substances against activated factor II. It therefore serves to identify quantitative and qualitative abnormalities in factor II, fibrinogen, antithrombin, heparin cofactor II and probably also other, as yet unknown, substances or mechanisms.

PO-157 A NEW TEST (TG-At) USED IN THE SCREENING OF THROMBOPHILIC PATIENTS

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We recently proposed a new analytical test (TG-At), able to evaluate global functionality of the anticoagulant system activated factor II-antithrombins. The aim of this study was to use the TG-At test in the screening of thrombophilic patients in our Centre, with a special attention to carriers of the 20210 GA mutation of the prothrombin gene. Ninety-one patients were recruited, forty of them with 20210 GA mutation, and 167 blood donors as a normal control group. We found a resistance to activated factor II inhibition ($\Delta N < 0.80$) 50% only in (20/40) of the patients with 20210 GA mutation; moreover, in the same group only 57% (20/35) showed high levels of coagulant activity of factor II (>125%). In contrast, in the group of patients without 20210 GA mutation, 45% (23/51) showed a high resistance to activated factor II inhibition. These findings show that the new proposed test is able to reveal a resistance to activated factor II inhibition in the presence of high levels of factor II (quantitative response), but of yet unknown dys functional reasons.

PO-158**MONITORING THE HAEMOSTATIC EFFECTS OF DESMOPRESSIN WITH THE PFA-100® SYSTEM IN PATIENTS WITH CONGENITAL DISORDERS OF PRIMARY HAEMOSTASIS**

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Background. Desmopressin shortens the prolonged bleeding times (BT) of patients with defects of primary haemostasis by complex mechanisms. Because of its complex mechanism of action and of the lack of global *in vitro* tests evaluating platelet function at high shear, therapeutic monitoring of desmopressin is based on the BT. **Aim.** We evaluated whether the effects of desmopressin infusions in patients with defects of primary haemostasis can be monitored by a technique evaluating platelet function at high shear. **Methods.** The PFA-100® system measures the time needed to form a platelet plug which stops the blood flow at high shear (closure time, CT) through cartridges coated with collagen and ADP (C-ADP) or collagen and epinephrine (C-EPI). Twenty-six patients with von Willebrand's disease (vWD) and 17 with congenital platelet secretion defects (delta-storage pool deficiency [delta-SPD]:n=7; primary secretion defect[PSD]:n=10) were studied before, 1 h and 4 h after the i.v. infusion of desmopressin (0.3 mg/Kg). The table shows the effects of desmopressin on the median BT and CT of the PFA-100® system:

Patients	Bleeding time (min)		CT (C-ADP) (sec)			CT (C-EPI) (sec)			
	Before 1 h	4 h	Before 1 h	4 h	Before 1 h	4 h	Before 1 h	4 h	
vWD type1	7	5	6*	243	64	118*	255	115	207*
vWD type2A	>30	>30	>30	254	218	226	217	240	219
vWD "Vicenza"	6	4	6*	>300	58	>300*	>300	79	>300*
delta-SPD	13	8	12*	100	59	69*	175	106	169*
PSD	8	6	8*	102	59	80*	180	89	136*

(*: p<0.05)

Therefore, the PFA-100® system and the bleeding times performed after desmopressin infusions gave comparable results. As the PFA-100® test is less invasive and more reproducible than the BT, it appears to be a good alternative for monitoring patients with defects of primary haemostasis treated with desmopressin.

PO-159**ATIII/D-DIMER (AT/D-d) RATIO: A PROGNOSTIC INDEX IN PATIENTS WITH MULTIPLE ORGAN DYSFUNCTION SYNDROME**

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Disseminated intravascular coagulation (DIC) is an important cause of multiple organ dysfunction syndrome (MODS) in patients with sepsis. The imbalance between coagulation and fibrinolysis, evaluated by the ratio of thrombin-antithrombin (TAT) and plasmin-antiplasmin (PAP) complexes, is a significant prognostic factor. A high TAT/PAP ratio indicates a coagulation process not counterbalanced by fibrinolysis; it is higher in non-survivors, therefore indicating a poor prognosis. TAT and PAP are not routinely available. AT, constantly decreased in sepsis, and D-dimer (D-d) (indicative of fibrinolytic activation) are widely available, specific and sensitive. We derived a prognostic index from the values of AT and D-d observed in patients included in a placebo-controlled study to evaluate the eventual benefit of AT concentrates in patients admitted to an intensive care unit (ICU) (Intens Care Med. 24:336,1998). **Material and methods.** One hundred twenty patients with AT<70% were randomised to receive AT or placebo treatment for 5 days; 56 patients were in septic shock. MODS was graded according to Marshall (Crit Care Med 1995,23). AT activity and D-d were measured daily for the first 7 days. The AT/D-d normalised ratio was utilised to formulate a prognostic index according to the formula: $\frac{AT/100}{D-d/0.5^*}$

(*normal value).

The reported data refer to all patients regardless of treatment. Data are presented as mean + SE; a p level<0.05 was considered statistically significant (ANOVA for repeated measures).

Results.

		MODS	AT%	D-d mg/ml	AT/D-d
Survivors (S)	Day 0	6.31+0.45	66.9+13.2	2.01+0.28	0.57+0.1
	Day 7	3.35+0.45#	90.6+17.2##	1.97+0.24	0.47+0.11
Non survivors (NS)	Day 0	8.78+0.48*	54.4+14.5**	1.34+0.14*	0.58+0.9
	Day 7	8.85+0.58^	71.7+25.5^^	1.39+0.18	0.96+0.2F

#, *, ^, F p<0.05; ##, **, p<0.01. S at day 7 vs day 0 = #, ##; NS vs S at day 0 and day 7 = *, ^, **, ^^; NS at day 7 vs day 0 = F

Conclusions. Both levels of D-d and the AT/D-d ratio were significantly different from day 0 to day 7 in relation to outcome (p<0.05). Our results indicate that a high AT/D-d ratio is a poor prognostic factor and is associated with progression to irreversible multiple organ failure. The AT/D-d ratio is a useful prognostic index.

PO-160**DETECTION OF PLASMA FIBRINOGEN USING HIGH-PERFORMANCE CAPILLARY ELECTROPHORESIS**

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Congenital fibrinogen abnormalities can be divided in quantitative defects, termed hypo- or afibrinogenemia, depending on the severity of the deficiency.

cy, and qualitative defects, designed dysfibrinogenemia. Congenital quantitative defects of plasma fibrinogen must be distinguished from those of congenital dysfibrinogenemia. Currently, methods to quantify plasma fibrinogen concentrations rely on clotting and immunologic assays. High-performance capillary electrophoresis (HPCE) allows for a rapid and efficient analysis of components present in small sample volumes. In view of the fact that very small volume flow rates and on-column detection applied provide reliable results on very small amounts of sample (nanolitres per run), HPCE-based methods have increasingly gained popularity as alternative tools for a variety of routine analytical applications. In addition, one of the main advantages of HPCE is that it requires only simple instrumentation so solvent usage and disposal costs are reduced. We aimed to achieve a HPCE-based method to measure quantitatively fibrinogen circulating concentrations, comparable to those obtained employing immunologic methods. HPCE analysis of plasmas from normal subjects revealed the presence of a series of peaks with a retention time (RT) ranging from 5.6 to 6.0 min. Comparison with electropherograms containing standard amounts of reduced fibrinogen, handled in the same fashion, enabled us to identify peaks corresponding to different fibrinogen chains. The possibility of utilising plasma samples from subjects with the complete absence of detectable fibrinogen, with hypofibrinogenemia, and carrying qualitative abnormalities of fibrinogen allowed us to obtain an *ad-hoc* reference samples. Plasma levels of all the components were well above the detection limit of the proposed HPCE method. Moreover, a linear correlation was observed, for each component, between the concentration of the peak area observed in plasma and those obtained after the addition of different amounts to human plasma. Determinations in 20 normal healthy individuals revealed a high reproducibility of the relative RT, less than 5% change of the relative run-to-run, and day-to-day standard deviation being observed. In conclusion, the method presented here is simple, fast, and accurate technique to achieve a selective and sensitive identification of fibrinogen chains meaning the HPCE is suitable for routine determinations of plasma fibrinogen levels. The proposed method may be useful for distinguishing between congenital quantitative and qualitative defects of plasma fibrinogen.

PO-161

HR2 HAPLOTYPE IS ASSOCIATED WITH SIGNIFICANTLY INCREASED RESISTANCE TO ACTIVATED PROTEIN C ONLY WHEN ASSOCIATED WITH FACTOR V LEIDEN MUTATION

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As a part of a study of possible factors influencing activated protein C (APC) phenotype in non factor V (FV) Leiden carriers, we assessed the prevalence of

the R2 allele in 102 unrelated subjects randomly selected from the VITA project. Twelve out the 102 subjects (11.8%) were heterozygous for the HR2 haplotype, with an overall allele frequency of 5.9%. Surprisingly, the mean n-APC ratio was not different in these subjects compared to the 90 negative subjects (n-APC 1.03 ± 0.09 vs 1.04 ± 0.10 ; $p=0.5$). We decided to confirm this result in our series of patients referred for thrombophilia screening during 1995-1999. During this period we identified at our centre 9 families in which the R2 allele and FV Leiden were present. We measured APC resistance in the 52 available family members (including index cases). Patients on oral anticoagulant treatment were not included. All the subjects were screened for FV activity and for the presence of additional inherited defects predisposing to thrombophilia.

	Normal relatives (n = 11)	Heterozygous HR2 subjects (n = 15)	Heterozygous FV Leiden subjects (n = 12)	Heterozygous HR2/ FV Leiden (n = 14)
n-APC (M±SD)	1.03±0.06	1.04±0.05	0.74±0.09	0.62±0.09*
(range)	(0.91 - 1.13)	(0.93 - 1.1)	(0.5 - 0.88)	(0.44 - 0.76)

* $p = 0.0033$ versus heterozygous F Leiden (by ANOVA)

Interestingly, in the group of patients with isolated heterozygosity for FV Leiden, the subject with n-APC of 0.5, but without R2, and the two subjects with compound heterozygous HR2/FV Leiden having n-APC ratio of 0.44 and 0.45 had mild FV deficiency (FV 45-52%), whereas none of the remaining subjects with compound heterozygosity had FV levels below 75%. Thus, we confirm that coinheritance of HR2 haplotype and heterozygous FV Leiden induces a significantly decreased sensitivity to APC in comparison to FV Leiden alone, thus providing further support to the relative increased risk of venous thrombosis observed in these patients. However, the presence of both alleles does not invariably determine a degree of APC resistance similar to that observed in homozygous FV Leiden. It appears that the cosegregation of HR2 haplotype and mild FV deficiency or FV deficiency alone in patients with heterozygosity for FV Leiden could be the most important variable in inducing this effect. Finally, on a population basis, the HR2 haplotype *per se* does not cause different ratios of n-APC in comparison to non-carriers. Further studies are needed to clarify the possible genetic reasons for this phenomenon.

PO-162

ACCURACY OF D-DIMER TEST FOR EXCLUDING SYMPTOMATIC ACUTE VENOUS THROMBOEMBOLISM IN AN EMERGENCY SETTING

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If misdiagnosed or left untreated, acute venous thromboembolism, constitutes a potentially fatal disease. In emergency medicine it is crucial to confirm

or rule out an acute and potentially life-threatening situation: it is therefore important to deal with tests having high sensitivity and high negative predictive value. Management of venous thromboembolism may require multiple diagnostic approaches, due to the low accuracy of clinical judgement; in particular, signs and symptoms of haemodynamically stable pulmonary embolism (PE) are often confused with those presented by other respiratory and cardiac disorders. Pulmonary angiography represents the gold standard test for diagnosing PE, but it is an invasive and expensive approach. Deep vein thrombosis (DVT) requires ultrasound examination to exclude the presence of potentially embolic thrombi located in the proximal segment. SimpliRED, (Agen Biomedical Ltd, Brisbane, Australia) is a rapid plasma test for measuring D-dimer, a marker of thrombin activation. Patients referred to an emergency department usually present with critical signs and symptoms and may therefore represent a population different from patients who attend outpatients clinics. Studies have been applied to the diagnosis in outpatients but none of these considered emergency situations. In order to improve the management of patients in an emergency setting, we evaluated the clinical accuracy of SimpliRED, in patients with a clinical suspicion of DVT and/or PE. Patients clinically suspected of having a DVT underwent compression ultrasonography (CUS) and D-dimer testing; patients with clinically suspected PE underwent bilateral CUS and D-dimer testing; with a positive CUS the patients were considered as having venous thromboembolism, otherwise they underwent additional tests (chest ray, pulmonary V/Q scan and/or pulmonary angiography and/or spiral CT scan). During 18 months follow-up, we evaluated 265 patients (191 for DVT and 74 for PE); DVT was confirmed in 45 patients (23.5%) and PE in 21 (28.3%). Among patients with confirmed PE, 3 patients only (14.8%) had concomitant DVT (1 isolated distal thrombosis). The D-dimer test was normal in all but 2 patients with DVT, in 1 with superficial phlebitis and in all but 2 patients with PE. The sensitivity, specificity, negative and positive predictive values of SimpliRED, were 93.3%, 45.2%. 95.6% and 34.4%, respectively for patients with a clinical suspicion of DVT and 90.4%, 62.2%. 94.2% and 48.7% respectively for patients with a clinical suspicion of PE. This study confirms the need for a rapid plasma test in the management of patients with suspected venous thromboembolism. On the other hand, despite a relatively high sensitivity and negative predictive value, SimpliRED, should not be proposed as the only test for screening patients with a clinical suspicion of venous thromboembolism in the emergency department.

ORAL COMMUNICATIONS

Genetic risk factors for venous thromboembolism

CO-163

COMBINATIONS OF FOUR MUTATIONS (FV R506Q, FV H1299R, FV Y1702C, PT 20210 G/A) AFFECTING THE PROTHROMBINASE COMPLEX IN A THROMBOPHILIC FAMILY

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We report here the study of the molecular bases of thrombophilia in a large family with four symptomatic members. Three thrombophilic genetic components (FV R506Q, FV H1299R and PT 20210G/A), all affecting the activity of the prothrombinase complex, were detected alone or in combination in various family members. In addition, a newly identified missense mutation (FV Y1702C), causing FV deficiency, was present in the family and appeared to enhance APC-resistance in carriers of FV R506Q or FV H1299R, by abolishing expression of the counterpart FV allele. The relationships between complex genotypes, coagulation laboratory findings and clinical phenotypes were analysed in the family. All symptomatic family members were carriers of combined defects and showed APC-resistance and elevated F1+2 values. Evidence for the causative role of the FV Y1702C mutation, which affects a residue absolutely conserved in all three A domains of FV, factor VIII and ceruloplasmin, relies on 1) the absolute co-segregation between the mutation and FV deficiency in the family and in the general population; 2) immunoblot studies showing the presence of only R506Q FV molecules in the FV Y1702C/R506Q doubly heterozygous propositus, in spite of normal levels of the FV Y1702C mRNA; 3) molecular modelling data which support a crucial role of the mutated residue in the A-domain structure. These findings help to interpret the variable penetrance of thrombosis in thrombophilic families and to define the molecular bases of FV deficiency.

CO-164

THE RISK OF RECURRENT DEEP VENOUS THROMBOSIS AMONG HETEROZYGOUS CARRIERS OF THE G20210A MUTATION IN THE PROTHROMBIN GENE

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Background. The G20210A mutation in the prothrombin gene is a common cause of inherited thrombophilia and is associated with an increased risk of first venous thrombosis. It is not established whether or not carriers of the mutation should be recommended lifelong anticoagulant treatment after a first venous thrombosis, since the long-term risk of recurrence is still unknown. **Patients and Methods.** In order to determine the risk for recurrent venous thromboembolism in heterozygous carriers of the prothrombin gene mutation we retrospectively investigated a cohort of 624 patients referred for previous objectively documented venous thromboembolism. **Results.** After exclusion of other inherited (antithrombin, protein C, protein S deficiency and factor V Leiden) or acquired (antiphospholipid antibody syndrome) causes of thrombophilia and exclusion of patients receiving oral anticoagulants for more than six months after the first thrombosis, the overall cumulative probability of recurrence in the remaining 470 patients was 12.2% (95% CI 9.2 to 15.3) at 2 years, 18.8% (95% CI 14.8 to 22.8) at 4 years, and 32.4% (95% CI 26.6 to 38.2) at 8 years after the first thrombosis. We compared 52 heterozygous carriers of the prothrombin gene mutation (M/F 26/26) to 283 patients with normal genotype (M/F 120/163). The two groups did not differ in age at the time of first DVT (median age 42 vs. 44), length of the interval between the first DVT and the referral to the Thrombosis Centres (median 3 years, range 1 to 36, vs. 3 years, range 1 to 41), and rate of first spontaneous thrombotic events (40% vs. 40%). The patients with the mutation had an overall risk of recurrent venous thromboembolism similar to that of patients with normal genotype (hazard ratio 1.2, 95% CI 0.7 to 1.9); the risk was similar both considering only the overall spontaneous recurrences (hazard ratio 1.3, 95% CI 0.7 to 2.3) or spontaneous recurrences after a first spontaneous thrombosis (hazard ratio 1.5, 95% CI 0.7 to 3.1). **Conclusions.** Carriers of the prothrombin gene mutation are likely to be treated with oral anticoagulants after a first deep vein thrombosis as long as patients with a normal genotype.

CO-165

THE INCIDENCE OF THROMBOTIC MANIFESTATIONS IN THROMBOPHILIC CHILDREN: A PROSPECTIVE STUDY

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Background. Antithrombin, protein S, protein C defects and FV Leiden mutation are well recognised risk factors for venous thrombosis in adults. The relevance of these defects in predisposing children to thrombosis is still undefined. A clear definition of a thrombophilic state in children however is necessary to establish whether or not a prophylaxis has to be given. **Methods.** Asymptomatic children (aged 1 to 18 years), who were family-members of probands with an objectively diagnosed thromboembolic event and

a documented AT, PS, PC deficiency or FV Leiden mutation were included in the study. After laboratory diagnosis of thrombophilic defect the children come for a visit every six months to our Centre. Thrombotic events were diagnosed by objective tests. **Results.** One-hundred and fifteen children, from 52 families, were enrolled. Sixty-four (55.6%) were carriers of inherited defects: 9 heterozygous carriers of AT deficiency, 15 of PC, 3 of PS and 37 carriers of FV Leiden mutation (35 heterozygous and 2 homozygous). The mean observation time in the two groups was 5 years. Twenty-two risk periods occurred in the carriers group and six in non-carriers. No thromboembolic events occurred in the two groups even during risk periods. **Conclusions.** The thrombotic risk in children with a single identified thrombophilic defect appears to be very low. The data from this prospective follow-up of thrombophilic children appears to be in agreement with those from retrospective studies.

CO-166

FREQUENCY OF HFE MUTATIONS IN ITALIAN PATIENTS WITH VENOUS THROMBOSIS

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Background. The hypothesis of a role of iron in atherosclerosis and thrombosis has been suggested by *in vitro* and clinical evidence and has prompted several studies. Evaluation of the body iron status by the usual parameters, serum ferritin, serum iron and total iron-binding capacity, however, has the limitation of being influenced by short-term effects such as inflammation, iron intake, blood loss, and diurnal variation. The identification of HFE, the gene involved in genetic haemochromatosis, provides a new approach to this issue. Heterozygotes for HFE mutations are frequent in Caucasian populations; they have mild but significant alterations of iron parameters but are clinically silent. HFE mutations may be therefore considered markers of a lifelong exposure to mild iron overload. **Aim.** To investigate whether or not the distribution of HFE mutations in patients with thrombosis stratified by a common marker of a prothrombotic state, factor V Leiden (FVL), provides evidence that a genetically determined alteration of iron status can influence the risk for thrombosis. **Methods.** We studied 195 Italian patients with venous thrombosis (VT) and 197 age- and sex-matched controls. FVL and the C282Y and H63D mutations of HFE were detected by appropriate restriction enzyme analysis of DNA fragments amplified by the polymerase chain reaction. **Results.** FVL was detected in 35 patients (17.8%) and in 4 controls (2.0%). The allelic frequency of the main HFE mutation (C282Y) was identical in patients and controls (0.015) and also the second HFE mutation, H63D, was similarly distributed in the two groups (0.114 in patients vs. 0.172 in controls). Since an association between FVL and

HFE mutations in patients with thrombosis was previously suggested, we investigated the relationship between the two genetic markers. At least one HFE mutation was found in 25.9% of thrombotic patients, in 31.4% of thrombotic patients carriers of FVL, and in 35.9% of controls. None of the observed differences was statistically significant, and similar findings were obtained when subjects were sorted by sex. **Conclusion.** Our data do not support the hypothesis that HFE mutations, or the association of FVL and HFE mutations, enhance the risk of VT, although environmental and genetic backgrounds may result in different combinations of genetic prothrombotic factors prevailing among patients with thrombosis from different populations.

CO-167

REDUCED INHIBITION BY HEPARIN OF ACTIVATED PROTHROMBIN: A POSSIBLE MARKER OF THROMBOPHILIC ALTERATION

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A new method based on prothrombin activation by *Echis Carinatus* snake venom in the absence/presence of unfractionated heparin has recently been designed. In the normal response, the addition of heparin induces a prolongation of the clotting time because of the antithrombin activity exerted by the antithrombin/heparin complex. In a not negligible number of patients who suffered from venous thromboembolism (VTE), the addition of heparin did not result in the expected prolongation of the clotting time. The aim of this study was to evaluate the results of this new test ("Activated Prothrombin Heparin-Inhibition test") in 489 (206 males, median age 45y, 11-98y) unselected, unrelated patients who had suffered from at least one objectively confirmed VTE episode compared with those obtained in a large group of healthy controls (n=408, 218 males, median age 40y, 10-79y). The results of the test are expressed as normalised ratio. Significant sex-related differences were observed both for patients and controls; therefore, separate 95th percentiles were calculated for men (ratio cut-off value = 0.85) and women (0.79) in the control group. In 54 (11.0%) of the 489 patients the ratio was below the cut-offs (19/35 males/females), as compared with 19 (5% by definition) in the control group (10/9 males/females). The crude Odds Ratio (OR) for VTE in subjects with altered vs those with normal results was 2.54 (95% CI: 1.48-4.37). ORs did not change significantly after adjustment for age (2.47, 95% CI: 1.44-4.26) and age/sex (2.30, 95% CI: 1.32-4.00) by logistic regression. After adjustment for antithrombin III, fibrinogen and prothrombin levels the risk associated with altered results remained clearly elevated. The overall OR for VTE in females (2.84; 95% CI: 1.33-6.05) was higher than

that in males (2.11; 95% CI: 0.96-4.66). However, for both sexes there was a sharp increase in the risk of VTE associated with altered results in patients aged less than 45 years (crude OR 7.73; 95% CI: 2.66-22.5). The finding of 11% prevalence of altered results among patients who had suffered from VTE and of an OR of 2.5 leads to the conclusion that the alteration in this test may be a marker of a thrombophilic alteration associated with a moderate risk for VTE; the risk seems to be higher in subjects aged less than 45 years.

CO-168

RAS GENE POLYMORPHISMS ACCOUNT FOR INCREASED RISK OF DEEP VEIN THROMBOSIS IN SUBJECTS WITHOUT ACQUIRED OR INHERITED THROMBOPHILIA

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In addition to vascular effects the renin angiotensin system has been implicated in the regulation of fibrinolysis as well as blood clotting activation. The I/D ACE gene polymorphism has been recently found to be a risk factor for deep vein thrombosis (DVT) in orthopaedic surgical patients. The aim of the study was to evaluate the role of ACE and AT1R gene polymorphisms in DVT patients without acquired and inherited thrombophilic risk factors. Sixty consecutive patients (31 females and 29 males; age: range 14-77 yrs) with a history of primary or secondary DVT referring to the Centre for Thrombosis, and 140 control subjects were enrolled in this study. Thrombophilic risk factors were ruled out by the following assays: AT, PC, PS by chromogenic assay, homocysteine by HPLC, ACL, anti β 2 by ELISA, LAC by clotting methods, prothrombin (FII) G20210A and factor V (FV) Leiden gene mutations by molecular biology techniques. ACE I/D and AT1R A1166C polymorphisms were analysed by PCR and RFLP methods. ACE D allele frequency and genotype distribution were significantly different between patients and controls ($p=0.0007$ and $p=0.007$ respectively). ACE DD genotype was significantly associated with DVT (OR=2.34; $p=0.007$). As AT1R A1166C polymorphism is concerned, no difference was found between the two groups. The contemporary presence of ACE D and AT1R C allele significantly increased the risk of a thrombotic event (OR= 14.7; $p=0.001$). The D allele frequency was higher in patients both with idiopathic (73%) and secondary DVT (66%) than in controls. Our results highlight that RAS gene polymorphisms have a possible relevance not only for atherogenesis and thrombogenesis in the arterial bed, but as a risk factor for venous thrombosis in the absence of classic thrombophilic risk factors.

CO-169**INHERITANCE OF THE HR2 HAPLOTYPE IN THE FACTOR V GENE AND RECURRENT VENOUS THROMBOEMBOLISM**

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Although there is evidence of a series of major risk factor for the development of venous thrombosis, there still exist many thrombotic events whose pathogenesis is unclear. The risk of recurrent venous thrombosis still remains considerably high, up to 30% over a period of eight years, despite adequate treatment with heparin and oral anticoagulants. The haplotype HR2 in the factor V gene is associated with increased resistance to activated protein C (APC) both in normal subjects and in thrombophilic patients, independently of carriership of factor V Leiden (FV) mutation. The co-inheritance of HR2 and FV Leiden mutation determines a degree of functional resistance to APC comparable to that observed in patients homozygous for FV Leiden mutation. In relatives of patients who suffered from at least one episode of deep vein thrombosis, double heterozygosity for FV Leiden mutation and HR2 haplotype conferred a three- to four-fold increase in relative risk of venous thromboembolism compared to factor V R506Q alone. Among 502 patients with a documented diagnosis of deep venous thrombosis in one leg consecutively referred for a thrombophilic work-up, we retrospectively assessed the rate of objectively documented previous recurrence in carriers of HR2 haplotype. DNA was extracted from peripheral blood leukocytes according to standard protocols. The FV Leiden mutation and FV HR2 haplotype were detected as previously described. In this setting, 426 patients (84.9%) did not have the HR2 haplotype, 74 (14.7%) were heterozygous, and in 2 subjects (0.4%) both chromosomes carried the HR2 haplotype. Sixty-nine patients had experienced 85 episodes of recurrent venous thromboembolism. Among them, 10 patients (14.5%) had the HR2 haplotype, whereas 66 out of 433 patients (15.2; *p*: n.s.) without recurrence carried the HR2 haplotype. The probability of having a recurrent deep venous thrombosis was similar in patients carrying the FV Leiden mutation (OR: 1.8; 95% CI: 1.0-3.2), and in subjects with both FV Leiden mutation and HR2 haplotype (OR: 2.1; 95% CI: 0.4-10.4). We conclude that, in the present setting, the rate of previous recurrent venous thromboembolism is not significantly higher in subjects carrying both FV Leiden mutation and FV HR2 haplotype and is comparable to that observed in subjects with the FV Leiden mutation.

CO-170**ESTABLISHMENT OF OPTIMAL REFERENCE LIMITS FOR THE DIAGNOSIS OF FREE PROTEIN S DEFICIENCY**

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Background. Diagnosis of protein S (PS) deficiency is difficult due to the interaction of PS with C4Bp and to the influence of different factors on PS levels. **Aim of the study.** To establish the optimal reference limits for the diagnosis of PS deficiency. **Methods.** We measured free PS on a random sample of 506 subjects enrolled in the Vicenza Thrombophilia and Atherosclerosis (VITA) Project, without personal or family history of venous thromboembolism. Free protein S was measured with an ELISA method, following PEG precipitation, using commercial polyclonal antibodies to PS (Dako, Glostrup, Denmark). A calibration curve was obtained from pooled plasma obtained from 106 blood donors, assumed to contain 1 U/dL of free PS and serially diluted from 1:50 to 1:400 after PEG precipitation. The interassay coefficient of variation for the free protein S assay was below 8%. **Results.** On a multivariate model, males had a mean free PS level 23 U/dL higher than females; in these latter, contraceptive pill use resulted in lower free PS values (-11 U/dL) and menopause in slightly higher levels (+7 U/dL). A significant interaction between free PS, gender, cholesterol and triglycerides was observed, with cholesterol influencing free PS in males but not in females, and triglycerides influencing free PS in females but not in males. Separate reference ranges were established for gender and hormonal status:

	Percentiles	
	Lower (90% CI)	Upper (90% CI)
Crude estimate	54.6 (49.1-57.0)	161.7 (153.0-176.2)
Male	64.0 (57.0-71.1)	179.0 (166.2-211.5)
Female	49.4 (43.6-54.6)	138.9 (128.6-148.7)
Fertile, no pill	50.1 (35.9-55.6)	124.2 (118.3-142.9)
Fertile, pill	46.0 (45.0-53.1)	128.0 (93.2-128.0)
Menopause	49.5 (37.0-61.0)	149.6 (139.1-153.0)

Conclusions. These data extend the recent work of Liberti *et al.* (1999), showing that an interaction between hormonal status and lipids strongly influences free PS level. Partition of reference ranges for free PS between males and females is warranted, whereas it is not necessary to differentiate the reference range of females by contraceptive pill use and menopausal status.

CO-171**SUBJECTS WITH HETEROZYGOUS A20210 PROTHROMBIN MUTATION HAVE SIGNIFICANTLY INCREASED ACTIVATED PROTEIN C RESISTANCE: A POSSIBLE ADDITIONAL RISK FACTOR FOR VENOUS THROMBOSIS**

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It has been demonstrated that activated protein C (APC) resistance is a risk factor for venous thrombosis independently of the presence of FV Q506 (FV Leiden). The role of additional genetic factors in inducing such a phenotype is still under investigation. We measured APC in 894 normal subjects from the VITA project and found significantly different mean n-APC in normal subjects compared to heterozygous carriers of an isolated G20210A mutation of the prothrombin gene (1.01 ± 0.12 vs 0.95 ± 0.11 ; $p=0.01$). We hypothesised that increased APC resistance may contribute to increase the risk of venous thrombosis (VT) in patients with G20210A referred for VT. Twenty families with VT and isolated G20210A consecutively diagnosed between June 1998 and October 1999 were enrolled in the study together with their relatives. There were 44 normal subjects and 57 subjects heterozygous for the prothrombin mutation. The male to female ratio and mean age were not differently distributed in the two groups. Twenty-nine out of 57 (52 %) prothrombin carriers had had VT objectively documented or at least two episodes of superficial VT. Mean n-APC was significantly higher in normal relatives compared to carriers, even after sex and age adjustment (1.04 ± 0.12 vs 0.91 ± 0.06 ; $p<0.0001$). Mean n-APC was even lower in the 29 symptomatic carriers than the 28 asymptomatic carriers (0.93 ± 0.06 vs 0.88 ± 0.06 ; $p<0.0001$). When grouping APC values in tertiles, the OR for VT for subjects in the lowest tertile (n-APC 0.73 - 0.9) was 4.8 (95 % CI 1.4 - 16.3) compared to the other tertiles (n-APC 0.91 - 1 and 1.01 - 1.3). In conclusion APC resistance without FV Leiden mutation is significantly increased in subjects with the prothrombin mutation in comparison to normal subjects and the carriers with the lowest APC resistance values have a significantly increased risk of VT.

ORAL COMMUNICATIONS
Clinical aspects of inherited
coagulation disorders

CO-172**EVALUATION OF THE *IN VITRO* REACTIVITY OF INHIBITOR ANTI-FVIII WITH 3 DIFFERENT CONCENTRATES**

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Objectives. 1) To evaluate, *in vitro*, factor VIII inhibitor reactivity with 3 different factor VIII concentrates. **Patients.** Twenty-two patients with severe haemophilia A (FVIII:C<2%) were enrolled in 7 Italian haemophilia centres. Inclusion criteria in the study were: a) presence of inhibitor for at least 2 years; and b) a history of an inhibitor titre at least of 2 UB. Factor VIII preparations. We used 3 different factor VIII preparations: 1 plasma derived, monoclonal antibody purified (Hemophil M-Baxter); 1 plasma derived, chromatographically purified (Emoclot D.I.-ISI) and 1 recombinant product (Kogenate - Bayer). Each product was reconstituted according to the manufacturers' instructions. Dilutions were adjusted to 1 unit/mL. **Methods.** Samples were tested with each FVIII concentrate, and after two hours of incubation FVIII:C was measured by the standard Bethesda method. The specificity of anti-FVIII inhibitors was identified by immunoblotting. **Results.** For 17 patients, the type of chain was identified: 5 (29%) heavy chains (HCs), 9 (53%) light chains (LCs) and 3 (18%) HC/LC chains (HLCs). At enrolment, the inhibitor titre was 54.3 UB (range 4.8-200) in the HB group, 16.4 UB (range 4.4-56.0) in the LC group and 19.4 UB (range 8.4-40.0) in the HLC group. Patients were stratified by type of chains we evaluated the reactivity of inhibitor for each concentrate and analyzed data comparing the 3 different FVIII preparations. The mean inhibitor titre of both plasma derived concentrates was similar ($p=0.44$), while the mean inhibitor titre using the recombinant product was really double that of the plasma derived concentrates ($p=0.44$). These differences were higher in the patients with LCs or HLCs than in patients with HCs. **Conclusions.** Even if the analysis did not show any statistically significant difference, we observed a lower inhibitor capacity when plasma derived concentrates were used. Moreover, this phenomenon was increased in patients with inhibitor to LCs and HLCs. This trend could be explained by the different biochemical characteristics of the plasma derived products: in Hemophil M, the presence of phospholipids, and in Emoclot D.I. the presence of phospholipids and of von Willebrand factor.

CO-173**VERY HIGH RISK OF DEVELOPMENT OF INHIBITOR IN PATIENTS WITH HAEMOPHILIA A AND LARGE FACTOR VIII GENE DELETION**

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In patients with haemophilia A the formation of antibodies against the transfused coagulation factors still remains a major problem for replacement therapy. The development of inhibitors has been correlated in the past with the type and the amount of therapy as well as with the MHC status, but up to now it is not fully understood. Recently some authors have described different proportions of antibody formation in patients carrying different FVIII gene mutations. Large gene deletions, factor VIII gene inversions and stop mutations have an incidence of inhibitor formation of approximately 35%, while antibodies are present in only 5-7% of patients with missense mutations or small deletions. In our Centre we confirmed these results. In a cohort of 96 severely affected patients we found 38 inversions (39%) and 17 out of the 38 (45%) had a history of inhibitors compared with 17% of patients without an inversion. These are not yet fully characterised for mutations. Now we are screening patients for mutations by conformation sensitive gel electrophoresis (CSGE). The whole coding region of the FVIII gene is analysed by PCR from genomic DNA in 34 fragments for haemophilia A. Among the 17 patients not carrying the inversion to date we have found the mutation responsible for the disease, we have identified and characterised three relative haemophiliacs (two brothers and one nephew) with a large gene deletion. This is due to lack of amplification of the region spanning from exon 2 to exon 25 of the factor VIII gene. By using the expand long template PCR system (Boehringer) with a forward primer in exon 1 and reverse primer at the 5' end of exon 26, we have been able to obtain a 21 kb band which is specific for this mutation. All three patients now aged 45, 30 and 8 years respectively, have a very similar story. They each developed inhibitors at very young age, after less than 10 exposure days and with a very high and stable titre (>100 BU). In addition, in the case of the two adults, they have a very severe arthropathy. In the haemophilia A database, 42 multiple-exon large deletions are reported. For 35 patients data on inhibitors are available and are present in 20 (57%). In conclusion we think that this large FVIII gene deletion represents a very high risk factor for development of inhibitors, but other still unknown familial factors, which could be genetic as well, may play an additional role. As in these patients the risk of development inhibitors is very high this should be taken into consideration for accurate genetic counseling and to evaluate the possibility of a preventive immunotolerance program at birth or, likely, even before.

CO-174**TOTAL HIP REPLACEMENT (THR) IN HAEMOPHILIACS WITH HIGH TITRE INHIBITORS BY RECOMBINANT FVIIa (NOVOSEVEN) CONTINUOUS INFUSION**

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We have performed THR's using rFVIIa CI in two haemophilia A patients with high titre of antibodies to FVIII. For the second patient, we modified the protocol previously adopted, by using a similar amount of replacement therapy, but in a different way of administration. Patient 1: 32 years old male with severe sporadic haemophilia A (FVIII: C<1%, with FVIII gene inversion). The titre of inhibitors was 14 BU at the time of surgery with a peak level in the past of 150 BU. rFVIIa therapy started with a bolus dose of 120 µcg/kg which was administered at the beginning of the operation and after two hours. At the end of the operation, a 30 µcg/kg/h CI was started for the first 24 hours, 22 µcg/kg/h for the following two post-operative days, 18 µcg/kg/h for days 4-5 and 11 µcg/kg/h from the 6th day. On the 8th day a major bleed occurred at the site of the surgery, so the rFVIIa infusion rate was increased to 18 µcg/kg/h until the day 13th. From the day 14th a 11 µcg/kg/h CI was administered until the 29th day. The total dose of rFVIIa used was 9.93 mg/kg. During his inpatient stay he required 16 units of red blood cells. The coagulant FVII level ranged from 24 U/ml, which was the peak after the bolus injection, to 8 U/ml on the seventh day. Tranexamic acid was administered i.v. at a dose of 30 mg/kg twice daily for all the period of rFVIIa CI. Patient 2: 29 years old male with severe haemophilia A (FVIII: C<1% with large FVIII gene deletion). When the operation was performed the titre of inhibitor was 700 BU. A program to collect autologous red cells units was started three week before and the patient was able to donate three units. Therapy with rFVIIa started with a bolus injection which was repeated three times until the patient returned to the haemophilia ward four-hours later. CI was with a dose of 42 µcg/kg/h for days 1-4 followed by 30 µcg/kg/h for days 5-8 and 21 µcg/kg/h for days 9-12. Plasma FVII:C ranged from 56 to 13 U/ml. The total dose of rFVIIa used was 9.32 mg/kg. He started a mobilisation on the 8th day. On the 14th day he developed a bleed at the site of the wound and was treated successfully with two bolus injections of 90 µcg/kg of rFVIIa. Throughout the post operative course this patient was treated with tranexamic acid by continuous infusion at a dose of 5 mg/kg/h. *Conclusions.* The total amount of rFVIIa administered was similar (9.93 mg/kg and 9.32 mg/kg, respectively) but the first patient received therapy for longer, but with a lower dosage while second was treated intensively for 12 days only. In our opinion the crucial point is to obtain full haemostatic control during the early perioperative phase. In conclusion we recommend maintenance the FVII:C level above 10 U/ml, especially if there is a significant trauma of the soft tissues as during THR.

CO-175**A PROSPECTIVE STUDY ON LONG-TERM SAFETY OF PORT-A-CATH USE IN CHILDREN WITH HAEMOPHILIA: WHICH ALTERNATIVES?**

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Indwelling catheters are often required in haemophilic children to assure long-term venous access for home treatment and prophylaxis or immune tolerance treatment (ITT). To assess the risk associated with the use of the implantable device (Port-A-Cath, Pharmacia Deltec Inc., USA), 21 children with severe haemophilia and 1 with severe FVII deficiency (median age 4.3 years, range: 0.1-10.1) have been prospectively evaluated. Port insertion was required for ITT in 3 inhibitor patients and for prophylaxis in 19 patients. Of these, 4 subsequently required ITT for inhibitor development. The ports were implanted under general anaesthesia and antibiotic prophylaxis was given on day 0 and post-operative day 1. In non-inhibitor patients surgery was covered by specific replacement treatment through an external vein catheter for one week. Two patients with high titer inhibitors were given recombinant activated FVII (rFVIIa) for one week by continuous infusion. Another inhibitor patient (titer: 7 BU/mL) received high-dose rFVIII for 3 days by continuous infusion. After training in the use of the port all patients continued their infusion programme at home. All ports have remained in place for a median period of 2.6 years (range: 0.5-5.5). Port-site haematoma occurred in 7/22 patients (30%): 5 with inhibitors and 3 of these latter, with concomitant infection of the port. All the haematomas were controlled by FVIII or rFVIIa administration. Infectious complications occurred in as many as 11/22 patients (50%) after a median of 1.8 years from port insertion (range: 0.02-5.5 years). Four of 11 patients had inhibitors when infections occurred. Six Port-A-Cath were removed and 2 reservoirs replaced due to recurrent infections in spite of 1-3 courses of parenteral antibiotics. The port infection rate was 0.73 per 1,000 patient-days. Since we consider these results unsatisfactory, alternative vascular accesses are under investigation. In particular, we have considered the feasibility of an internal arterio-venous (A-V) fistula. Therefore, A-V fistulas have been surgically created in forearms of 3 children, who needed port removal, and, subsequently, adopted in further 5 haemophilic children as first option. No complications have been observed after a median follow-up of 3 months. In summary, long-term use of indwelling devices in hemophiliacs is associated with a high risk of infections. Short-term data on safety and feasibility of an internal A-V fistula in haemophilic children seem promising. Although long-term follow-up is required, this preliminary experience encourages us to include internal A-V fistula among the options for achieving permanent venous access in haemophilic children.

CO-176**COCIS (COST-OF-CARE INHIBITOR STUDY): EVALUATION OF THE SOCIO-ECONOMICAL IMPACT OF INHIBITORS IN HAEMOPHILIACS**

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In order to evaluate the socio-economical impact of inhibitors in a cohort of adult haemophiliacs, 44 haemophilia. A patients (mean age: 36.8 years, range: 17-63) with inhibitors were enrolled in the Italian Cost-of-Care Inhibitor Study (COCIS). A medical questionnaire was used to collect information on bleeding episodes, surgical procedures, physicians' appointments, hospitalisations, WFH orthopaedic score, type of treatment, concentrates used, work days lost. All data concerned the 6-month period before enrolment into the study. Over this period, a total of 179 bleeding episodes were reported: 133 haemarthroses (74.3%), 41 haematomas (22.9%) and other bleedings (2.8%), with a rate of 0.7 bleedings per patient-month. Three patients had undergone knee arthroplasty, treated with recombinant FVIIa (rFVIIa). Three of 44 patients were on immune tolerance treatment: 1 with recombinant FVIII (rFVIII) and 2 with plasma-derived FVIII (pdFVIII). Six low-titre inhibitor patients were on treatment with FVIII (2 with rFVIII and 4 with pdFVIII). Of the remaining 35 high-titre inhibitor patients, 8 were treated with activated prothrombin complex concentrate (APCC) only, 6 with rFVIIa only and 4 with APCC and rFVIIa, whereas 17 patients did not receive any treatment. Overall 347,500 U of rFVIII (115,800 U/treated patient), 1,297,000 U of pdFVIII (216,500 U/treated patient), 469,000 U of APCC (39,000 U/treated patient) and 2815 mg of rFVIIa (216 mg/treated patient) were used. As for rFVIIa, 1,781 mg were used for surgery (594 mg/treated patient) and 1,034 for spontaneous bleedings (86 mg/treated patients). The overall direct costs of treatment of 44 inhibitor patients for 6 months were about 6.5 billion ITL (3.5 million US\$) with a mean cost of 24.5 million ITL/patient/month (13,260 US\$). The major component of direct costs was rFVIIa (60.5%), followed by pdFVIII (18.7%), APCC (13.9%), rFVIII (6.4%), hospital (0.4%, drugs excluded) and physicians' appointments (0.1%). The exclusion of rFVIIa usage attributable to surgical procedures would lower overall costs to about 4 billion ITL (2.1 million US\$) with a mean cost of 15.1 million ITL/patient/month (8,185 US\$). Even in this scenario, rFVIIa would remain the major component of costs (36.5%), followed by pdFVIII (30.4%), APCC (22.7%), rFVIII (10.2%) and physicians' appointments (0.2%) respectively. In conclusion, inhibitor treatment of adults has a dramatic impact on costs, which has to be counterbalanced by increased quality of life achieved by these patients.

CO-177**SUSTAINED COMPLETE RESPONSE TO INTERFERON/RIBAVIRIN TREATMENT IN MORE THAN HALF OF HAEMOPHILIACS WHO DO NOT RESPOND TO OR RELAPSE AFTER INTERFERON TREATMENT**

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Treatment of chronic hepatitis C with interferon (IFN) in haemophiliacs has yielded limited results (complete sustained responses in 10-20%). Combined treatment with ribavirin (RBV+IFN) in non-haemophiliacs is promising, since it has been shown to give complete sustained responses in 50-65%. *Aim.* To evaluate safety and efficacy of IFN/RBV treatment in HIV- haemophiliacs who were non-responders (NR) or relapsers (Rel) to IFN only. *Patients.* Thirty-nine hemophiliacs (median age:39 yrs, range:23-65), who had been unsuccessfully treated with IFN, 3 or 6 MU thrice weekly and showing abnormal ALTs and HCV-RNA positivity, entered this multicentre open trial after a wash-out period of at least 6 months. *Treatment.* 5 MU IFN α 2b sc. were given thrice weekly for 6 months followed by 3 MU thrice weekly for a further 6 months and oral RBV, 1.000 mg daily for 12 months. IFN/RBV was interrupted if a biochemical response was not observed after 4 months. *Virological evaluation:* at study entry, serum HCV-RNA was quantified by 2nd-generation branched-DNA assay and the HCV genotype was determined by LIPA. Biochemical parameters were monitored monthly during treatment and during the following 6 months. At the end of treatment and after 18 months serum HCV-RNA was assayed by RT-PCR. *Efficacy.* ALT normalisation was achieved in 27 patients (69%) at month 4, complete response (ALT normalisation and negative HCV-RNA) in 21 (54%) at month 12 and in 9/16 (56%) at month 18. According to an intention to treat analysis, 6/39 patients (15%) who interrupted IFN/RBV treatment (2 with side-effects and 4 not compliant to the protocol) were considered NR. According to the response to the 1st IFN course, 14/15 Rel (93%) showed a biochemical response to IFN/RBV at month 4 compared to 13/21 NR (62%), $p=0.05$. Side-effects: a mean decrease in hemoglobin of 2.9g/dL (range:0.2-5.2) was observed in all patients during treatment. Other side-effects were: fatigue (21%), headache or insomnia (15%), and nausea (13%). The occurrence of haemolytic anaemia required RBV dose reduction in 4 cases. IFN dose was reduced in 4 cases. In 1 case IFN/RBV was interrupted for severe fatigue. *Conclusions.* Combination treatment was reasonably well tolerated. IFN α 2b/RBV therapy was associated with a sustained complete response in more than 50% of haemophiliacs with chronic hepatitis C resistant to IFN only. A higher chance of having a primary response to IFN/RBV therapy was shown by relapsers compared to non-responders to IFN alone.

CO-178**CONTINUOUS INFUSION OF RECOMBINANT ACTIVATED FACTOR VIIa FOR TREATMENT OF PATIENTS WITH HIGH TITRE INHIBITOR**

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Introduction. Recombinant activated factor VIIa rFVIIa (Novoseven, Novo Nordisk, Denmark) is used in the hospital setting for the treatment of serious bleeding or for prophylaxis prior to surgery in patients with high titre inhibitor. Due to the short half-life, continuous infusion represents an attractive and convenient administration method for prolonged treatments. However, the optimal maintenance dose and the adequate monitoring of treatment are still debated. *Methods.* Four centres treated 18 patients (15 with haemophilia and 3 with acquired inhibitors, historical inhibitor peak: 20-3,860 BU/mL) on 23 different occasions (10 bleedings, 13 surgical procedures, of which 7 minor and 6 major surgery). The mean inhibitor titre at the time of rFVIIa treatment was 160 BU/mL (range: 3-1,100 BU/mL). All treatment courses consisted of a bolus injection with 90-150 μ g/Kg (median 90 μ g/Kg) followed by a mean maintenance dose of 18.1 μ g/Kg/hr (range: 10.0-50.0). No additional bolus injections were administered. The mean duration of rFVIIa treatment course was 8 days (median: 6 days; range 1-34). Seventeen rFVIIa courses were administered using a minipump (Walkmed 440 PIC, Medfusion Inc. USA), whereas syringe pumps were used in the remaining courses. Parallel infusion of saline was adopted in 21 of 23 courses. Tranexamic acid was additionally administered in 5 of 23 rFVIIa courses. The conventional one-stage FVII:C assay used to monitor rFVIIa continuous infusion detected a mean plasma level of 17.4 U/mL (median:16.0 U/mL, range: 6.0-59.0). *Results.* rFVIIa treatment was judged effective in 9/10 bleeding episodes (90%) and 12/13 surgical procedures. Of the 2 failures, one (pace-maker implantation) occurred with FVII:C level of 6 U/mL and was resolved by adopting a more accurate surgical haemostasis. The other one (retroperitoneal bleeding) occurred with FVII:C levels of 11-14 U/mL and it was solved with porcine FVIII concentrate. A mean infusion rate ranging between 16.5 and 20 μ g/Kg/hr after a bolus dose of 90-150 μ g/Kg was successful in 14 of 14 bleedings (100%). Infusion rates lower than 16.5 μ g/Kg/hr were successful in 6 of 8 cases (75%). Local thrombophlebitis was observed on one occasion, in spite of parallel saline infusion. *Conclusions.* Continuous infusion is a safe and cost-saving way of administering rFVIIa. A mean infusion rate ranging between 16.5 and 20 μ g/Kg/hr after a bolus dose of 90-150 μ g/Kg appears to have the best cost/effect ratio. FVII:C monitoring does not seem to be a sensitive method for predicting success in this cohort of patients.

ORAL COMMUNICATIONS

Platelets II

CO-179

INTEGRIN $\alpha_2\beta_1$ ACTIVATION BY COLLAGEN PRODUCES H_2O_2 IN HUMAN PLATELETS

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The interaction between collagen fibers and platelets is involved in the early stages of haemostasis. Collagen is able to adhere to blood platelets and to induce platelet-platelet interaction. Adhesion and activation of platelets by collagen seem to be mediated by distinct receptors: adhesion occurs via integrin $\alpha_2\beta_1$, while activation occurs via glycoprotein VI. Previous studies showed that collagen-induced platelet activation is associated with H_2O_2 production. In the present work we suggest that only $\alpha_2\beta_1$, and not glycoprotein VI, is involved in platelet H_2O_2 production by collagen. In fact, platelet stimulation with a specific agonist of glycoprotein VI, the snake venom toxin convulxin, failed to induce H_2O_2 production. Moreover, an antibody anti α_2 chain of the integrin inhibited H_2O_2 production induced by collagen. In addition, platelet adhesion to collagen was dose-dependently inhibited by catalase, an enzyme which reduces cytosolic concentration of H_2O_2 . Our experiments demonstrated that catalase also inhibited in a dose dependent fashion the collagen induced increase in tyrosine phosphorylation of both phospholipase $C\gamma_2$ and p72Syk. This inhibitory effect was not evident when tyrosine phosphorylation was induced by convulxin. Platelet treatment with catalase resulted in a marked decrease in the amount of actin in the low speed cytoskeleton fraction induced by collagen; moreover the formation of filament actin induced by collagen was reduced dose-dependently by catalase. These results suggest that a cellular activation depending on the link of an agonist to the platelet integrin $\alpha_2\beta_1$ involves H_2O_2 production, which leads to a cascade of second messengers, essential for the cell response. The reduction of H_2O_2 production causes, not only the inhibition of cell adhesion to collagen, but also alterations of cell motility. This finding may be useful for identifying a new therapeutic strategy to prevent platelet activation at the site of an atherosclerotic plaque.

CO-180

PAR-1 HYDROLYSIS BY THROMBIN IS ACCELERATED BY GLYCOPROTEIN Ib ON INTACT PLATELETS

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Platelet activation by thrombin is mostly mediated

by at least two protease activated receptors, PAR1 and PAR4, whose hydrolysis by the enzyme induces signal transduction. Platelet glycoprotein Ib (GpIb) is also involved in platelet activation by α -thrombin through mechanisms that are still unknown. Whether GpIb interaction with α -thrombin may influence, on intact platelets, the hydrolysis of PAR molecules by the enzyme has never been evaluated. In this study we measured the kinetics of PAR-1 hydrolysis by thrombin with and without thrombin binding to GpIb. The PAR1 hydrolysis was monitored by using a PE-conjugated anti-PAR1 MoAb (SPAN12) which binds to a domain of the receptor spanning the cleavage site and thus recognises only intact molecules. Gel filtered platelets from normal volunteers were exposed to 1 nM α -thrombin in the presence of EDTA. At different times (15 to 300 sec) aliquots of stimulated platelets were drawn in tubes containing 100 nM hirudin to stop thrombin activity and the tubes placed in ice to inhibit receptor internalisation. After 30 min incubation with PE-SPAN 12, the geometric mean of fluorescence value was obtained by a Becton-Dickinson FACScan instrument. The k_{cat}/K_m value for PAR1 hydrolysis was $3.2 \pm 0.88 \times 10^7$ M sec (mean \pm SE), in agreement with the k_{cat}/K_m value of $1.96 \pm 0.47 \times 10^7$ M sec previously found for the hydrolysis of the thrombin receptor peptide 38-60, which contains the cleavage site of PAR-1 (Thromb Haemost 1997, 77:735). The inhibition of thrombin ligation to GpIb reduced the velocity of PAR1 hydrolysis by roughly ten fold. In fact, the k_{cat}/K_m value obtained in the presence of an anti-GpIb MoAb, SZ2, which binds to the GpIb domain involved in thrombin ligation, was $2.85 \pm 0.6 \times 10^6$ M sec, and the value obtained in the presence of HD22, a DNA aptamer which inhibited the thrombin binding to GpIb by binding to the heparin binding site of the enzyme, was $4 \pm 0.88 \times 10^6$ M sec. Since glyocalicin in solution had been previously demonstrated not to affect the hydrolysis of PAR1 38-60 peptide, the observed effect must be attributed to reciprocal interaction of GpIb and PAR1 molecules on the membrane of intact platelets. Whether GpIb affects PAR1 hydrolysis by increasing its availability to thrombin on the platelet membrane, or by transducing a signal which modifies the PAR1 and/or the membrane characteristics, is currently being investigated.

CO-181

THE D272-E282 REGION OF PLATELET GpIb α INTERACTS WITH THE HEPARIN BINDING SITE OF α -THROMBIN AND PROTECTS THE ENZYME FROM THE HEPARIN-CATALYZED INHIBITION BY ANTITHROMBIN III

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Platelet glycoprotein Ib (GPIb) interacts with both von Willebrand factor and thrombin. Thrombin binds to GPIb *via* its heparin binding site (HBS). To identify the thrombin-binding domain on GPIb α , we examined the effect of GPIb α_{1-282} , a GPIb α fragment released by the cobra venom mocoarhagin, on the heparin-catalyzed rate of thrombin inhibition by antithrombin III (AT). GPIb α_{1-282} inhibited the reaction in a dose-dependent and competitive fashion. In contrast, the GPIb α_{1-271} fragment, produced by exposing GPIb α_{1-282} to carboxypeptidase Y, had no effect on thrombin inhibition by the heparin-AT complex. Measurements of the apparent equilibrium constant of the GPIb α_{1-282} binding to thrombin as a function of different salts (NaCl and tetramethylammonium chloride) concentration (0.1-0.2 M) indicated a large salt-dependence ($\Gamma_{\pm}=4.5$), similar to that pertaining to the heparin binding to thrombin. The involvement of thrombin HBS in its interaction with GPIb α was confirmed using DNA: aptamers, which specifically bind to either HBS (HD22), or the fibrinogen recognition site (FRS) of thrombin (HD1). HD22, but not HD1, inhibited thrombin binding to GPIb α_{1-282} . Furthermore, the proteolytic derivative γ_{τ} -thrombin, which lacks FRS, binds to GPIb α *via* its intact HBS in a reaction that is inhibited by HD22. Neither α nor γ_{τ} -thrombin bound to GPIb α_{1-271} suggesting that the D272-E282 region of GPIb α may act as a *heparin-like* ligand for the thrombin HBS, thereby inhibiting heparin binding to thrombin. It was also demonstrated that intact platelets may dose-dependently inhibit the heparin-catalyzed thrombin inhibition by AT, at enzyme concentrations <5 nM. This effect was not observed with GPIb-depleted platelets. Moreover, HD22 inhibits in a saturable fashion the thrombin-induced calcium increase in intact platelets, whereas this inhibitory activity was lost for GPIb-depleted platelets. Altogether, these findings show that thrombin HBS binds to the region of GPIb α involving the D272-E282 segment, protecting the enzyme from the inactivation by the heparin-AT system.

CO-182

α -THROMBIN BINDING TO PLATELET GLYCOPROTEIN IB IS MEDIATED BY ITS ANION BINDING EXOXITE II

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We have previously demonstrated that platelet glycoprotein (GP) Ib possesses moderate affinity and relatively high capacity binding sites for thrombin (JBC 273, 1880-1887, 1998). In the present study, to investigate the GPIb-thrombin interaction further, we have used two well characterised mutant thrombins: R68E, a mutant with a substitution in exosite I and R89E a mutant with a substitution in exosite II. Binding studies of I¹²⁵ α -thrombin to purified glyocalicin (GC) bound to an anti-GPIb (LJ-P3) monoclonal antibody coupled to sepharose beads were then performed. Competition experiments with wild type

(WT) and the two mutant thrombins gave the following results: the IC₅₀ of the WT was 2.25 \pm 0.25 E-7, similar to that found with mutant R68E (3.00 \pm 0.4 E-7); in contrast, with mutant R89 E-7 a very decreased affinity was observed, since it was not possible to obtain a 50% inhibition even when the mutant R68E was used at 600 fold excess concentration over radiolabelled WT thrombin. Additional experiments, by using binding of I¹²⁵-thrombin to washed platelets at 4°C for 10^o min., a condition in which α -thrombin binding to platelets is mostly GPIb dependent, demonstrated a very decreased affinity of mutant R89E compared to WT (13.6 E-9 | 27 E-9 versus 3.3 E-9 | 1.6 E-9). Data were analysed with *Drug option of the LIGAND* program. Our results demonstrate that α -thrombin binds to GPIb through its anion binding exosite II and therefore anion binding exosite I remains available to act as an active enzyme. The significance of thrombin binding to high capacity binding sites on GPIb may be that of increasing the concentration of thrombin onto the platelet membrane for subsequent proteolytic cleavage of appropriate substrates and therefore has a pro-thrombotic effect.

CO-183

ADP POTENTIATES PLATELET DENSE GRANULE SECRETION INDUCED BY U46619 OR TRAP THROUGH ITS INTERACTION WITH THE P2cyc RECEPTOR

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Background. ADP is a weak agonist. As such, it does not induce secretion of the platelet dense granule constituents directly, but through the aggregation-mediated synthesis of thromboxane A₂ (TxA₂), which is greatly enhanced when [Ca²⁺]_{out} is decreased by sodium citrate. However, we recently showed that ADP that had been secreted by platelets potentiates platelet secretion independently of aggregation and the synthesis of TxA₂. *Aims.* To assess which of the 3 platelet receptors for ADP (P2X1, P2Y1 or P2cyc) is involved in the potentiation by ADP of platelet secretion. *Subjects.* Four normal volunteers and patient VR (congenitally deficient in platelet P2cyc receptor). *Methods.* [¹⁴C]5HT secretion was measured 2 min after the addition of U46619 (1 mmol/L) or TRAP (20 mmol/L, which stimulates the PAR1 thrombin receptor) to pre-labelled aspirin-treated washed platelets suspensions containing 2mmol/L CaCl₂, which were not stirred (to prevent platelet aggregation). The effects of adrenaline (10 mmol/L) and the following nucleotide analogues was investigated: AR-C69931MX (P2cyc antagonist, 0.1 mmol/L), MRS-2179 (P2Y1 antagonist, 50 mmol/L), a,b-me-ATP (P2X1 agonist, 10 mmol/L). *Results.* ADP and adrenaline, when added alone to platelet suspensions, did not induce platelet secretion. The table shows the mean percent platelet [¹⁴C]5HT secretion induced by

Additions	U46619		TRAP	
	Controls	V.R.	Controls	V.R.
Tyrode	41.2	6.9	38.9	18.5
AR-C69931MX	6.6	6.6	24.3	18.4
MRS-2179	31.2	4.5	39.3	17.5
AR-C + MRS	5.4	5.9	24.5	18.7
AR-C + MRS + a,b-me-ATP	4.5	4.3	22.7	17.4
AR-C + MRS + adrenaline	28.8	25.1	32.8	25.1

Conclusion. P2cyc, which is negatively coupled to adenylyl cyclase (AC) mediates the potentiation of platelet secretion by released ADP. Adrenaline, which inhibits AC, also potentiated secretion. Therefore, the Gi pathway must be stimulated for full platelet secretion.

CO-184

EFFECTS OF NITROASPIRIN ON PLATELET ACTIVATION: POTENTIATION BY NITRIC OXIDE OF THE INHIBITORY EFFECTS OF CYCLO-OXYGENASE INHIBITION

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Nitro-aspirin (NCX 4016) is a nitroderivative of aspirin (ASA) capable of releasing nitric oxide (NO). NCX 4016 displays an inhibitory profile on human platelets *in vitro* which is similar to aspirin but also to NO-donors. The aim of the present study was to distinguish the relative roles of the aspirin- and NO-effects in the platelet inhibitory action of NCX 4016. NCX inhibited the aggregation of washed platelets induced by arachidonate (AA), collagen, U46619 and thrombin (IC₅₀=7.7±11.5; 24.0±5.14; 106±17.9; 112±20mM), similarly to the NO-donor SNP (IC₅₀=4.6±2.4; 1.8±0.56; 0.5±0.14; 138±56mM), while ASA only inhibited AA and collagen induced aggregation (IC₅₀ 4.5±0.44; 32.5±11.5mM). NCX 4016 inhibited the formation of TxB₂ in serum (IC₅₀ 0.06 μM) similarly to ASA (IC₅₀ 0.058μM) while SNP was inactive (IC₅₀>100). Both NCX 4016 and SNP inhibited shear-stress induced platelet activation (IC₅₀=215 and 22mM) while aspirin was inactive (IC₅₀>500mM); the inhibition was completely suppressed by the soluble guanylate cyclase inhibitor ODO (10μM). Similarly, the inhibition by NCX 4016 or SNP of thrombin-induced aggregation in washed platelets was abolished by ODO (NCX100μM = -40.3±15%; ODO + NCX100μM = -5.1±2.1%; SNP 1mM = -97.8±2.1%; ODO + SNP = -6.25±3.2%). The addition to ASA of non-inhibitory concentrations of SNP led to a striking potentiation of thrombin-induced washed-platelet aggregation (ASA 300uM = -9.8±6.7%; SNP 68μM = -10.8±4.7%; ASA+ SNP = -92.2±7.7%). On the other hand, the preincubation of washed platelets with salicylate (SA), an antagonist of the cyclo-oxygenase-blocking activity of ASA, partly reversed the inhibitory activity of NCX 4016 on thrombin-induced aggregation (NCX 4016 100μM = -60.4%; NCX 4016 + SA2mM = -27.7%). Finally, when

dose-response curves of the inhibition of AA-induced washed-platelet aggregation and TxB₂ formation were built for ASA, SNP and their combination it turned out that the addition of a non-inhibitory concentration of SNP to ASA shifts to the left the dose-response curve to ASA for aggregation (about forty-fold) while it only slightly affects that for TxB₂ formation (leftward shift of around two-fold). In conclusion, the effects of NCX 4016 on platelets *in vitro* are due to both ASA-like and NO-like activity; the addition of a NO donor to aspirin potentiates the inhibitory effects on aggregation without increasing the inhibition of cyclo-oxygenase.

CO-185

MATRIX METALLOPROTEINASE 2 IS RELEASED BY ACTIVATED PLATELETS AND POTENTIATES AGGREGATION BY A MECHANISM LINKED TO PROTEIN KINASE C ACTIVATION

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Matrix metalloproteinases (MMPs) are a class of zinc-dependent enzymes involved in the degradation of extracellular matrix. MMPs are released by macrophages or smooth muscle cells (SMCs) present in atherosclerotic lesions and contribute to the fissuring of plaques. Recently, it has been shown that also platelets can release MMPs and one of these (MMP-2) exerts a proaggregatory effect, however no data are available on the mechanism of this activity. The aim of our study was to explore the signal transduction mechanisms activated by MMP-2 in human blood platelets. Washed platelets stimulated with collagen (0.3-1 mg/mL), thrombin (0.05-0.07U/mL) or U46619 (0.3-0.7 μM) released MMP-2 (measured by SDS-PAGE zymography) in the supernatant (0.0084 to 0.075 ng/10⁹ plts). When recombinant, human MMP-2 (0.05 ng/mL, kindly donated by Dr. Stetler-Stevenson) was added to washed platelets two minutes before stimulation with subthreshold concentrations of collagen, thrombin, U46619 or ADP, aggregation (+94.9%±4.5 to +224%±6.9) as well as platelet secretion (+35% to +200%) were significantly potentiated. The inactive proenzyme (pro-MMP-2) did not enhance aggregation. Two specific inhibitors of MMP-2 (recombinant human TIMP-2 or the zinc chelating agent *o*-phenanthroline) significantly reduced aggregation induced by collagen or U46619, but not by thrombin, suggesting that endogenously released MMP-2 contributes to platelet activation. Inhibition of protein kinase C (Ro31-8220 10μM) markedly reduced the proaggregatory effect of MMP-2 (with thrombin: -96±0.43%; with U46619: -89±3.05%). In contrast, the protein tyrosine kinase inhibitor tyrphostine A23 did not affect the potentiating effect of MMP-2 on aggregation. Aspirin (ASA) did not affect the potentiating activity of MMP-2 on thrombin- (without ASA +176±24.3%, with ASA +180±17.08%) or U46619-induced aggregation (without ASA +129±14.7%,

with ASA +137±16.3%). In conclusion, our data show that human platelets release MMP-2 at a concentration which is able to facilitate the platelet activation response to several physiologic agonists. This effect appears to be exerted at the level of a second messenger system common to different agonists and data with Ro31-8220 suggest that PKC is involved. Platelet-released MMP-2, and perhaps also the MMP-2 liberated by macrophages and/or SMCs at the site of ruptured atheromas, may contribute to platelet activation *in vivo*.

CO-186 INTRACELLULAR SIGNAL TRANSDUCTION MECHANISMS REGULATING THE RELEASE OF LYSOSOMES BY ACTIVATED HUMAN PLATELETS

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Platelets contain, besides α - and δ -granules, lysosomes (λ -granules) which store several glycohydrolases able to degrade glycoproteins, glycolipids and glycosaminoglycans. While several studies have explored the signal transduction mechanisms involved in the release of α - and δ -granules, very little information is available on the biochemical mechanisms regulating lysosomal release. We composed the mechanisms regulating α -, δ - and λ -granule release by stimulated, washed human platelets. The following markers of granule release were used: α = bTG (ELISA), P-selectin (flow cytometry); δ = ATP (luminometry), 5HT (3 H-5HT release); λ = Hex (fluorimetry), LIMP and LAMP-2 (flow cytometry). Platelets released their lysosomal contents only after stimulation with strong agonists (thrombin or collagen), differently from α - and δ -granules which could be liberated also by weak stimuli (ADP, adrenaline). The direct PKC activator PMA induced aggregation (81.1±5.1%) and the release of Hex (12.3±1.4% of total), bTG (39.5±3.5% of total) and ATP (65.6±13.3% of total). The calcium ionophore A23187 (1.0mM) also caused a release of Hex (11.6±2% of total) and bTG (42.9±10% of total). The association of A23187 and PMA caused a larger release of Hex (23.6±3.1%) and bTG (55.8±5.6%) than the single agonists. After PKC inhibition (Ro 31-8220 10mM) the release of all platelet granules was greatly reduced (with thrombin = Hex -98.2±0.4%, bTG -44.7±18.4%, ATP -39.3±4.9%; with collagen = Hex -65±14.7%; bTG -49.7±11.6%, ATP -76.2±17%; with PMA = Hex -86±14.2%; bTG -87.5±12%, ATP -73.5±26%). After tyrosine-kinase inhibition (Genistein 100mM) the release of platelet granules was not significantly modified. The chelation of extracellular Ca^{2+} (EGTA) reduced and that of intracellular Ca^{2+} (BAPTA-AM) almost abolished the release of platelet granules. When cyclo-oxygenase was inhibited by aspirin (100mM) the release of all platelet granules was significantly reduced if the agonist was collagen (2mg/mL) (Hex = -70±2.7%, bTG -81.6±2.6%, ATP -88.6±4), while with thrombin or U46619 it was

not modified. In conclusion, platelets release λ -granules only after stimulation with strong agonists such as thrombin or collagen. The release of λ -granules depends on PKC activity and on intracellular calcium movements. PTK activity is only marginally involved. The mechanisms regulating the release of λ -granules seem to be quite similar to those regulating the release of α - and δ -granules, although, especially when thrombin is the agonist, PKC appears to be more strictly required.

CO-187 PLATELET/PMN ADHESION: ROLE OF SRC KINASES IN P-SELECTIN INDUCED MAC-1 ADHESIVENESS

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PMN adhesion to activated platelets represents an important event that allows PMN recruitment at the site of vascular damage. We showed that, in cell suspensions subjected to high speed rotatory motion, activated platelet/PMN interaction can be modeled as an adhesion cascade in which an initial P-selectin-dependent interaction triggers PMN β 2-integrin Mac-1 adhesiveness, that mediates an adhesion-strengthening interaction. P-selectin-induced Mac-1 function was strictly dependent on intact tyrosine kinase activity and was accompanied by tyrosine phosphorylation of a prominent protein of 110 kD in PMN (Evangelista V. *et al.* Blood 93; 876:1999). In the present study, using a double colour cytofluorimetric assay, we evaluated the effect of inhibitors of different families of tyrosine kinases on PMN adhesion to PFA-fixed activated platelets in mixed cell suspensions. PP1, a specific inhibitor of tyrosine kinases belonging to src family, dose dependently (IC_{50} =15.6±5.2 mM) inhibited PMN/platelet adhesion that was completely abolished at 50 mM. At similar concentrations, PP1 blocked tyrosine phosphorylation of P110 that occurs in PMN adhering to activated platelets. In contrast, neither adhesion nor phosphorylation was significantly modified by piceatannole, an inhibitor of the syk/Zap-70 tyrosine kinase family, or PD98059, an inhibitor of MAPK kinase. Similar results were obtained for PMN adhesion and protein phosphorylation triggered by CHO-P. Interestingly PP1, while completely inhibiting P110 tyrosine phosphorylation, failed to significantly modify PMN homologous aggregation induced by fMLP. *In vitro* kinase assay of immunoprecipitated kinases showed that the activity of lyn and hck was strongly enhanced in PMN adherent to activated platelets or CHO-P, being the activity of fgr unaffected. Blockade of P-selectin or β 2-integrins by monoclonal antibodies reduced the functional upregulation of both kinases. Our results suggest that src kinases, not only mediate the outside-in signalling transduced by β 2-integrins, but may also play an important role in the regulation of Mac-1 adhesiveness triggered by P-selectin.

ORAL COMMUNICATIONS

Venous thromboembolism

CO-188

ANTIPLATELET FACTOR 4 ANTIBODIES AFTER CARDIAC SURGERY AND HLA EXPRESSION

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Heparin administration results in the formation of antibodies against the heparin-platelet factor 4 complex in about 3% of cases. The formation of the same antibodies is also induced by contact with heparin in the course of cardiopulmonary bypass surgery. The pathogenetic mechanisms underlying the onset of the typical thrombocytopenia symptoms have been only partially explained. This study was carried out to assess the formation of anti heparin/platelet factor 4 antibodies before and after surgery using cardiopulmonary bypass. In addition, tissue typing was performed to try to determine whether there is a relationship between HLA status and the development of heparin-induced thrombocytopenia. *Methods and results.* The plasma of 69 patients was analysed by ELISA immunoassay before (T1), six days after (T2), and three/four weeks after (T3) heart surgery to detect the presence of anti-heparin/platelet factor 4 antibodies. Ten patients (14.4%) were found to be positive on one of the three occasions, but none of them developed the typical symptoms of heparin-induced thrombocytopenia. The pattern of the average values of the antibody titre, as assessed on the three occasions, showed statistically significant differences. HLA tissue typing highlighted the presence of the HLA DR3 antigen in 50% of the positive patients over 11.8% of negative patients (RR=7.4; X² Yates=6.2; p=0.01) and 15.9% of the normal population of our region (RR=5.3; X² Yates=6.1; p=0.01). *Conclusion.* Our study confirms that a high percentage of subjects undergoing cardiopulmonary bypass surgery develop anti-heparin/platelet factor 4 antibodies although few of them (none in our study) develop symptoms that are typical of heparin-induced thrombocytopenia. Moreover HLA-DR3 antigen was found to be present in a significantly higher percentage of positive patients than either negative ones or the general population. Whether this antigen is a factor predisposing to antibody formation could be confirmed by a larger study group.

CO-189

A NOVEL ULTRASOUND PROTOCOL FOR THE DIAGNOSIS OF SYMPTOMATIC RECURRENT DEEP VEIN THROMBOSIS

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Diagnosis of recurrent deep-vein thrombosis (DVT) is a challenge for clinicians. We have recently developed an ultrasound (US) method for diagnosis of recurrent ipsilateral DVT, which relies on repeated measurements of the diameters of the common femoral and popliteal veins. The purpose of the current investigation was to assess the safety of withholding anticoagulation from patients with suspected recurrent DVT but improved or stable compression vein diameters. Two hundred five consecutive patients presenting with suspected recurrent ipsilateral DVT were evaluated. The vein diameter was measured under compression with the transducer and compared with earlier results. Patients with abnormal US findings (i.e., incompressibility of a previously normal (ised) venous segment, or increase >2 mm of the vein diameter) had confirmatory venography. Patients with stable or improved US findings had repeated US assessments after 2 (+1) and 7 (+1) days. Patients with repeatedly normal test results were followed-up for six months to determine the incidence of recurrent venous thromboembolism. Abnormal test results on the day of referral were found in 52 patients (25%). Venography confirmed the diagnosis in 38 of the 42 patients in whom adequate venograms could be obtained (PPV, 90%; 95% CI, 77 to 97). Of the remaining 153 patients with stable or improved US findings who were scheduled for repeat US assessment, the test became abnormal in 3, and recurrence was confirmed by venography in all. Of the remaining 150 patients with repeatedly normal US test who had six months of follow-up, 2 (1.3%; 95% CI, 0.02 to 4.7) had confirmed non-fatal venous thromboembolic complications. It is safe to withhold anticoagulant treatment from patients with suspected recurrent ipsilateral DVT but improved or stable vein diameter distentions on ultrasound.

CO-190

CLINICAL CHARACTERISTICS OF PATIENTS WITH HEPARIN-INDUCED THROMBOCYTOPENIA WITH OR WITHOUT THROMBOSIS

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Background. About 2% of patients receiving unfractionated heparin (UFH) develop heparin-induced thrombocytopenia (HIT), and 30-40 % of those develop thrombosis (HITT), which can involve both the arterial and the venous systems; the thrombotic complications are fatal in about 20% of patients, and an additional 20% have to undergo limb amputations. The aim of this study was to look for the clinical characteristics of the patients with HIT to predict those who will develop thrombotic complications. *Patients and Methods.* We retrospectively studied clinical records of 39 patients. HIT was diagnosed by means of a clinical score (>3) and *in vitro* demonstration of IgG/IgM against PF4/heparin complex (in house ELISA method). Twenty-five of the HIT

patients developed thrombotic complications (17 venous thromboses, 3 arterial thromboses, 1 DIC, 2 skin necrosis, 2 limb amputations, 2 deaths related to thrombosis). The patients who developed HIT were compared to those with HIT alone. **Results.** Comparing HIT versus HIT patients, we did not find a significant difference in sex distribution (M/F 8/17 vs. 6/8), age (63 ± 12 vs. 59 ± 11 years $M \pm DS$), heparin dosage (16.036 ± 6.768 vs. 17.678 ± 7.873 U), duration of therapy (12.18 ± 7.15 vs. 12 ± 6), route of administration UFH (subcutaneous 16/25 vs. 10/14; intravenous 9/25 vs. 4/14) and time to develop thrombocytopenia (9.06 ± 3.12 vs. 7.84 ± 3.60 days). Previous exposure to heparin was not significantly prevalent in HIT patients (6/25 vs. 1/14). We observed a significant association between severity of thrombocytopenia and the appearance of thrombotic complications ($43.000 \pm 32.000/\text{mm}^3$ vs. $105.000 \pm 86.000/\text{mm}^3$ $p < 0.05$). Orthopaedic patients were prevalent in the HIT group (9/25 vs. 0/14 $p < 0.05$) whereas medical patients were prevalent in the HIT group (5/25 vs. 8/14 $p < 0.05$). IgG anti-heparin antibodies were most frequent in HIT patients (17/25 vs. 5/14 $p < 0.05$) and IgM antibody in HIT patients (2/25 vs. 6/14 $p < 0.05$). Nine patients (6 HIT and 3 HIT) had IgG+IgM antibodies. **Conclusions.** 1) HIT patients have a more severe thrombocytopenia than patients with HIT 2) Orthopaedic patients receiving heparin for prophylaxis are at higher risk of HIT.

CO-191

THE "HOME TREATMENT PROGRAM" FOR DEEP VEIN THROMBOSIS AT THE EMERGENCY DEPARTMENT: PRELIMINARY RESULTS

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Background. Previous studies suggested that low-molecular weight heparins (LMWHs) might be used for the treatment at home of patients with confirmed deep vein thrombosis (DVT); nevertheless, authors reported different criteria for selecting patients, thus giving an effective rate of inclusion from 22% to 83%. In the absence of standardised guidelines, each institution should evaluate a safe and efficacious approach for treating patients with DVT at home. A successful "home treatment programme" requires careful patient selection and education, daily follow-up and 24h emergency room facilities; the emergency department (ED) might be an appropriate institution for the initial management of patients selected. **Objective and methods.** In order to evaluate the safety and efficacy of "the home treatment programme", we investigated, during the period February-October 1999, 102 patients with a clinical suspicion of DVT. All patients, admitted to the ED for a few hours, underwent diagnostic tests for DVT (pre-test clinical probability, compression ultrasonography and D-dimer test) along with baseline diagnostic examinations (chest X-ray, electrocardiography, blood gas,

and routine blood tests). In the case of confirmed DVT, patients were considered as eligible for the "home treatment programme"; exclusion criteria were symptoms of pulmonary embolism (PE), risk of major bleeding, pregnancy, low compliance or any other condition for which the ED physician suggested prolonged hospitalisation. In case of eligibility for home treatment, patients were educated in the self-injection of LMWH; a full dose of LMWH (enoxaparin 100 UI/Kg/bid) plus oral anticoagulant (warfarin 7.5 mg/once daily for the first 2 days and then according to the INR value) was initiated. An informative letter for the General Practitioner was given to the patients; they were asked to return immediately to the ED if they developed signs and symptoms of venous thromboembolism or bleeding. All patients gave their consent. **RESULTS.** The overall prevalence of DVT was 18.6% (19/102); 7 patients (36.8%) were enrolled for the "home treatment programme". The mean time of hospitalisation was 4.6 h; the mean time to reach a stable INR was 6.7 days (range 4 to 9 days). After 3 months of follow-up, no cases of recurrent DVT or PE occurred; one patient had an episode of epistaxis on the 5th day of therapy (LMWH plus warfarin, INR 2.2). One patient had an episode of vertigo, headache and fever on the 45th day of warfarin (INR 2.3); the cerebral CT scan excluded intracranial haemorrhage. **Conclusions.** LMWHs may permit the home treatment of acute confirmed DVT; this choice should be made in selected patients after a complete baseline medical screening. In this respect, the initial management of home therapy might be problematic at clinics. Our approach, applied to the ED, permitted almost one-third of patients to be safely managed at home. Further examinations must confirm these initial results.

CO-192

RECOMBINANT SOLUBLE THROMBOMODULIN PREVENTS THROMBIN-INDUCED THROMBOEMBOLISM IN MICE INDEPENDENTLY OF DIRECT THROMBIN INHIBITION

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Thrombomodulin (TM) is a membrane glycoprotein expressed by endothelial cells. It binds thrombin thereby reducing its procoagulant properties and greatly enhancing its capacity to activate protein C (PC) but also TAFI (thrombin activatable fibrinolysis inhibitor). *In vivo*, in conditions of increased thrombin generation, TM may thus exert both anti- and pro-thrombotic activities. We have previously shown that thrombin-induced mortality in mice is dependent on a positive feed-back leading to the generation of additional (endogenous) thrombin which, in turn, makes fibrin resistant to lysis. Using this model, we tested the effect of solulin (recombinant soluble TM) on mortality and evaluated the relative importance of

thrombin inhibition, PC- and TAFI-activation. Solulin, given i.v. 2 min before a lethal dose of human thrombin (1250 U/kg i.v.), reduced mortality in a dose-dependent way, with 80% protection at the dose of 10 mg/mouse. The marked consumption of circulating fibrinogen produced by thrombin injection (from 318±17 to 18.1±2 mg/dL) was not attenuated by solulin (22±3 mg/dL), in agreement with the *in vitro* observation that thrombin was poorly inhibited by this cofactor ($IC_{50} = 50 \mu\text{g/mL}$). Solulin (up to 20 mg/mouse) failed to prevent death in warfarin-treated mice (PT ratio >10) given 2500 U/Kg of thrombin (minimum dose producing >80% mortality). These findings indicate that solulin does not act by inhibiting the injected thrombin but, probably, comes into action in a later stage by reducing the generation of additional thrombin. This is supported by the observation that mortality can be prevented also when solulin is given 1 or 2 min after thrombin. Plasma obtained 2 min after thrombin injection contained an APC-like anticoagulant activity (prolongation of APTT but not of TT), that was more pronounced in the solulin group. On the contrary, measurement of carboxypeptidase activity (by means of furoylacroleyl-alanyl-arginine substrate) showed that extensive TAFI activation (50 to 70% of total) occurred in both control and solulin-treated mice. Our data suggest that, in thrombin-induced thromboembolism in mice, solulin enhances PC but not TAFI activation and thus protects from mortality by preventing additional thrombin formation.

CO-193

RISK FACTORS FOR EARLY RECURRENCE OF PULMONARY EMBOLISM: RESULTS FROM THE ICOPER REGISTRY

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Background. Early recurrence of pulmonary embolism (PE) is associated with an increased short-term mortality. No data are currently available concerning the risk factors for early recurrence of PE. **Aim of the study.** To identify the risk factors associated with early recurrence of PE in patients included in the ICOPER registry. **Methods.** Frequencies distribution were calculated on clinical and physical features. c2 test were performed (p value <0.05) to evaluate the correlation among patients' features and PE recurrence. **Results.** An overall 7.9% incidence (190 out of 2403) of PE recurrence was observed during the three-month follow-up. A reduced echo LV ejection fraction (p=0.046), chronic heart failure (p=0.024), COPD (p=0.009) and a low platelet count (p=0.038) were associated with an increased likelihood of PE recurrence. The rate of recurrence was higher in patients with echo RV dysfunction (53.5% versus 38.9%; p=0.0025). The incidence of recurrent PE was 14.1% and 6.5% in patients receiving thrombolysis

and heparin, respectively (p<0.001). Concomitant DVT was present in 54.1% of thrombolysis patients and in 50.5% of heparin patients. No correlation was found between concomitant DVT and recurrence of PE (p=N.S.). **Conclusions.** Chronic heart failure, reduced echo LV ejection fraction, echo RV dysfunction, COPD and low platelet count are significantly associated with increased likelihood of recurrence of PE. Whether the higher risk of recurrence of PE observed in patients receiving thrombolysis is related to the greater severity of the disease in thrombolysis patients or whether it is due to the treatment itself remains to be established.

	RECURRENCE		p
	Yes (%)	No (%)	
EF<0.40	10.0	5.9	0.046
CHF	15.3	10.1	0.024
COPD	18.4	11.9	0.009
RV Dysfunction	53.5	38.9	0.002
Plt<100.000	7.4	4.2	0.038
Concomitant DVT	56.1	50.2	0.120
Previous PE	11.9	8.8	0.166
Previous DVT	21.9	20.7	0.721
VenaCaval Filter	1.1	0.8	0.756
Cancer	24.1	22.4	0.603
Chemotherapy	13.3	23.8	0.110

	RECURRENCE		p
	Yes (%)	No (%)	
CVC	7.9	7.4	0.818
Surgery	29.5	28.8	0.855
Immobilization	43.1	39.2	0.299
Creatinine>2.0	5.3	5.1	0.901
Trauma	14.7	10.9	0.104
Oral contraceptives	8.4	6.7	0.498
Estrogens	3.8	5.9	0.104
Hypercoagulability	5.9	5.2	0.690
Idiopathic patients	22.1	24.8	0.403
Age (≥50; <50)	27.0	23.38	0.215
Age (≥70; <70)	65.8	63.8	0.590

CO-194

RISK FACTORS FOR VENOUS THROMBOEMBOLISM IN PATIENTS UNDERGOING ELECTIVE NEUROSURGERY

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Background. Enoxaparin associated with compression stockings is effective and safe for the prevention of venous thromboembolism in patients undergoing elective neurosurgery, but it is associated with a residual DVT incidence of 17%. More aggressive prophylaxis

lactic regimens are limited by the fear of intracranial bleeding and should be first evaluated in patients at very high risk for venous thromboembolism. *Aim of the study.* To identify a subgroup at high risk for venous thromboembolism from the general population of neurosurgery patients, by finding out the risk factors for prophylaxis failure. *Study methodology.* Data base was derived from 260 elective neurosurgery patients. Over 20 potential risk factors were analyzed. Multiple logistic regression analysis was performed using maximum-likelihood estimation and stepwise forward selection of possible predictors. Treatment (placebo or enoxaparin) was forced as the first variable into the model, once verified that the P-value for the interaction of treatment with every other variable was >0.05 . The other factors were kept in the model if their P-value was <0.05 . *Results.* An increased risk for venous thromboembolism was associated with increased age (OR 2.5, CI95% 1.4-4.3, $p<0.01$), over-weight (OR 1.6, CI95% 1.1-2.5, $p<0.05$), increased pre-surgery hospital stay (OR 1.7, CI95% 1.1-2.6, $p<0.05$), increased degree of pre-surgery immobilization (OR 3.3, CI95% 1.9-5.6, $p<0.001$) increased surgery duration (OR 2.3, CI95% 1.4-3.7, $p<0.001$), and decreased platelet count on the first day after surgery (OR 0.35, CI95% 0.16-0.75, $p<0.01$). Treatment with enoxaparin resulted in a reduced risk (OR 0.39, CI95% 0.20-0.79, $p<0.01$). Retrospectively applied to the data base, assuming a critical 25% (65/260) probability of venous thromboembolism, the model would correctly predict 50/65 cases with thromboembolism (sensitivity 77%) and 145/195 cases without thromboembolism (specificity 74%). *Conclusions.* The analysis of risk factors allows the identification of a neurosurgery population at high risk for venous thromboembolism that is potentially candidate for clinical studies with more aggressive anticoagulant prophylactic regimens.

CO-195 CLINICAL OUTCOME OF PATIENTS WITH PULMONARY EMBOLISM, NORMAL BLOOD PRESSURE AND ECHOCARDIOGRAPHIC RIGHT VENTRICULAR DYSFUNCTION

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Background. The role of echocardiographic right ventricular (RV) dysfunction to predict the clinical outcome of patients with pulmonary embolism (PE) and normal blood pressure is still undefined. *Objective.* To assess the prevalence and short-term prognosis of normotensive patients with RV dysfunction among a cohort of PE patients with a broad spectrum of clinical presentation. *Design.* 209 consecutive patients (age 65 ± 15 years; male 40%) with PE confirmed by lung scan, spiral CT scan, and/or pulmonary angiography, were included in a prospective

clinical outcome cohort study. Echocardiographic RV dysfunction was defined as: RV dilatation in absence of RV hypertrophy (end-diastolic diameter >30 mm or R/L ventricular end-diastolic diameter ratio >1); paradox septum systolic motion; pulmonary hypertension (doppler pulmonary acceleration time <90 ms or RV/atrial gradient >30 mmHg). *Results.* 4 groups of patients were identified: 1) 28 patients presenting with shock or cardiac arrest (13%); 2) 19 hypotensive patients (systolic blood pressure <100 mmHg) without shock (91%); 3) 65 normotensive patients with RV dysfunction (31%); and 4) 97 normotensive patients without RV dysfunction (47%). Among the normotensive patients with RV dysfunction, 6 (10%) developed PE-related shock within in-hospital stay: 3 of them died, while 3 were successfully treated with thrombolytic agents. The PE-related mortality of these patients was similar to that of the hypotensive patients without shock (5%). None of the 97 normotensive patients without RV dysfunction developed shock or died due to PE. *Conclusions.* A significant proportion (31%) of normotensive patients with acute PE has echocardiographic RV dysfunction; these patients have a short-term rate of PE-related shock and mortality of 10% and 5%, respectively and may require more aggressive therapeutic strategies, including thrombolytic treatment.

CO-196 PREVALENCE AND CLINICAL DETERMINANTS OF NON-FATAL VENOUS THROMBOEMBOLISM IN THE ACTIVE POPULATION

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Background. The prevalence and clinical determinants of venous thromboembolism (VTE) in the active population are still undefined, and cost-effective strategies for the identification of subjects at-risk are still lacking. *Aim of the study.* To ascertain the prevalence and risk factors for VTE in active population. *Methods.* We analyzed the data from 15,055 subjects enrolled in the Vicenza Thrombophilia and Atherosclerosis (VITA) Project, a cross-sectional epidemiological investigation on venous thrombophilia. VTE cases were identified with a validated questionnaire with 71% sensitivity and 99% specificity. Data regarding height, weight, smoking, use of oestrogen-progestins, previous superficial thrombophlebitis (SVT), family history of VTE at age of first thrombosis were investigated by direct interview. Logistic regression with correction for non-differential classification bias was used to identify risk factors for VTE; multinomial logistic regression was used to account for differences in VTE inciting factors. *Results.* We identified 116 cases of non-fatal venous thromboembolism among 15,055 subjects: 93 deep vein thromboses (prevalence: 61.7/10,000, 95% CI 49.8-75.6), 3 upper deep vein thromboses (prevalence: 1.9/10,000, 95% CI 0.4-5.8) and 20 pulmonary embolisms (prevalence: 13.2/10,000, 95% CI 8.1-

20.5). Risk factors statistically associated with subsequent VTE were: history of superficial vein thrombosis (OR=6.9), use of oestroprogestins (OR=4.1), family history of VTE (OR=2.6), smoking (OR=1.7) and body-mass index above the third tertile (OR vs. first tertile 5.6). All reported values are age and sex adjusted. When VTE cases were stratified into three categories (pregnancy related, trauma/surgery related, idiopathic), the degree of association of some risk factors was different (OR for oestroprogestin use: 1.08 for trauma/surgery vs. 13.5 idiopathic; OR for positive family history: 0.5 surgery/trauma, 4.1 idiopathic, 8.4 pregnancy; OR for smoking: 1.9 surgery/trauma, 0.9 pregnancy). *Conclusion.* This study shows that non-fatal VTE affects about 0.7% of the subjects below 65 years belonging to an active population. Clinical assessment of risk factors for VTE remains the mainstay for the identification of patients at risk.

ORAL COMMUNICATIONS

Pregnancy, complications and hypercoagulability

CO-197

GENETIC MUTATIONS IN COAGULATION FACTORS IN YOUNG WOMEN WITH UNEXPLAINED LATE FOETAL DEATH

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Background. A factor V gene mutation (factor V Leiden) is an independent risk factor for venous thrombosis and has been associated with miscarriage, possibly due to thrombosis of the uteroplacental circulation. Recently, a mutation in the gene encoding the coagulation factor prothrombin has been discovered as a common cause for venous thrombosis. To establish whether or not these coagulation factor mutations are also associated with an increased risk of late fetal death, a case-control study was carried out in women with a first unexplained late fetal death. The role of a mutation in the gene encoding the enzyme methylenetetrahydrofolate reductase, involved in homocysteine metabolism, was also evaluated. *Methods.* We studied 67 women aged 35 years or less who had a first episode of unexplained late fetal death and 232 women who had one or more normal pregnancy and no late fetal deaths. All women were tested for the presence of the factor V, prothrombin and methylenetetrahydrofolate reductase gene mutations. Women with other known thrombophilic conditions were excluded from the study. Odds ratios and 95% confidence intervals (CI) were used as a measure of the association between late fetal death and each gene mutation. Odds ratios were adjusted

for the effect of parity using multiple logistic regression model. *Results.* Overall, 11 (16.4%) of the 67 women with late fetal death and 13 (5.6%) of the 232 controls had either factor V or prothrombin gene mutation, with an odds ratio of 3.3 (95% CI, 1.4 to 7.8). Among cases, 5 (7.5%) had the factor V and 6 (9.0%) had the prothrombin gene mutation, compared to 6 (2.6%) and 7 (3.0%) in controls. The adjusted odds ratios for late fetal death in carriers of the factor V and prothrombin gene mutations were 3.2 (95% CI, 1.0 to 10.9) and 3.3 (95% CI, 1.1 to 10.3), respectively. Examination of the placenta obtained in 62 cases showed features of thrombosis in 77.8% of those with and in 75.5% without factor V or prothrombin gene mutations. Homozygosity for the methylenetetrahydrofolate reductase gene mutation was found in 9 cases (13.4%) and 43 controls (19.8%), with an odds ratio of 0.8 (95% CI, 0.5 to 1.2). *Conclusions.* Both the factor V and prothrombin gene mutations expose women to an approximately 3-fold increased risk of late fetal death. Although this finding may provide a rationale for anticoagulant prophylaxis, several issues, including risk of recurrence and that of bleeding, need to be addressed by specifically designed studies before implementation of this treatment.

CO-198

EFFECT OF HEPARIN FOR PREVENTING FOETAL LOSS DUE TO PLACENTAL INSUFFICIENCY IN WOMEN WITH INHERITED THROMBOPHILIA

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The inherited thrombophilias are a group of genetic disorders of blood coagulation resulting in an increased risk of thrombosis; thrombosis of placental vasculature and/or increased fibrin deposition in intervillous space may result in a number of gestational pathologies, including intrauterine growth retardation, pre-eclampsia, first and second trimester miscarriages, intrauterine fetal death and placental abruption. We studied 340 patients (776 pregnancies), referred to our outpatient clinic for previous episodes of placental insufficiency; 65 (19.11%, 103 pregnancies) were carriers of factor V Leiden mutation, 44 (12.94%, 73 pregnancies) carriers of factor II G20210A mutation; 15 (4.41%, 18 pregnancies) carriers of inherited coagulation inhibitors defect; 35 (10.29%) carriers of 677T MTHFR mutation; 55 (16.17%) with antiphospholipid syndrome (APLS); 90 (26.47%) with other autoimmune diseases; 14 (4.11%) with myeloproliferative syndrome and 22 (6.47%) with undefined thrombophilia. From 1995, pregnant women with inherited thrombophilia received standard antithrombotic therapy, normally used for pregnant women with APLS: low dose aspirin 50mg/die from the 6th to the 26th week of pregnancy, s.c. heparin 5000Ux3/die or s.c. LMWH 4000U/die from the 6th week to delivery. The treat-

ment was applied to 73 pregnancies in women with inherited thrombophilia: 32 pregnancies in carriers of V Leiden mutation; 28 pregnancies in carriers of factor II G20210A mutation and 13 pregnancies in carriers of inherited coagulation inhibitors defect. Therapy was adjusted according to coagulation parameter variations. *Results.* We observed that the percentage of fetal loss in patients with heparin treatment, before the 20th week of pregnancy, decreased from 26% to 9% in carriers of factor V Leiden mutation, from 33% to 7% in carriers of factor II G20210A mutation and from 55% to 8% in patients with inherited coagulation inhibitors. After the 20th week of pregnancy the percentage of fetal loss decreased from 6% to 0% in carriers of factor V Leiden mutation; from 14% to 0% in carriers of factor II G20210A mutation and from 11% to 0% in patients with inherited coagulation inhibitors defect.

CO-199

PREVALENCE OF THROMBOPHILIC GENOTYPES IN WOMEN WITH A HISTORY OF PRE-ECLAMPSIA OR HELLP SYNDROME

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Background. Pre-eclampsia and HELLP syndrome are multifactorial diseases in which the presence of a hypercoagulable state has been suggested. Whether these pregnancy complications are related to an increased frequency of thrombophilic mutations is still uncertain. The aim of our study was to estimate the prevalence of the most common gene polymorphisms associated with inherited thrombophilia. *Patients and methods.* We studied 65 women with a history of severe preeclampsia (n=59) or HELLP syndrome (n=6). The median age at the obstetric event was 31 years (median age 30, range 20 to 40). The control group consisted of 132 healthy women with no history of complicated pregnancy (mean age 55, median 54, range 40 to 93). All individuals were genotyped for the presence of factor V Leiden and the G20210A mutation in the prothrombin gene. *Results.* We found 6 heterozygous carriers of factor V Leiden (9.2%) and 3 heterozygous carriers of the G20210A prothrombin mutation (4.6%) among the cases; an additional case carried both mutations (1.5%). No patient with a history of HELLP syndrome had a mutant genotype. Two controls (1.5%) carried factor V Leiden and 4 (3%) carried the prothrombin mutation. No homozygous individual was found. The relative risk for preeclampsia/HELLP syndrome associated with factor V Leiden was significantly increased in comparison with controls (adjusted odds ratio 6.9, 95% CI 1.3 to 35.1); no increase in risk was found associated with the G20210A mutation in the prothrombin gene (adjusted odds ratio 1.7, 95% CI 0.4 to 7.9). *Conclusions.* Factor V Leiden is associated with an increased risk of pre-eclampsia, whereas no

increase in risk was found associated with another common thrombophilic genotype (G20210A prothrombin). The reason for this discrepancy could be due to an enhancement of the gene-induced resistance to activated protein C by the physiological pregnancy-induced resistance to activated protein C.

CO-200

MUTATIONS IN THE THROMBOMODULIN GENE IN WOMEN WITH LATE FOETAL LOSS

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Background. A successful outcome of pregnancy requires an efficient uteroplacental vascular system that may be compromised by disorders of haemostasis associated with a prothrombotic state. Thrombomodulin (TM), an integral membrane protein crucial for normal activation of the protein C anticoagulant pathway, has also been localised to the placental syncytiotrophoblast. *Aim of the study.* We performed a study in 106 Caucasian women with late foetal loss (>20 weeks), to elucidate whether TM gene mutations could be a risk for this complication of pregnancy. Cases were excluded from the study if they had independent risk factors for foetal death. Controls were women who gave birth to one or more healthy babies, without a history of late fetal death or obstetrical complications. *Methods.* The entire TM gene, including the promoter region, was amplified in 20 overlapping fragments by PCR and screened by single strand conformation polymorphism (SSCP). When aberrant band patterns were identified, the respective TM fragment was sequenced. Finally, all the detected point mutations were confirmed with restriction enzyme digestion. *Results.* Three different mutations in the heterozygote form were identified in 4 of 106 patients (3.7%):

- a silent point mutation at nucleotide position 282 (C 282 to G) that did not predict any amino acid change at Pro 136 in the lectin-like domain;
- a G 1208 to A substitution, predicting an Arg 385 to Lys replacement in the IV EGF-like domain;
- 2 C 1502 to T substitutions, predicting a Pro 483 to Leu change.

Moreover, the previously identified dimorphism C 1418 to T leading to Ala 455 Val substitution in the sixth EGF-like domain was investigated: an allelic frequency of 13% for Val 455 and 87% for Ala 455 was found. *Controls.* No G 1208 to A mutations were identified in the control group (0/234). The C 1502 to T mutation was identified in 2/234 subjects (0.8%). The analysis of the C 282 to T mutation in the controls and women with uncomplicated pregnancies's under investigation. No differences in the allelic frequencies were detected in the C 1418 to T dimorphism compared to the patients. *Conclusions.* Muta-

tions in the TM gene are more frequent in women with a history of late foetal loss than in women with uncomplicated pregnancies.

CO-201

THE EFFICACY OF BOTH LOW-DOSE ASPIRIN AND CALCIUM HEPARIN TREATMENTS IN ANTIPHOSPHOLIPID ANTIBODIES-ASSOCIATED RECURRENT PREGNANCY LOSS

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Unexplained recurrent pregnancy losses are strongly correlated with increased levels of anticardiolipin antibodies (ACA) and/or the lupus anticoagulant (LA) activity. Several regimens have been proposed for the treatment of antiphospholipid syndrome (APS) including aspirin alone, prednisone and aspirin, heparin and aspirin and more recently intravenous immunoglobulin. The aims of the present study were, therefore, 1) to evaluate the effect of both the low-dose aspirin and calcium heparin treatments, 2) to compare the obtained results. We performed investigations on 261 healthy women, mean age 30 years, with a documented history of two or more consecutive negative foetal outcomes and with no evidence of any underlying connected disease. The control group consisted of 50 healthy women, mean age 29 years, recruited among LA and/or ACA- subjects. The search for ACA was performed using a MELISA test (Byk-Gulden). The diagnosis of LA was based on criteria according to the ISTH-recommendations (95). In order to exclude other possible causes of recurrent foetal loss a series of laboratory studies were made: history and physical examination, hysterosalpingography, hormonal status, infections and immunologic tests, karyotypes. The results showed that of the 261 pts tested 154 (59.1%) were APL-. As to the remaining 107 pts (40.9%) who were APL+ 73 were ACA+ LA-, 10 were LA+ ACA-, 24 were ACA+ and LA+. All the other parameters were normal in all pts. All LA and/or ACA+ subjects were treated with prednisone (0.5-1 mg/Kg body weight for 20 days) and were submitted to routine coagulation tests with an interval of at least 4 weeks. Following treatment, we found a downward trend for IgG and IgM ACA levels and, subsequently, 45 women became pregnant. Four women withdrew from the study (3 delivered healthy infants at term and the fourth aborted between the 9th and the 11th week of gestation). In the pts treated with heparin, platelets and PTT were checked. Each woman was followed-up with repeated coagulation examinations and β -HCG was also monitored fortnightly. Given the high risk of foetal loss in these pts, foetal vitality was documented at each obstetric appointment by a complete pregnantly evaluation. *Results.* There were 12 live births in the calcium heparin treatment group (1 is ongoing, 7 resulted in the first trimester miscarriages); 19 live infants were born to the women treated with low-dose aspirin (2 pregnancies are ongoing). All

neonates were delivered by Caesarean section. None of them suffered from intra-and/or post-partum bleeding complications. In conclusion, our data confirm the significant effectiveness of both treatments and suggest that for APL-associated recurrent pregnancy loss, low-dose aspirin is better.

CO-202

ACE DD GENOTYPE INCREASES FIRST TRIMESTER FOETAL LOSS SUSCEPTIBILITY

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The causes of obstetrical complications are not completely elucidated, but recently they have been associated with abnormal placental vasculature and disturbance of haemostasis. Inadequate maternal-foetal circulation and complications of pregnancy have been found to be related with thrombophilic polymorphisms that explain about 30% of obstetrical complications. The aim of this study was to evaluate angiotensin converting enzyme (ACE) and angiotensin type 1 receptor (AT1R) polymorphisms as possible risk factors for the occurrence of foetal loss. Fifty-nine women with a history of three or more first trimester (7-12 wks of gestation) foetal losses and 70 healthy women with a history of normal pregnancies, after exclusion of APL syndrome, were enrolled in this study. Thrombophilic risk factors were analysed: antithrombin (AT), protein C (PC), protein S (PS), plasminogen activator inhibitor-1 (PAI-1) by chromogenic assay and homocysteine by HPLC. ACE I/D and AT1R A1166C polymorphisms, prothrombin (FII) G20210A and factor V (FV) Leiden mutations were analysed by molecular biology techniques. A significant association between the ACE DD and AT1R CC genotype and foetal loss was observed [OR DD/ID+II = 2.26 (CI95% 1.09-4.67) $p=0.03$ and OR CC/AC+AA = 5.62 (CI 95% 1.75-18.08) $p=0.002$ respectively]. The effect of the ACE DD genotype on the risk of foetal loss was higher in AT1R C allele carriers ($p=0.04$). The prevalence of FII and FV mutations were 1.7% and 10% in patients, and 1.4% and 3% in controls. The prevalence of hyperhomocysteinaemia was significantly ($p=0.016$) higher in women with foetal loss than in controls, and an association between hyperhomocysteinaemia and foetal loss was detected [OR= 4.21 (CI 95% 1.28-13.88) $p=0.02$]. All patients showed normal values of AT, PC, PS, PAI-1. The presence of ACE DD or AT1R CC genotype in the absence of other risk factors was found in 33 out of 59 patients (56%). In conclusion, our results identify a new possible predictive marker for foetal loss in RAS gene polymorphisms. Large-scale studies are warranted to attribute clinical relevance to these polymorphisms as risk factors for complicated pregnancies.

ORAL COMMUNICATIONS**Biochemistry and genetics of coagulation factors****CO-203****OXIDATION OF HUMAN FIBRINOGEN BY THE MYELOPEROXIDASE-H₂O₂-CHLORIDE SYSTEM: BIOCHEMICAL AND FUNCTIONAL CHANGES**

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The myeloperoxidase- H₂O₂-Cl⁻ system (MPOS) is exploited by polymorphonuclear cells (PMN) and monocytic white blood cells to generate reactive oxygen species, which have the capacity to oxidise or modify lipids, proteins and nucleic acids. This study investigated the biochemical and functional effects of oxidation of human fibrinogen by the MPOS. This system caused in the fibrinogen molecule loss of tryptophan residues and formation of chloramine and dityrosine groups. Similar changes could be directly induced by fibrinogen incubation with reagent HOCl, but not with H₂O₂ alone. These biochemical changes of the fibrinogen molecule were associated with functional abnormalities, such as reduction of its clotting activity. Static laser light-scattering studies showed that the initial phase of protofibril formation was strongly inhibited in oxidised fibrinogen (ox-fibr) compared to the untreated molecule, while the process of thicker fibre formation followed normal kinetics. The thrombin-catalyzed release of fibrinopeptide A from ox-fibr is characterized by a k_{cat}/K_m value similar to that of normal fibrinogen ($2 \pm 0.2 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}$). In addition, fibrin obtained from ox-fibr showed an impaired capacity ($\approx 50\%$ inhibition) to enhance the tPA-mediated hydrolysis of plasminogen activation. In contrast, MPOS treatment did not cause a change in the fibrinogen capacity to support platelet aggregation, since only a 10% inhibition was observed in this case. In SDS-PAGE studies the α , β and γ chains of ox-fibr showed a different pattern compared to that of the untreated sample, suggesting the occurrence of an homolytic process, likely mediated by radical-catalyzed phenomena. In conclusion, this study showed that fibrinogen is most sensitive to oxidative modifications by the MPOS leading to severe impairment of its clotting activity and profibrinolytic properties whereas the platelet aggregating activity is conserved. Thus, oxidative modification of normal fibrinogen by the MPOS causes dysfibrinogenemia, that may occur in inflammatory clinical settings, where the myeloperoxidase system is activated.

CO-204**TWO NEW MISSENSE MUTATIONS IN THE FACTOR XIII_A GENE OF AN ITALIAN FACTOR XIII DEFICIENT FAMILY**

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Factor XIII (FXIII) is a transglutaminase essential for normal haemostasis. It functions during the last stages of the coagulation pathway when it cross-links fibrin to produce clots of high mechanical strength. We carried out molecular analysis of the FXIII_A gene of an Italian FXIII deficient patient, and his family. This patient lacks plasma FXIII_A subunit and plasma FXIII activity. A heterozygous C>T mutation was identified at codon 310 in exon 7, and at codon 408 in exon 10. This CGG>TGG DNA sequence change at both these loci corresponds to an Arg>Trp missense mutation. The codon 310 mutation was inherited through the maternal line and was also carried by the patient's son and two of his siblings. The codon 408 mutation segregated via the paternal line. The patient was also homozygous for nucleotide A at position -246 in the promoter region upstream of exon 1, and heterozygous at codon 650 (GTT/ATT corresponding to Val/Ile). These latter are, however, known to be normal polymorphisms which do not cause FXIII deficiency. The Arg310Trp and the Arg408Trp mutations were absent from 110 individuals who are normal with respect to FXIII. Computer modelling was employed to assess the effects of these missense mutations on FXIII_A subunit structure. The side-chain of Trp is highly hydrophobic and this residue is not ideal at a surface location. Residue 310 is located at the surface of FXIII_A, while residue 408 is on the surface of a small pocket within the FXIII molecule. It is probable that both mutations result in localised rearrangement of the surrounding residues such that the mutant molecule is not folded properly and is thus unstable. In view of these predicted effects on FXIII structure, the absence of the mutations from the normal population, and the segregation of the mutations with disease, it is likely that Arg310Trp and Arg408Trp mutations are responsible for FXIII deficiency in this family.

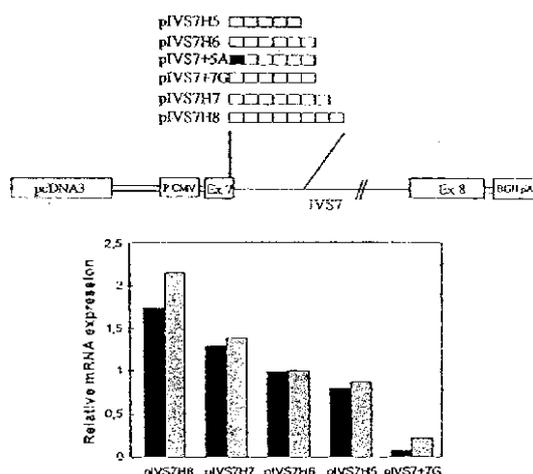
CO-205**MODULATION OF FACTOR VII LEVELS BY INTRON 7 POLYMORPHISMS: POPULATION AND *IN VITRO* STUDIES**

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Although previous population studies have established that FVII gene polymorphisms (5'F7, R353Q) contribute about one third of the FVII level variation

in plasma, FVII genotyping in patients with cardiovascular disease has produced conflicting results. To provide elements useful to address these discrepancies, we investigated, by population and expression studies, the role of intron 7 (IVS7) polymorphisms, including repeat and sequence variations, in controlling FVIIa and FVIIag levels. Genotype-phenotype studies performed in 438 Italian subjects suggested a positive relation between IVS7 repeat number and plasma FVII levels. The lowest values were associated with the IVS7+7G allele. Screening of 52 patients with mild FVII deficiency showed an eight-fold increase in frequency (8%) of this allele and among heterozygotes for identical mutations lower FVII levels were observed in the IVS7+7G carriers. This frequent genetic component participates in the phenotypic heterogeneity of FVII deficiency. The evaluation of the individual contribution of polymorphisms, hampered by genetic factors, was assisted by the expression of each IVS7 variant, as a minigene, in eukaryotic cells. The novel quantitative analysis revealed that alleles with higher numbers of repeats were associated with higher mRNA expression levels and that the IVS7+7G allele, previously defined by us as a functionally silent polymorphism, was responsible for the lowest relative mRNA expression



Taken together these findings indicate that the IVS7 polymorphisms contribute to the plasma variance of FVII levels via differential efficiency of mRNA splicing. These studies provide further elements towards the understanding of the control of FVII levels, which could be of importance for ensuring haemostatic balance under pathological conditions.

CO-206

THROMBIN-DEPENDENT ENHANCEMENT OF CLOT LYSIS *IN VITRO*

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In preliminary experiments we observed that serum enhanced t-PA-induced clot lysis *in vitro* by several

fold. The present study was undertaken to characterise this phenomenon. Serum was obtained from recalcified blood after 1 h incubation at 37°C. ¹²⁵I-fibrin blood clots were prepared from the same donor and immersed in serum or plasma. After addition of t-PA, the extent of lysis was determined at intervals from the radioactivity released in solution. Greater lysis of clots submerged in serum was observed at all tested concentrations of t-PA (100-1000 ng/mL). With 250 ng/mL of activator, clot lysis at 2 h amounted to 62% in serum and to 41% in plasma. This difference was not abolished when PTI (50 mg/mL), a specific inhibitor of TAFIa (thrombin activatable fibrinolysis inhibitor), was added to the system. The stimulating effect of serum was also observed with other fibrin-specific PAs (DSPA, t-PA/u-PA chimera) and with u-PA. Blood cells do not appear to be involved in this phenomenon since similar results were obtained with plasma-derived serum. Moreover, enhanced lysis in the presence of serum was observed with clots made from purified fibrinogen. If during serum preparation clotting activation was prevented by heparin or hirudin, the resulting sample failed to enhance clot lysis. The same was true if "serum" was prepared from plasma made deficient in vitamin K-dependent proteins by BaSO₄ adsorption. In the latter, however, clot lysis enhancing activity was restored if thrombin (20U/mL), but not FXa or batroxobin (thrombin-like enzyme), was added prior to serum formation. The following data suggest that thrombin exerts its profibrinolytic activity indirectly, likely via a second messenger generated in serum. 1) Serum contains very little thrombin activity; 2) addition of anticoagulant amounts of hirudin to serum did not prevent enhancement of clot lysis; 3) thrombin (up to 40 U/mL) failed to enhance the lysis of clots made of purified components. Zymographic analysis revealed that neutralisation of t-PA was slower in serum than in plasma. This, however, could not explain the effect of serum on clot lysis at short intervals (<1h), when most t-PA was active both in plasma and serum. Thrombin plays a pivotal role in coagulation by both stimulating and inhibiting (via protein C) fibrin formation. Our findings suggest that thrombin may play a similar role in fibrinolysis thanks to its capacity to both up-regulate and down-regulate (via TAFI and FXIII) the process of fibrin removal.

CO-207

FIVE NEW POINT MUTATIONS IN THE FIBRINOGEN ALPHA-CHAIN GENE CAUSING AFIBRINOGENEMIA

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Afibrinogenaemia is a rare autosomal recessive disease characterised by the complete absence or

extremely reduced levels of plasma fibrinogen and by bleeding manifestations of various severity. The genetic bases of this disease have been elucidated for the first time as a homozygous 11 Kb deletion of the alpha-chain gene. More recently, we reported the identification of two different homozygous missense mutations in the beta-chain gene, responsible for an impairment of fibrinogen secretion resulting in afibrinogenemia. By sequencing the entire coding region as well as the splicing junctions and about 300 bp of the promoter of the three fibrinogen genes in seven unrelated afibrinogenemic patients, we identified five new point mutations in the fibrinogen alpha-chain gene. Three of them were in the homozygous state (Ser100term, Arg110term and Arg149term). The remaining two were present in the heterozygous state (Gly13term and 1215delT, the latter leading to a frameshift followed by a premature stop at codon 50) and we could not detect a second mutation in the fibrinogen cluster neither in the patients nor in the available relatives. All mutations were localised within the first four exons of the alpha-chain gene and gave rise to truncated alpha-chain polypeptides because of the presence of premature-termination codons (PTCs). Even though in the analysed patients plasma fibrinogen was unmeasurable by a functional test (Clauss), the immunologic levels were very low but comprised in a wide range, between 0.02 and 4.9 mg/dL. Since PTCs are known to affect the metabolism of the corresponding mRNAs frequently, the existence of a correlation between the degree of stability of each mutant mRNA and plasma fibrinogen levels is under investigation.

CO-208

SEVERE FACTOR V DEFICIENCY CAUSED BY TWO NOVEL MUTATIONS IN EXON 13 OF FACTOR V GENE

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Factor V deficiency is a rare autosomal recessive bleeding disorder with an incidence of one in one million. Affected individuals usually present moderate bleeding manifestations such as ecchymoses, epistaxis and menorrhagia, while heterozygotes usually remain asymptomatic. Up to now only one mutation responsible for severe factor V deficiency has been reported: a 4 bp deletion in exon 13 that introduces a frameshift followed by a premature stop codon at position 1303. We studied three unrelated patients showing factor V plasma levels lower than 1% of normal and with bleeding manifestations ranging from moderate to severe. In these patients factor V antigen levels were also unmeasurable (<0.2% of normal). Sequencing of the factor V gene of these probands revealed two new mutations, both occurring in exon 13. The first was a

2 bp deletion at position 2833-2834 of the cDNA introducing a frameshift and a novel premature stop at codon 900. The proband was homozygous for the mutation and the proband's mother, the only available parent, was heterozygous. The second mutation occurred in two probands with different clinical symptoms, coming from the same geographical area (Southern Italy). This mutation is a C to T transition at cDNA position 3571 producing a stop codon. Both probands resulted to be homozygous also for the Leiden mutation. Their parents were heterozygous for both the Leiden and the newly identified null mutation. In the two patients the nonsense mutation was associated with different haplotypes thus excluding the existence of a common ancestor. Since these two novel mutations in exon 13 of factor V gene introduce premature termination codons, it will be interesting to verify the stability of mutant mRNAs.

CO-209

A NEW MUTATION IN IVS-3 OF THE HUMAN GAMMA FIBRINOGEN GENE CAUSING AFIBRINOGENAEMIA DUE TO ABNORMAL SPLICING

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Congenital afibrinogenemia is a rare autosomal recessive disorder characterised by the complete absence of detectable fibrinogen. Since the first case reported in 1920, approximately 150 cases have been described. However, to date only two responsible defects in the fibrinogen genes have been identified, a 11-kb deletion of the fibrinogen alpha-chain gene and two missense mutations in the fibrinogen beta-chain (Leu 353Arg and Gly400Asp) leading to an impaired fibrinogen secretion. We have investigated a six-year old girl who had suffered from major bleeding after trauma when she was 1-year old. At that time a very prolonged aPTT and PT, and undetectable fibrinogen levels (Modified Clauss' Method) were observed. As to her family history, no bleeding episode was reported. Her parents were consanguineous (first cousins). A family investigation confirmed undetectable levels of both functional and antigenic fibrinogen. Both parents of the proband, as well as her sister, had low levels of functional (160, 120, and 111 mg/dL, respectively) and antigenic (70, 65, and 45 mg/dL, respectively) fibrinogen. Isolation of DNA and PCR analysis were carried out according to standard procedures. Amplification of all coding regions fibrinogen genes and intron/exon boundaries was achieved using sense and antisense oligonucleotides. A homozygous G-to-A transition at the fifth nucleotide of the third

intervening sequence (IVS-3) of the gamma fibrinogen gene was identified in the propositus' sample. The same mutation was found, in a heterozygous form in samples from both parents and the sister. The G-to-A transition is within the consensus sequence of the donor splice site, a region that is believed to be critical for accurate mRNA splicing. Using the "Splice site prediction by neural network" program to predict changes induced by mutations in mRNA splicing, we observed that the mutation causes the disappearance of a donor splice site. The effect of the IVS-3 A-to-G mutation on mRNA is similar to those documented in thalassaemia and in protein C and factor IX deficiencies. In conclusion, we suggest that, in addition to the mutations in the alpha- and beta-chain genes, a molecular variation in the fibrinogen gamma-chain gene locus cause congenital afibrinogenemia by impairing the IVS removal from mRNA precursors.

CO-210 IDENTIFICATION AND THREE-DIMENSIONAL STRUCTURAL ANALYSIS OF NINE NOVEL MUTATIONS IN PATIENTS WITH PROTHROMBIN DEFICIENCY

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Prothrombin deficiency is an autosomal recessive disorder associated with a moderately severe bleeding tendency. We have identified a further 9 novel mutations in 13 patients with prothrombin deficiency, 11 patients with hypoprothrombinaemia and 2 with dysprothrombinaemia. Eight of nine mutations are missense associated with single amino acid substitutions in the propeptide (Arg-1Gln, Arg-2Trp), the kringle-1 (Asp118Try) and kringle-2 (Arg220Cys) domains and the catalytic serine protease domain (Gly330Ser, Ser354Arg, Arg382His and Arg538Cys). The remaining mutation is an in-frame deletion of 3 bp that results in the omission of one amino acid (del Lys 301/302). In order to clarify the effect of the single residue mutations on the prothrombin molecule, we have examined the location of 7 of 9 novel and of 12 previously described missense mutations on experimentally determined crystal structures. The effect of the two dysprothrombinaemia mutations at Ser354Arg and Arg382His were explained in terms of their location at the active site crevice or at exosite 1 within the serine protease domain. Most dysprothrombinaemia mutations result in substitutions within the cleavage sites of FXa and the serine protease domain of prothrombin, these mutations are dispersed throughout the serine protease domain surrounding the catalytic triad, but are generally not located near the interface between the serine protease domain and the Gla and kringle domains or the A chain of α -thrombin. This is as expected if the consequence of these mutations is to affect catalytic function. In contrast, the hypoprothrombinaemia mutations identified here and previously are all associated with this interface, and are distributed primarily in regions close to Gla and kringle domains

and the A chain. They fall into three groups, namely those that affect either the cleavage of the propeptide from the Gla domain, the correct folding of the kringle-1 and kringle-2 domains, or the correct association of the A and B chains of α -thrombin. The first description here of mutations that involve the extensive structural association of the A and B chains with a large buried surface area of 1200 Å² indicates that this may constitute an important event in the folding and stability of prothrombin.

CO-211 THREE-DIMENSIONAL STRUCTURAL AND *IN VITRO* EXPRESSION ANALYSIS OF TWO MUTATIONS LOCALISED ON THE CATALYTIC CLEFT OF FVII GENE

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Factor VII is a vitamin K-dependent glycoprotein that plays a vital role in the initiation of coagulation and its deficiency is a rare autosomally inherited recessive disorder. Sixty-nine mutations in the human FVII gene have been reported previously, however only a few have been functionally characterised. We studied two Iranian families with severe FVII deficiency type II. The FVII antigen (VII:Ag) level was normal in both patients but was associated with a reduction of FVII coagulant activity (FVII:C) to less than 1%. We found a homozygous mutation in each patient causing the substitution of residues Ser363Ile and Trp364Cys respectively. Serine 363 and tryptophan 364 residues are conserved in several serine proteases, which suggests that they possess a structural role that has been conserved during evolution. In order to clarify the effect of the single residue substitution on the FVII molecule, we examined the location of these two amino acids on experimentally determined crystal structure (pdb.1dan). Ser 363 and Trp364 occur at the C-terminus of the β -strand N at the active site cleft, and form four mainchain hydrogen bonds to the substrate analogue D-Phe-L-Phe-Arg chloromethyl ketone that was used to stabilise that FVII active site during crystallisation. To confirm this data wild type FVII (FVII WT) and both mutant FVIIcDNAs (FVIIIM363 and M364) were expressed transiently in COS-1 cells and stably in CHO cells. The FVII:C was measured in conditioned media and FVII:Ag in cell lysates and conditioned media of cells transfected with each of the three constructs (WT, M363 and M364). The results of expression studies reflected the patient's data. A similar FVII:Ag level was present in cell lysates and conditioned media of all three constructs. However, while the secreted WT recombinant protein was biologically normal expressing 100% of coagulant activity, the mutant recombinant proteins produced from both constructs in both systems were not proteolytically active, expressing only less than 1% of coagulant activity. The detrimental effect of these two mutations is therefore attributable to the role of Ser 363 and Trp364 at the substrate-binding site of FVIIa.

POSTERS

Genetic risk factors for venous thromboembolism**PO-212****RISK OF SUBSEQUENT VENOUS THROMBOEMBOLIC COMPLICATIONS IN CARRIERS OF THE PROTHROMBIN OR THE FACTOR V GENE MUTATION WITH A FIRST EPISODE OF DEEP-VEIN THROMBOSIS**

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Carriers of a mutation in the prothrombin (clotting factor II) or factor V gene have a two to four-fold increased risk of venous thromboembolism as compared to subjects without the mutations. Whether both mutations also predispose to recurrent venous thromboembolism is unclear. Outpatients with a first episode of venography proven symptomatic deep-vein thrombosis and who had long-term follow-up were studied. Patients with malignant disease, other thrombophilic conditions, and those who needed vitamin K antagonist therapy for reasons other than venous thromboembolism were excluded. The outcome measure was the cumulative incidence of confirmed venous thromboembolic complications. Two hundred and fifty-one patients were enrolled in the study. Five years of follow-up was completed in 232 patients (92%), and 10 years in 100 patients (40%), for a mean duration of follow-up of 8.3 years. The prothrombin gene mutation was demonstrated in 27 patients (prevalence, 10.8%; 95% CI, 6.9 to 14.6) and the factor V gene mutation in 41 patients (prevalence, 16.3%; 95% CI, 11.8 to 20.9). The cumulative incidence of venous thromboembolic complications after 10 years was 61.3% (95% CI, 35.7 to 87.9) in patients with the prothrombin gene mutation, and 55.2% (95% CI, 36.4 to 74.0) in patients with the factor V gene mutation, as compared to 23.1% (95% CI, 16.2 to 30.1) in patients without these mutations. The hazard ratios for recurrent venous thromboembolism were 2.4 (95% CI, 1.3 to 4.7; $p=0.004$) and 2.4 (95% CI, 1.4 to 4.1; $p=0.001$) for the presence of prothrombin or factor V gene mutation, respectively. We conclude that prothrombin and factor V gene mutations occur frequently in patients with venous thrombosis and are associated with an increased risk of recurrent venous thromboembolic complications. It remains to be assessed whether prolonged anticoagulant therapy is indicated in carriers of these mutations.

PO-213**THROMBOPHILIC FACTORS IN INFLAMMATORY BOWEL DISEASES**

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Background. Patients with inflammatory bowel diseases (IBD) are at increased risk of thromboembolic events (TEE). TEE in IBD are important because they occur in young people, often in unusual sites. The mechanism of enhanced procoagulant activity is not clear: approximately half of TEE pts have no identifiable risk factors. Hyper-homocysteinaemia (HHcy) is a newly established risk factor for TEE. **Aims.** To evaluate total plasma Hcy (tHcy) mean values and prevalence of HHcy in IBD pts compared to in a healthy control group; to assess differences within ulcerative colitis (UC) and Crohn's disease (CD) and to evaluate the presence of correlation with the stage of disease; to assess, in a subset of pts and control subjects, the presence of thrombophilic factors (TF) (FV1691A; PT20210A; APC resistance; ATIII, PC, PS deficiency; LAC and anticardiolipin antibodies). **Methods.** In 60 consecutive IBD pts, staged by Rachmilewitz score (CAI) and CD activity index (CDAI), (28F; mean age 49 ± 18 ; 34 UC, 26 CD) and 95 healthy sex and age-matched subjects a blood sample was taken to assess tHcy, serum folate (FOL) and vitamin B12 (B12). A subset of 20 of the last consecutive IBD pts (S1) (10f, mean age 45 ± 12 ; 11UC, 9CD) and 20 age and sex-matched controls (S2) were also tested for TF. **Results.** Mean concentration of tHcy was higher in IBD pts than in controls (14.3 ± 12.7 vs. 9.9 ± 3.1 , $p=0.011$), as was the prevalence of HHcy (43.3% vs. 20%, $p=0.002$); no significant differences were observed in FOL and B12; no significant differences were observed between mean tHcy levels in UC and CD pts, whereas HHcy prevalence was higher but not statistically significantly so in CD rather than UC pts (53.8% vs. 35.3%, $p=0.120$). In CD patients there was a significant correlation ($r=.578$, $p=0.002$) between tHcy and CDAI score, which remained statistically significant also after correction for vitamin status ($r=.538$, $p=0.012$), whereas no significant correlation with CAI score was found in UC pts. Neither in S1 (11/20 HHcy) nor in S2 (3/20 HHcy) was TF found or TEE recorded. **Conclusions.** Our data suggest no increased prevalence of TF in IBD pts, as previously described. We observed higher prevalence of HHcy in IBD pts than in controls, apparently not deriving from lower serum vitamin levels. tHcy correlates with disease activity index only in CD. Data about the incidence of TEE in our population will be collected and analysed in a prospective study.

PO-214
ASSOCIATION OF POLYCYTHAEMIA VERA WITH HETEROZYGOUS FACTOR V MUTATION

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A 55-year old woman was admitted to hospital in January '99 because of an ischaemic ulcer of the right foot. She had two episodes of deep venous thrombosis after deliveries 25 and 28 years ago. A lower extremity arterial occlusive disease was diagnosed many years ago, after a long history of *intermittens claudicatio*. She began therapy with prostanoids and unfractionated heparin and improved rapidly. Laboratory investigations revealed heterozygous factor V mutation. The woman was then discharged on warfarin therapy. After six months, she followed the family physician's advice and stopped anticoagulant therapy until November, when she restarted warfarin. One month later, she developed a haemarthrosis of the right knee and was again admitted to our hospital. After local treatment, she again began warfarin therapy. The tests showed polycythaemia vera (erythrocytosis, splenomegaly, low erythropoietin, increased red cell mass). We reviewed the previous laboratory investigations, when only thrombocytosis (500,000 platelets) was present. The association of polycythemia vera with heterozygous factor V mutation is unusual, but, because it could increase the risk of thrombosis, should be looked for.

PO-215
PREVALENCE OF FACTOR V (FV) R506 Q AND PROTHROMBIN 20210 A MUTATION IN THE CALABRIAN POPULATION

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Poor anticoagulant response to activated protein C (APC - resistance), due to the point mutation Arg 506 Gln in the factor V gene (FV R506 Q), has been identified as the most frequent hereditary disorder associated with venous thrombophilia. Recent studies have also demonstrated that a mutation in the gene encoding coagulation prothrombin (20210 A) predisposes individuals to a substantially increased risk of venous thrombosis. We studied the prevalence of both genetic variants in Calabria, a region of Southern Italy, where the majority of people share a Greek genetic background which differs from that of Northern Italians. We present the data of 216 healthy subjects. Six out of 216 subjects carried the FV R506 Q mutation, all of them in the heterozygous form, which gives a prevalence of 2.7% with a corresponding allele frequency of 1.38%. We found the prevalence of the 20210 G. A mutation in the prothrom-

bin gene in Calabria to be higher than that observed for factor V Leiden. Seven out of 216 healthy subjects were shown to carry this genetic variant, six of them in the heterozygous form, and one in the homozygous form, which gives a prevalence of 3.2% with an allele frequency of 1.8%. The mutations R506 Q of the prothrombin gene are, also in our experience, highly prevalent in the general population. This may be important in the evaluation of specific settings of patients, such as women candidates for oestrogen treatment, in whom acquired risk factors increase the thrombotic risk remarkably.

PO-216
FACTOR V LEIDEN AND PROTHROMBIN G20210A MUTATION: A SINGLE INSTITUTION EXPERIENCE

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In recent years two important inherited disorders, the Leiden mutation of coagulation factor V (FVL) and the prothrombin G20210A mutation, have been identified as risk factors for venous thromboembolism. It is controversial whether these defects are risk factors for early arterial thrombosis. The coexistence of the defects is not infrequent. From January 1997 to December 1999, 74, 72, 15 subjects were identified as carriers of FVL, prothrombin mutation or both respectively (71 males and 90 females, 82 with an objectively confirmed thrombotic episode or recurrent fetal loss, 68 asymptomatic, 11 women screened for thrombophilia prior to oral contraceptive use). The median age at the first thrombotic episode in the symptomatic subjects with FVL and prothrombin mutation was 38 yrs. (range 20-72) and 40 (range 16-75) years, respectively. In 17 symptomatic patients (20.7%) an associated risk factor was present (oral contraceptive use, surgery, pregnancy). The symptomatic subjects with FVL had a higher prevalence of deep venous thrombosis (24/47) than symptomatic subjects with the prothrombin mutation (9/35). This latter (isolated or associated with FVL) was more prevalent among the patients with an unusual site of venous thrombosis (3 patients with venous cerebral thrombosis and 1 patient with a portal thrombosis) and in patients with myocardial infarction in young age (< 45 years). In both groups of patients there was a significant prevalence of cerebral vascular disease and "unexplained" recurrent fetal loss (12 and 7 respectively in the FVL group, 7 and 8 in the prothrombin mutation group). However the patient selection and the small size of the samples do not allow determination of whether the FVL and the prothrombin mutation are risk factors for cerebral vascular disease and myocardial infarction in young age. In our opinion, a complete evaluation for inherited thrombophilia is appropriate in young subjects under 50 years with venous and arterial thrombosis. In elderly patients this evaluation will be suggested by the family and clinical history.

PO-217**PROTEIN C AND PROTEIN S LEVELS IN COMBINED TREATMENT WITH FLUOXETINE AND ORAL CONTRACEPTIVES**

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It's well known from literature the relationship between protein C/protein S level and oral contraceptives. On the other hand some authors have recently described modified corticosterone and progesterone levels during administration of fluoxetine in animal models and in humans. We describe a case of undetectable PC and normal PS levels, when measured by functional clotting methods, in a young woman in therapy with fluoxetine and oral contraceptives. *Case report.* A 32 year old woman, with no personal or family history of thrombosis, was chronically treated with fluoxetine (for depression) and oral contraceptives. Coagulation control (requested by gynaecologist) showed normal values for PT, APTT, fibrinogen, AT III, APC resistance, negative LAC and ACA, undetectable PC by clot-based assay, and functional PS 88%. PC and PS activity measured at a further control were 8% and 90%. PC tested on a diluted sample (1:2 and 1:4) showed the same value, but when measured by chromogenic assay, the activity resulted to be 104%. Antigenic PC and PS were normal. Two months after suspension of both drugs PC (clot assay) was 146% and PS activity 101%. *Discussion.* The functional activity of PC can be measured using either [a] synthetic chromogenic substrate or [b] a clot based assay. Chromogenic assay detects only abnormalities of PC activation or of the enzymatic active site, while clot assay also detects defects in substrate binding or the inability of PC to bind PS or phospholipids. In our patient liver enzymes were normal, lupus inhibitors were absent and PC clot-assay level was normal two months after suspension of fluoxetine and oral contraceptives. We suspect an acquired deficiency of PC due to fluoxetine treatment combined with oral contraceptives, possibly based on fluoxetine's influence on progesterone levels or due to competitive binding to phospholipid.

PO-218**INHERITED THROMBOPHILIA AND INFLAMMATORY BOWEL DISEASE: A CASE REPORT**

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Patients with inflammatory bowel disease (IBD) are at increased risk of systemic thromboembolic events; two thirds of the episodes manifest as deep venous thrombosis or pulmonary embolism. We report a case of recurrent venous thrombosis in a young woman affected by ulcerative colitis. At the age of 17 years the patient had her first episode of

bowel disease; after a month or a neurologic syndrome characterised by hemiparesis, diplopia, and aphasia occurred; the diagnosis was left sagittal sinus venous thrombosis. On that occasion, the patient received i.v. heparin therapy and then for the three next months oral anticoagulation therapy (OAT), with complete resolution of neurologic symptoms. Three months later after therapy interruption, during an acute episode of IBD, a new episode of deep venous thrombosis (femoral region) occurred on this occasion the patient was treated with LMWH and then with OAT; after three months the therapy was stopped because of the hemorrhagic risk due to IBD. We observed, for the first time, the patient during the third episode of deep venous thrombosis (popliteal region), which occurred nine months after the previous thrombosis, during a new episode of IBD; the patient was treated with LMWH therapy followed by OAT. Thrombophilic study revealed that the patient was a heterozygous carrier of factor V gene mutation (factor V Leiden), of prothrombin gene mutation (PT20210A) and of MTHFR gene mutation (677T); homocysteine plasma levels were normal. Based on laboratory results, we decided to continue OAT; at present after eleven months the patient has not had any more deep venous thrombosis episodes. A thrombophilic state in patients with IBD is the subject of many scientific studies, but no significant correlation between IBD and inherited thrombophilia has been found; however thrombophilic evaluation is justified in these patients after thrombotic events for correct evaluation of the individual's thrombotic risk and for the consequent therapeutic management.

PO-219**INHERITED PRO-THROMBOTIC CONDITIONS IN PATIENTS WITH RETINAL VEIN OCCLUSION**

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We screened 82 patients (47 males, 35 females, mean age 56.37 ± 11.53 years; mean age at first thrombotic event, 52.7 ± 12.52) with a history of retinal vein occlusion (OVR), referred to our Centre between January and December 1999. Fluorangiography showed that 41 patients had experienced occlusion of the central retinal vein (OVCR); 45, branch retinal vein occlusion (OVBR), and 4 patients had experienced both thromboses. Hypertension was found in 67% (55/82) of the patients; cigarette smoking in 45% (37/82); hypercholesterolaemia in 62% (51/82); hypertriglyceridaemia in 22% (18/82); diabetes mellitus in 14.6% (12/82) and hyperhomocysteinaemia in 27.5% of the subjects (19/69). No antithrombin III, protein C or protein S deficiencies were found, nor was any lupus anticoagulant posi-

tivity observed. The frequency of FV Leiden in the OVR group was 7.3% (6/82) and was not significantly different (5.7%, 12/211) from that of a healthy control population comparable for sex, age and genetic background. The same lack of significance was found when the PRTH mutation was analysed: 4.97% (4/82) vs 6.7%. Most evidence suggests that retinal vein occlusions have a series of similarities with deep venous thromboses of other segments. However, in a sex and age-matched population of 193 patients with a history of venous thrombosis, the frequency of factor V Leiden was 12.8% (26/193) and that of PRTH mutation 12.9% (24/191), meaning that PRTH frequency was significantly ($p < 0.03$ vs OVR, Fisher's exact test) higher in subjects with a history of deep vein thrombosis and the same was true when the two mutations predisposing to hypercoagulability were pooled together ($p < 0.005$ vs OVR, Fisher's exact test). These data suggest that inherited prothrombotic conditions are more common in patients with deep vein thrombosis than in OVR patients. In the latter, hypertension and hypercholesterolaemia or cigarette smoking seem to play a dominant role.

PO-220

INHERITED PRO-THROMBOTIC CONDITIONS IN PULMONARY EMBOLISM PATIENTS WITH AND WITHOUT A HISTORY OF DEEP VENOUS THROMBOSIS

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We screened 64 patients (29 males, 35 females, mean age 42.31 ± 15.04 years; mean age at first thrombotic event, 37.11 ± 13.88) with a history of pulmonary embolism (PE), confirmed by lung scan. All had been referred to our Centre between January and December 1998. Twenty-three subjects (group A) (14 M and 9 F, age at first event, 37.65 ± 13.76) experienced PE in the course of an episode of deep venous thrombosis (DVT). Forty-one patients (group B) (15 M and 26 F, age at first event 37.83 ± 13.56) at a longer interval (>3 mo) or in the absence of a detectable DVT ($n=12$). None of the 64 individuals had antithrombin III, protein C or protein S deficiencies. When the sample was analysed as a whole, the frequency of factor V (FV) Leiden in group A was 13% (3/23) and was not significantly different (9.7%, 4/41) from the frequency found in group B individuals. The same was true for the prothrombin (PRTH) mutation: 21.7% (5/23) in group A and 9.7% (4/41) in group B. Thus, the overall frequency of either mutation was 26% (7/23, one patient had both mutations) in group A and 17% (7/41 one patient had both mutations) in group B. When only patients without circumstantial risk factors (oral contraceptives, pregnancy/puerperium, surgery/trauma, can-

cer) were analysed, FV Leiden mutation was present in 21% (3/14) of patients in group A and in 7.6% (2/26) of group B. PRTH G20210A mutation was present in 29% (4/14) in group A and in 11.5% (3/26) in group B. Thus, the overall frequency of one or other of the mutations was 42% (6/14, one patient had both mutations) in group A and 19% (5/26) in group B individuals ($p=0.003$, Fisher's exact test on percentuals). In spite of the limited sample size, these data suggest that inherited pro-thrombotic conditions are more common in patients in whom DVT and PE coexist and/or occur in close proximity.

PO-221

INHERITED PRO-THROMBOTIC CONDITIONS IN SUBJECTS WITH A HISTORY OF RECURRENT VENOUS THROMBOSIS

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We screened 217 consecutive patients, referred to our Centre between January and December 1998, for the genotypes of factor V (FV) and prothrombin (PRTH). Of these patients 154 (73m/81f, mean age 45.6 ± 14 years) had suffered from one venous thrombotic event and 63 (31m/32f, mean age 40.7 ± 16) from more than one episode. Patients with antithrombin III ($n=2$), protein C ($n=1$) deficiencies or with lupus anticoagulant ($n=3$) were not considered further; none had protein S deficiency. When the sample was analysed as a whole, the frequency of FV Leiden in the "single event" group was 10.4% (16/154) and was not significantly different (15.9%, 10/63) from the frequency found in the "recurrence" group. The same lack of significance was found when the PRTH mutation was analysed: 11.7% (18/154) in the "single event" group and 14.3% (9/63) in the "recurrence" group. When only patients who had suffered from thromboembolic events in the absence of major/circumstantial risk factors (pregnancy/puerperium, oral contraceptives, surgery/trauma, cancers) were analysed [96/154 (62%) of the individuals in the "single event" group, and 29/63 (46%) in the "recurrence" group], the PRTH G20210A mutation was present in 12.5% (12/96) of individuals in the single event patients and in 27.6% (8/29) of those with recurrences ($p=0.052$). Despite the fact that FV Leiden was still not significantly different in the two groups [7.3% (7/96 individuals) in the group suffering a single event and 13.8% (4/29) in those with recurrences], one or other of the pro-thrombotic mutations was found in 18.8% (18/96) of individuals with single events and in 37.9% (11/29) of individuals with recurrences ($p=0.032$, Fisher's exact test). Thus, in this setting inherited pro-thrombotic mutations are more common in patients with recurrences than in those with single events."

PO-222**DIFFERENT CIRCUMSTANCES OF FIRST DEEP VENOUS THROMBOSIS IN YOUNG OR OLD CARRIERS OF THE G20210A MUTATION IN THE PROTHROMBIN GENE**

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Background. The G20210A mutation in the prothrombin gene is a common cause of inherited thrombophilia and is associated with a moderate increase in the risk of deep venous thrombosis. Scarce information is available about the thrombotic risk and the circumstances heralding thrombosis in different age groups. **Methods.** We investigated 426 patients with a first objectively documented episode of deep venous thrombosis of the legs or pulmonary embolism (M/F 201/225, mean age at the first event 38 years, median 36, range 2 to 84) and 509 healthy controls (M/F 266/243, mean age 45 years, median 44, range 7 to 93). After adjustment for other inherited causes of thrombophilia (deficiency of antithrombin III, protein C, protein S, or presence of factor V Leiden) we estimated the relative risk of venous thromboembolism associated with the G20210A mutation in the prothrombin gene as an odds ratio. The relative risk was further evaluated after stratification according to the circumstances and the age of the first deep venous thrombosis. **Results.** The increase in risk for venous thromboembolism associated with the G20210A mutation was 3.6 times higher than in the controls (95 percent confidence interval 1.8 to 7.1). Stratification according to the circumstances of the first deep venous thrombosis did not produce any variation in the thrombotic risk, being 3.9-fold (95 percent confidence interval 1.7 to 8.9) for spontaneous deep venous thrombosis and 3.5-fold (95 percent confidence interval 1.7 to 7.3) for secondary deep venous thrombosis. Further stratification according to age at the first thrombosis revealed an increased risk for spontaneous deep venous thrombosis only in patients older than 45 years in comparison with age-matched controls (odds ratio 6.2, 95 percent confidence interval 2.2 to 17.5); in individuals younger than 45 years the presence of the prothrombin mutation increased the risk of secondary deep venous thrombosis (odds ratio 4.7, 95 percent confidence interval 1.8 to 12.5) but not of spontaneous deep venous thrombosis. **Conclusions.** The G20210A mutation in the prothrombin gene is associated with a moderate risk of venous thromboembolism; yet the clinical penetrance of the defect is fully expressed in elder of individuals or in the presence of a circumstantial risk factor (oral contraceptives, pregnancy, surgery, trauma). Accordingly, such situations should not be considered a contraindication to laboratory screening.

PO-223**PREVALENCE OF THE C536T POLYMORPHISM OF THE TISSUE FACTOR PATHWAY INHIBITOR (TFPI) GENE AMONG PATIENTS WITH VENOUS THROMBOEMBOLIC DISEASE**

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Background. Tissue factor pathway inhibitor (TFPI) is an important regulator in the extrinsic blood coagulation pathway, playing a major role in the inhibition of factor VIIa-tissue factor complex proteolytic activity. A polymorphism was recently identified at locus 536 (536C → T) of exon 7 of the TFPI gene, leading to a proline 151 to leucine substitution (Pro151Leu). Our study was aimed at estimating the prevalence of the C536T transition among patients with venous thromboembolic disease and among healthy controls. **Patients and methods.** The polymorphism C→T at locus 536 of the TFPI gene was screened for in 180 patients (M/F 83/97) with previous deep venous thrombosis of the legs (DVT) and in 208 healthy controls (M/F 86/122, mean age 37 years, median 34, range 8 to 68). The mean age of the patients at the first episode of DVT was 38 years (median 35, range 1 to 84). Patients with cancer, myeloproliferative disorders, autoimmune diseases (including primary antiphospholipid syndrome) were excluded; pulmonary embolism was diagnosed in 56 patients (31%). The first thrombotic episode was associated with risk factors (surgery, pregnancy, puerperium, oral contraceptives, trauma, bed rest) in 108 cases (60%). Inherited thrombophilia was diagnosed in 64 cases (35%). **Results.** The distribution of the TFPI mutant genotype in the cases and in the controls is shown in the Table. No homozygotes were found. Three heterozygotes were identified in the patient group, 2 of them also affected by inherited deficiency of natural coagulation inhibitors (1 with antithrombin III deficiency and 1 with homozygous protein C deficiency); one healthy control was heterozygous. **Conclusions.** The C536T mutation in the TFPI gene is not associated with a significant risk of venous thromboembolism. Nevertheless, the mutant genotype seems to be overrepresented among probands with deficiency of natural coagulation inhibitors and previous DVT. Further studies on larger patient series are needed to confirm such suggestion.

Controls	1/208 (0.5%)	reference group
All DVT cases	3/180 (1.7%)	p = 0.34
DVT cases with AT,PC,PS deficiency	2/ 15 (13.3%)	p = 0.01
DVT cases with inherited thrombophilia	2/ 64 (3.1%)	p = 0.13

Prevalence of the C536T TFPI genotype in the cases and in the controls

PO-224**VENOUS THROMBOSIS OF UNUSUAL SITES: HIGH PREVALENCE OF THROMBOPHILIC GENOTYPES OR LATENT CHRONIC MYELOPROLIFERATIVE DISEASES**

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Background. Venous thrombosis of unusual sites (cerebral or splanchnic veins) is a recognised clinical manifestation of myeloproliferative disorders; recent investigations reported a close association also with inherited thrombophilia. **Patients and methods.** We investigated 72 patients with objectively documented venous thrombosis of unusual sites (M/F 37/35, median age at the moment of thrombosis 35 years, range 3-79): 8 with Budd-Chiari syndrome (BC), 38 with portal and/or mesenteric vein thrombosis (PM), 26 with cerebral vein thrombosis (CV). We also investigated a control group of 456 healthy individuals (M/F 266/190, median age 44 years, range 7-93). All patients underwent screening for inherited thrombophilia; in 40 cases we carried out a clonogenic assay for development of erythroid colonies (EC) in the absence of exogenous erythropoietin, which is considered a reliable sign of chronic myeloproliferative disease including the latent forms. **Results.** Thrombosis was associated with a circumstantial risk factor (pregnancy and puerperium, surgery, oral contraceptives) in only 18 cases (25%); all the 6 cases secondary to hormonal treatment had CV and had no inherited thrombophilic traits. We identified, from among the patients, 1 individual with AT III deficiency (PM), 2 with protein C deficiency (1 PM, 1 CV), 10 heterozygous carriers of factor V Leiden (FV-L) (2 BC, 5 PM, 3 CV) and 6 heterozygous carriers of the G20210A prothrombin mutation (FII-A) (1 BC, 2 PM, 3 CV); overall, 26.4% of patients had a thrombophilic genotype. Among the controls 10 were heterozygous for FV-L, 1 was homozygous for FV-L, and 12 were heterozygous for FII-A. The risk (odds ratio) for unusual venous thrombosis was 8.2 (95% CI 3.2-20.5) for heterozygous carriers of FV-L and 4.1 (95% CI 1.5-11.3) for heterozygous carriers of FII-A. The EC assay was positive in 19 cases (47.5%): 2 BC (40% of the checked cases), 12 PM (54% of the checked cases), 5 CV (38% of the checked cases). EC positive assay and FV-L genotype were found associated in 4 patients (2 PM and 2 CV). **Conclusions.** The prevalence of thrombophilic genotypes associated with unusual venous thrombosis was comparable to that found in patients with deep vein thrombosis of the legs; the assay for development of spontaneous erythroid colonies increases the diagnostic yield for that.

PO-225**THE INCIDENCE OF VENOUS THROMBOEMBOLISM IN CARRIERS OF THE PROTHROMBIN 20210A MUTATION. INTERIM REPORT**

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Background. The prothrombin 20210A mutation is a common coagulation defect associated with an increased risk of venous thromboembolism (VTE). A clear definition of the thrombotic risk in 20210A carriers is necessary to establish prophylactic guidelines. **Methods.** First-degree relatives of consecutive patients referred to our Center with a history of thrombotic disease and identified as carriers of 20210A prothrombin mutation were enrolled in the study. Before DNA testing, a medical history was taken with specific attention to previous VTE and concomitant risk factors. **Results.** A total number of 9 individuals belonging to 34 families were studied. Forty-six patients were carriers (2 homozygous) and 47 non-carriers of 20210A mutation. Out of the 46 affected family members, 5 (11%) had a well documented history of thrombotic manifestations. The thrombotic events were: 2 VTE occurred in the presence of associated risk condition (trauma, infection), 2 ischaemic stroke and 1 myocardial infarction. The two homozygous patients remained asymptomatic. No thrombotic events were documented in non-carriers. The annual incidence of thrombotic events in relatives of propositi was 0.40% in those with the mutation. If we consider only VTE the annual incidence was 0.16%. **Conclusions.** The absolute incidence of venous thromboembolism in carriers of prothrombin 20210A mutation appears to be low. Given the small number of observation-years the data should be convalidated by more extensive cohort studies.

PO-226**VENOUS THROMBOEMBOLISM AND LYMPHOPROLIFERATIVE DISORDERS: A POTENTIAL ROLE FOR FV LEIDEN?**

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Background. The association between venous thromboembolism (VTE) and haematological malignancies is still controversial; data reported in the literature are discordant regarding the overall incidence, the time of occurrence and the criteria used for the diagnosis. **Objective.** In order to evaluate the association between haematological malignancies and VTE, we prospectively investigated, during the period 1996-99, 212 patients referred to our institution for lymphoproliferative disorders (LP-D) (64%

NHL, 17% CLL, 11% MM, 8% HD). All patients were screened for congenital and acquired thrombophilia. The overall prevalence of objectively confirmed VTE was 5.6% (12/212):

Pts.	LP-D	Sex	Age	Thrombosis site/extension	FV Leiden	G20210A
1	NHL (IA)	M	47	Superficial VT (lower limbs)	Heterozygous	Absent
2	MM (I)	F	87	Popliteal DVT	Absent	Absent
3	AILD	F	67	Extensive DVT (lower limbs)	Absent	Absent
4	NHL (IVE)	F	50	Jugular VT	Heterozygous	Absent
5	NHL (IVB)	M	54	DVT + fatal PE + MI	Absent	Absent
6	MGUS	M	60	DVT (femoro-popliteal)	Absent	Absent
7	NHL (IIB)	F	65	DVT (femoro-popliteal)	Heterozygous	Absent
8	NHL (IVB)	F	60	DVT (femoral) + MI	Heterozygous	Absent
9	MM (III)	F	58	DVT (femoro-popliteal)	Not done	Absent
10	CLL (IV)	M	61	DVT (femoro-popliteal)	Absent	Absent
11	MM (II)	M	54	DVT (femoral)	Absent	Absent
12	CLL (IV)	M	56	DVT (femoro-iliac)+fatal PE+MI	Not done	Absent

Three patients had VTE at the moment of the diagnosis of LP-D; in 7 VTE occurred during the first six months after the diagnosis of LP-D and in 2 patients during the follow-up. All patients were treated with standard heparin/anticoagulant treatment for 3 to 12 months. Four out of 10 of patients were heterozygous for the FV Leiden mutation but in none of them was mutated prothrombin or other thrombophilic changes found. **Conclusions.** Our reports confirm the low prevalence of VTE in patients with LP-D; most of them developed VTE during the first six months of follow-up. Bulky disease was observed in 7 out of 12 patients with VTE; 3 of them were also heterozygous for the FV Leiden mutation.

PO-227

FACTOR V LEIDEN AND PROTHROMBIN 20210A MUTATION AND THE RISK OF THROMBOSIS IN INFLAMMATORY BOWEL DISEASE: PRELIMINARY RESULTS

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Background. Acute thromboembolism (TE) is a cause of morbidity in patients with inflammatory bowel disease (IBD). Whether this association is due to acquired or congenital thrombophilia is still not defined. Previous studies reported conflicting data on the potential association between the factor V Leiden mutation and the development of TE in patients with IBD. **Objective.** To evaluate the prevalence of the factor V Leiden and the prothrombin 20210A mutation in patients affected by IBD with and without TE. **Materials and methods.** Retrospective design. Whole blood was collected from Italian patients with a diagnosis of IBD made according to

currently validated scores. The diagnosis of venous or arterial TE had to be confirmed by objective tests. The presence of factor V Leiden and prothrombin 20210A mutation was determined by PCR amplification followed by restriction enzyme digestion of PCR products. **Results.** Fifty-four patients with IBD were investigated, thirty-seven having Crohn's disease and 17 ulcerative colitis. Fourteen out of 54 IBD patients had experienced objectively documented TE (9 venous thrombosis and 5 arterial thrombosis). Factor V Leiden mutation was detected in one patient who had not suffered from TE. No patients had prothrombin 20210A. **Conclusion.** Our preliminary results show a low prevalence of factor V Leiden and prothrombin 20210A among patients with IBD. No excess of these thrombophilic defects is present in IBD patients as compared to those without TE suggesting that other mechanisms may be responsible for the increased thrombotic predisposition. These results are apparently in contrast with those reported by Liebman *et al.* who showed a prevalence of factor V Leiden mutation of 36% among IBD patients with confirmed TE (versus 4% in IBD control patients without TE). The limited number of subjects investigated so far and different selection criteria of IBD patients may account for this discrepancy.

PO-228

ENDOTHELIAL PROTEIN C RECEPTOR GENE MUTATIONS IN PATIENTS WITH MYOCARDIAL INFARCTION OR DEEP VEIN THROMBOSIS

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Background. Endothelial protein C receptor (EPCR) is expressed on endothelial cells of large vessels (especially arteries). Protein C binding to EPCR increases the local protein C concentration and hence augments protein C activation. EPCR gene homology to the class I major histocompatibility complex molecules suggests a role of EPCR in inflammation. EPCR role in coagulation and inflammation mechanisms makes this molecule a candidate gene to look for mutations in patients with myocardial infarction (MI) and deep vein thrombosis (DVT). **Aim of the study.** To identify genetic mutations of the EPCR gene in patients with MI or DVT and to evaluate the association of the mutations with thrombotic events. **Methods.** Case groups: 198 patients with juvenile MI (<50 years) and 194 patients with a first episode of objectively documented DVT at the time of observation. Two control groups, matched for age and sex, were analysed. Screening technology: double gradient denaturing gradient gel electrophoresis and single strand conformational analysis were used for different DNA fragments.

Results.

n° of patients	mutation	fragment	Predicted AA sequence	controls
1 MI	T>G	Exon 2	Val>Gly	0/561
1 DVT				
4 MI	23bp insertion	Exon 3	YQFLstop codon	4/575
2 DVT				
1 MI	C>T	Exon 3	Arg>Cys	-
1 DVT	T>C	Promoter region		1/388
1 DVT	C>T(-342)	Promoter region		0/388
1 DVT	C>T(-313)	Promoter region		0/401
1 DVT	G>C	Promoter region		0/388

Conclusions. These preliminary data suggest a potential role of EPCR in the development of arterial and venous thrombosis.

PO-229**INCREASED PREVALENCE OF THROMBOSIS IN PATIENTS WITH MULTIPLE CAUSES OF THROMBOPHILIA**

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From 1984 to Jan 2000 we studied 417 patients with hereditary thrombophilia belonging to 172 families. Of these patients, 388 (93%) showed a single cause of thrombophilia (FVLeiden=199; FII20210=119; PC=20; ATIII =16; PS=34), whereas 29 had two or more associated causes of congenital or acquired thrombophilia (FVLeiden+ FII20210=11; FVLeiden+ HOCys=3; FVLeiden+ LAC=1; FII20210+PS=2; FII20210+PC=1; PC+HOCys=1; FII20210+LAC=6; FII20210+ HOCys=1; PC+LAC=1; FVLeiden+ FII20210+HOCys = 1; FVLeiden + LAC+ HOCys=1). In this study we compared the two groups of patients with single or multiple thrombophilic defects for the prevalence, type (arterial or venous) and recurrence of thrombosis and age of onset of the first thrombotic event. The prevalence and recurrences of thrombosis were significantly higher in patients with multiple defects than those with a single defect (prevalence: 79.3% vs 32.7%; see table, $p<0.0001$) (recurrence: 37.9% vs. 10.3%, $p<0.001$). The difference remained significant also when the prevalence was calculated only in the family members excluding propositi (60% vs 16.5%, $p<0.0001$). In contrast, significant differences between the two groups were not observed for the type of thrombosis (81.1% venous events in patients with multiple defects, 89.9% venous events in those with single defect), and median age of onset of the first thrombotic event (multiple: median=37 years, range=7-53; single: median=30 years, range=0-69). This study shows an increased thrombotic risk in patients with multiple causes of thrombophilia and, thus, confirms the importance of assessing other congenital and acquired thrombophilic factors in patients with an already diagnosed hereditary thrombophilia.

Patients n.	Venous thrombosis, n.			Arterial thrombosis, n.			Total n. (%)
	DVT+PE+ST	Cerebral	Abdominal	Cerebral	IMA	Peripheral	
single defect (388)	150	5	5	16	2	0	179(32.7%)
multiple defects (29)	26	1	3	6	1	0	37(79.3%)

DVT=deep vein thrombosis; PE=pulmonary embolism; ST=superficial thrombophlebitis; IMA-myocardial infarction.

PO-230**CENTRAL RETINAL VEIN THROMBOSIS: A STUDY OF 36 CASES**

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Background. Central retinal vein thrombosis (CRVT) is a rarely rare disease that may also affect young people and recur easily. The pathogenesis, risk factors and therapeutic possibilities of this often severe disease leading to a permanent visual loss, are not yet well understood. **Patients and Methods.** Thirty-six patients with CRVT (24 males, 12 females, median age 49 years, range 22-80) were submitted to the following screening for thrombophilic factors: protein C and antithrombin (chromogenic assays), total and free protein S (ELISA), activated protein C resistance (Chromogenix), lupus anticoagulant (aPTT sensitive, KCT,DRVVT), and anticardiolipin antibodies (MELISA Byk Gulden). The presence of factor V Leiden, A20210 mutation of prothrombin and genotype TT677 of methylene tetrahydrofolate reductase (MTHFR) were also investigated. **Results.** Four patients had recurrent disease; none had a history of thrombosis in other sites. A previous retinitis and a retinal vasculitis had occurred in 2 patients. Seven (19%) were cigarette smokers, 7 (19%) had hypertension, 1 (3%) hyperlipaemia, 1(3%) cardiac ischaemia; 2 (5%) used oral contraceptives. More than one risk factors were present in 3(8%) patients. No patients had lupus anticoagulant or deficiencies or protein C, S, antithrombin or factor V Leiden mutation. Five patients (14%) were heterozygous for the A20210 mutation of prothrombin (4 younger than 50 yrs and 1 with recurrent CRVT). This prevalence was higher, but not significantly so than (4.7%) in a group of 431 healthy people from the same geographic area and quite similar to that (14.9%) of our population of 222 patients with deep vein thrombosis. The TT677 MTHFR genotype was present in 25% of patients with CRVT and in 18% in the previously described group of healthy controls. **Conclusions.** The A20210 mutation of prothrombin may be considered a risk factor for CRVT as it is in patients with deep vein thrombosis.

PO-231**USE OF DENATURING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (DHPLC) FOR THE RAPID DETECTION OF UNKNOWN MUTATIONS OF PROTEIN C GENE**

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Background. Genomic abnormalities responsible for protein C (PROC) deficiency have been extensively studied and provide important insights into PROC structure-function relationships. The PROC gene abnormalities are heterogeneous, making it difficult and expensive to identify each mutation. Available techniques for screening large populations for sequence variations lack sensitivity and are labor intensive. DHPLC recently has been described as a rapid, automated, screening method for unknown mutations. **Objective.** To evaluate the sensitivity and specificity of DHPLC as a method for rapid screening for unknown mutations within the PROC gene of patients with PROC deficiency. **Study population:** Patients (n=31) with a history of venous thromboembolism referred to the Mayo Clinic Special Coagulation Laboratory and found to have type I or type II PROC deficiency. **Methods.** Protein C activity was determined by chromogenic assay using Protac and protein C antigen level was determined by ELISA. For each patient, a minimum of 4 kb of the PROC gene was amplified by PCR. Based on an optimal amplicon length of 250-500 bp, 10 amplicons were designed in order to screen all exons, the putative promoter region, and the 3' untranslated region. Each amplicon (n=338) was sequenced (both upstream and downstream) using fluorescence-based Perkin-Elmer ABI methodology. Twenty-five µl of each crude PCR product and 25 µl of a 50% mix of each PCR product with a known homozygous wild-type PCR product, underwent a denaturing and reannealing step to favour heteroduplex formation. After this step 5 to 10 µl of each PCR product were analyzed by DHPLC (Transgenomic Wave™ DNA Fragment Analysis System). A blinded analysis of the chromatograms obtained from the DHPLC was performed by an independent reader. Chromatograms were classified as either heterozygous or homozygous. These results were then compared to the sequence data. **Results.** DHPLC has a sensitivity and specificity of 100% for the detection of unknown mutations (before and after sample mixing with a known wild type sample) when compared to the automated sequencing analysis. **Conclusion.** We conclude that DHPLC is a highly sensitive and specific method for the rapid detection of unknown mutations, allowing screening of large patient populations.

PO-232**IDENTIFICATION OF FIVE NOVEL MUTATIONS OF PROTEIN C GENE IN PATIENTS WITH VENOUS THROMBOEMBOLISM AND PROTEIN C DEFICIENCY**

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Background. Protein C (PROC) is a vitamin K-dependent plasma zymogen which is activated to the serine protease, activated protein C (APC), by the thrombin-thrombomodulin complex on the endothelial cell luminal surface. APC exerts its anticoagulant activity by the cleavage/inactivation of cofactors Va and VIIIa. This proteolytic cleavage requires protein S as a cofactor as well as calcium (Ca⁺⁺) ions and a negatively charged phospholipid surface. More than two hundred different mutations of the PROC gene have provided important insights into PROC structure-function relationships. **Objective.** To extend the study of PROC structure-function relationships by screening patients with thrombosis and type I or type II PROC deficiency for novel mutations within the PROC gene. **Methods. Study Population.** Patients (n=11) with a personal or family history of venous thromboembolism (VTE) referred to the Mayo Clinic Special Coagulation Laboratory and found to have type I or type II PROC deficiency. **Methods.** Protein C activity was determined by chromogenic assay using Protac and protein C antigen level was determined by ELISA. For each patient, minimum of 4 kb of PROC gene was amplified by PCR and sequenced using fluorescence-based Perkin-Elmer ABI methodology (both upstream and downstream), including the putative promoter region, all exons (including splice junctions), and the 3' untranslated region. **Results.** The table describes the identified mutations, as well as the PROC antigen and activity levels. Five of these mutations are novel. **Conclusion.** We speculate that C3189G affects a high-affinity Ca⁺⁺ binding site important for PROC activation, G3245A creates an alternative splicing site, and 8496/7(del2nt) and T8731C disrupt PROC catalytic function.

Patient #	Plasma PROC(%) Activity+	Antigen++	Nucleotide Change	Amino Acid Change
1°	34	38	C1432T	R15W
2°	59	59	C1432T	R15W
3	47	58	G2873A*	41W, stop codon
4	58	104	C3189G*	S82R
5	61	68	G3245A*	Splicing error?
6	43	48	8496/7, del2nt*	L261A
7	25	26	G8514A	A267T
8	66	74	C8551T	P279L
9	Unav.		C8608T	T298M
10	54	85	T8731C*	V339A
11	Unav.		A8922C	I403L

**novel mutations; °unrelated patients; +normal range: 70-130%; ++normal range: 60-125%.*

PO-233 MULTIPLE THROMBOPHILIC FACTORS IN A PATIENT WITH BUDD-CHIARI SYNDROME

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Budd-Chiari syndrome is an uncommon but severe disease caused by obstruction of hepatic vein outflow. Many causes have been implicated in its pathogenesis and inherited thrombophilic factors seem to play a significant role among them. We describe a case of Budd-Chiari syndrome in which multiple hypercoagulable states were identified. A 28-year old Caucasian male developed Budd-Chiari syndrome in July 1999. He suffered from essential thrombocytemia diagnosed one year previously. After recovery from the acute onset the patient was submitted to the screening for protein C and antithrombin (Chromogenic assay), total and free protein S (ELISA) deficiencies, protein C and antithrombin (Chromogenic assay), total and free protein S (ELISA), activated protein C resistance (Chromogenic), lupus anticoagulant (LA) (aPTT sensitive, KCT, DRVVT), anticardiolipin antibodies (aCL) (MELISA Byk Gulden), the presence of factor V Leiden (FUL), A20210 mutation of prothrombin (PTHR) and genotype TT677 of the methylene tetrahydrofolate reductase (MTHFR). The results are shown in the table.

PT/PTT ratio	1.36/1.61
KCT/DRVVT ratio	2.30/1.33
aCL IgG/IgM U/mL	6.1/2.5
Protein C %	78
Protein S total/free %	100/116
AT %	99
aPCR	0.67

The activated protein C resistance due to heterozygous FVL was associated with heterozygous A20210 mutation of the prothrombin gene. The patient was started on oral anticoagulation. We think that the myeloproliferative disorder, the FVL and the A20210 mutation of prothrombin concurred in the pathogenesis of Budd-Chiari syndrome in this patient and suggest that thrombophilic states should be searched for in all patients with Budd-Chiari syndrome even if a known risk factor is already present.

PO-234 COMPARISON OF THROMBOTIC PHENOTYPES BETWEEN PATIENTS WITH PROTEIN S (PS) DEFICIENCY ONLY OR ASSOCIATED WITH FACTOR V G1691A, PROTHROMBIN G20210A OR MTHFR C677T POLYMORPHISMS

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Background. PS, a vitamin K dependent protein, is a cofactor to activated protein C. Hereditary PS deficiency is associated with an increased risk of venous thrombo-embolism. **Aim of the study.** Evaluation of thrombotic phenotype in probands with PS deficiency only or associated with factor V G1691A, prothrombin G20210A, MTHFR C677T polymorphisms. **Methods.** Plasma levels of total and free PS were determined by ELISA and/or functional tests. Factor V and factor II polymorphisms were determined by ARMS and MTHFR polymorphism by restriction analysis with Hind I. Thrombotic phenotype was evaluated as 1) age at first event 2) recurrent thrombosis. **Results.** Eighty-four patients were screened for factor V G1691A, factor II G20210A and MTHFR C677T polymorphisms. Sixty-five patients had only PS deficiency, 19 had PS deficiency and factor V G1691A or prothrombin G20210A. Fifteen patients were PS deficient and homozygotes for the MTHFR 677T allele. Asymptomatic patients (n=7) or patients with arterial events (n=2) were excluded from the phenotype analysis. Patients homozygotes for MTHFR 677T had a thrombotic phenotype similar to that of patients with PS deficiency only.

Age at first episode	N° of patients	Protein S deficiency Only (n=58)	Protein S deficiency + FV G1691A or FII G20210A (n=17)
≤ 20	16	10 (62 %)	6 (38%)
21-40	41	32 (78 %)	9 (22%)
> 40	18	16 (89 %)	2 (11%)

Twenty patients (35%) with PS deficiency only had recurrences compared with nine patients (53%) with PS deficiency and factor V G1691A or prothrombin G20210A. **Conclusions.** Patients with PS deficiency and factor V G1691A or prothrombin G20210A have venous thrombosis at a younger age and more recurrent events compared to patients with PS deficiency only.

PO-235
FIBRINOLYTIC PARAMETERS IN YOUNG ADULTS: THE FLOREN-TEEN (FLORENCE TEENAGER) STUDY

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Low fibrinolytic activity and PAI-1 and t-PA plasma concentrations have been identified as risk indicators for cardiovascular disease. Recently, high mass concentrations of PAI-1 have been found to predict a first myocardial infarction. Atherosclerosis is a chronic disease that initiates early in life and its progression relates not only to the presence and extent of risk factors but also to their persistence over time. Scarce information is available on young people with regard to haemostasis-related risk factors and in particular few data exist in the literature about fibrinolysis. In the frame of a longitudinal five-year study of Preventive Medicine and Education Program (Floren-teen Study) a number of cardiovascular risk factors have been investigated in apparently healthy students from two high schools in Florence. The aim of the present study was to determine of fibrinolytic parameters in adolescent students from a high school in Florence involved in the Floren-teen Study. A total of 144 healthy students (59 males and 85 females) aged 17-19 years were enrolled in the study. Venous blood was taken from fasting subjects, after 30 minutes of resting. Euglobulin lysis time (ELT) was measured on acidified plasma, PAI activity was measured by the chromogenic method, PAI-1 antigen and t-PA antigen were measured by ELISA. Lipid analysis was performed by enzymatic assays. We observed sex-related differences for ELT, which was shorter in males, and PAI-1 ag and t-PA ag levels which were significantly higher in males. No differences in the various parameters were observed according to smoking habits, alcohol consumption or practice of physical activity, in leisure time. A correlation was found between ELT and TC, HDL-C and TG. PAI-1 ag was correlated to weight, BMI, arterial blood pressure and HDL-C. PAI activity was directly related to blood pressure only in females. t-PA ag was correlated to arterial blood pressure and HDL-C and TG. Direct correlations were observed among ELT, PAI activity, PAI-1 ag, and t-PA ag. Our data show that the influence of classical risk factors on fibrinolysis-related risk factors takes place early in life.

PO-236
LIPOPROTEIN (a): A NEW RISK FACTOR FOR VENOUS THROMBOEMBOLISM?

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Elevated Lipoprotein (Lp(a)) plasma levels are associated with arterial thrombotic disease and the antifibrinolytic properties of this protein have been well documented. At variance, only scarce and conflicting data are available on the role of increased Lp(a) plasma levels in venous thromboembolism. The aim of this study was to evaluate Lp(a) plasma levels in 337 consecutive patients referred to the Centro di Riferimento Regionale per la Trombosi in Florence because of at least one episode of previous deep vein thrombosis (DVT) and/or pulmonary embolism (PE). Lp(a) plasma levels were detected by an ELISA method in plasma samples stored at -20°C . The assessed Lp(a) plasma levels were $226.4 \text{ mg/L} \pm 275.6 \text{ mg/L}$ (median 123 mg/L ; range $1-2,382 \text{ mg/L}$). Ninety-seven patients (28.8%) had Lp(a) plasma levels $>300 \text{ mg/L}$. Of the patients with elevated Lp(a) plasma levels, 30.2% had had more than one thromboembolic episode in comparison to 24.9% of the patients with normal Lp(a) plasma levels. Plasma levels higher than 300 mg/L were found in 33.3% of patients aged >65 years and in 26.9% of patients aged <65 years. Prevalences of elevated Lp(a) plasma levels were not significantly different in the various groups of patients as regards the localisation of thrombosis: 28.6% in patients with DVT in the upper limb; 31.6% in patients with DVT in the lower limb; 35.4% in patients with PE isolated or associated with DVT; 33.3% in patients with DVT in other localisations. Seventy-five patients (20%) had also had clinical manifestations of atherosclerotic disease (AMI, ischaemic stroke or POAD). Twenty-nine out of 337 patients (8.6%) did not have acquired risk factors (oestrogen therapy, surgery, immobilisation, trauma, neoplasia) or other haemostatic alterations (coagulation inhibitor defects, fibrinolytic alterations, activated protein C resistance, factor V Leiden mutation, factor II G20210A mutation, hyperhomocysteinemia, elevated factor VIII plasma levels) except for elevated Lp(a) plasma levels. In conclusion, increased Lp(a) levels play an important role in venous thromboembolism in adults. These results suggest the opportuneness of including Lp(a) measurement in the evaluation of patients with venous thromboembolism.

PO-237
NEW HAEMOSTATIC RISK FACTORS FOR VENOUS THROMBOEMBOLISM: ANALYSIS OF 758 UNSELECTED PATIENTS REFERRED TO A THROMBOSIS CENTRE

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The importance of evaluating haemostasis-related risk factors for venous thromboembolism (VTE) has become more obvious in the last years. In this study we examined 758 consecutive, unselected patients (mean age $54,1 \pm 16$ years) with at least one previous episode of objectively confirmed VTE, referred to the

Centro di Riferimento Regionale per la Trombosi in Florence. At least one acquired risk factor was documented in 238 patients (31.4%): 182 females (36%) and 56 males (22%). The following parameters were assessed: antithrombin, protein C, protein S, fibrinogen, plasminogen activator inhibitor type 1 (PAI-1), lupus anticoagulant, anticardiolipin antibodies, activated protein C resistance, factor V Leiden mutation, factor II G20210A mutation, homocysteine (base-line and after methionine loading levels), factor VIII, factor XII, plasminogen. In our study, most patients were 40 to 60-years old (36.1%) and 60 to 80-years old (37.5%). The patients aged >65 years did not show a greater prevalence of haemostatic risk factors than the patients aged less than 65 years. An AT-defect was found in 2.5% of patients, whereas a protein C deficit was observed in only 0.3% and a reduced protein S activity in 11.9% patients. About 5% of the patients showed at least one of the above mentioned defects. The prevalences of activated protein C resistance (32.9%) and factor V Leiden mutation (30.3%) are in agreement with published data. Only in 12.6 % of patients was the factor II G20210A mutation found. Hyperhomocysteinaemia was documented in 33.5 % of patients (males:27.8%; females: 37.3 %) and was not associated with acquired risk factors. Lupus anticoagulant and anticardiolipin antibodies were found in 12.6% and 13.7% of the patients respectively. Elevated PAI-1 plasma levels were observed in 13.5% of studied patients. Elevated plasma fibrinogen and factor VIII were present in about 25% of the patients. Only 16.9% of the patients show neither acquired risk factors nor alterations of the haemostatic system. In 3/4 of patients at least one haemostatic risk factor was observed. Our study, performed in consecutive, unselected patients has shown a higher prevalence of haemostatic risk factors in patients with VT, suggesting the importance of extensive screening for these risk factors in patients with VTE.

PO-238

NEW RISK FACTORS FOR CENTRAL RETINAL VEIN OCCLUSION

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Central retinal vein occlusion (CRVO) is one of the most common retinal vascular disorders affecting not only elderly individuals but also young patients. Few and contrasting data are available on the presence of haemostatic risk factors in patients with CRVO. The aim of our study was to investigate the metabolic and inherited risk factors for venous thrombosis in a group of 100 CRVO patients (median age: 59 yrs; range 18-77) and in 100 comparable controls (median age: 56 yrs; range 18-84). In no patients was a deficiency of physiological clotting inhibitors antithrombin, protein C, protein S or heparin cofac-

tor II found. No significant differences between patients and controls were observed for positivity for lupus anticoagulant (Pts: 2% vs Ctrl: 0%), anticardiolipin antibodies (Pts: 10% vs Ctrl: 5%) and the presence of G20210A factor II polymorphism (3.7% vs 1.8%). In patients median homocysteine levels were significantly higher than in controls (male: 12.6 $\mu\text{mol/L}$, range 3-51 vs 10.0 $\mu\text{mol/L}$, range 3-24; $p < 0.005$ / female: 10.0 $\mu\text{mol/L}$, range 5.2-35.2 vs 7.5 $\mu\text{mol/L}$, range 2-10; $p < 0.0001$). The presence of activated protein C resistance (19% vs 5%), factor V Leiden positivity (12% vs 4%), homozygosity for C677T MTHFR (30% vs 17%), Lp(a) levels above 300 mg/dl (19% vs 6%) and PAI-1 activity >15 IU/mL (24% vs 8%) was significantly higher in patients than in controls (p at least < 0.05). After adjustment for sex, age, and the other "classical" risk factors (hypertension, diabetes, hypercholesterolaemia, smoking, family history of CAD) only hyperhomocysteinaemia (OR 11, 95%CI. 3.6-36.2; $p < 0.0001$), elevated PAI-1 plasma levels (OR 4.2, 95%CI. 1.3-13.7; $p < 0.01$) and the presence of at least one of the thrombophilic genetic polymorphisms investigated (OR 3.4, 95%CI. 1.4-8.4; $p < 0.005$) were independent risk factors for CRVO. These data demonstrate a potential role of haemostatic risk factors in the pathophysiology of CRVO.

PO-239

FACTOR XIII LEU34VAL POLYMORPHISM IS NOT ASSOCIATED WITH THE RISK OF DEEP VENOUS THROMBOSIS

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The pathogenesis of venous thrombosis is multifactorial, involving acquired and genetic factors. Although there is evidence of a series of major risk factors for the development of venous thrombosis, there still exist many thrombotic events whose pathogenesis is unclear. The Leu34Val polymorphism within the coagulation factor XIII (FXIII) α -subunit gene has been associated with the occurrence of venous thrombosis, homozygosity for the Leu 34 allele conferring protection. Among 427 patients with a documented diagnosis of deep venous thrombosis in one leg consecutively referred for a thrombophilic work-up and 1045 healthy controls, we retrospectively assessed the allele and genotype frequencies of the FXIII Leu34Val polymorphism. Medical histories were collected. The FV Leiden and FII A²⁰²¹⁰ mutations, and the Leu34Val polymorphism anticoagulant factors and antiphospholipid antibodies were determined by standard techniques. The distribution of the Leu34Leu genotype was similar in patients and in controls (6.6% vs. 4.7%; $p = 0.313$). The risk of throm-

basis among carriers of this genotype was not significantly increased (odds ratio: 1.4 [95% CI: 0.9-2.3]). The estimated risk associated with the Leu34Leu genotype was 1.4 (95% CI: 0.8-2.4) in subjects with (n=272), and 1.6 (95% CI: 0.8-3.1) in those without (n=155) predisposing (hereditary, acquired, circumstantial) risk factors for venous thrombosis. Factor V Leiden and FII A²⁰²¹⁰ are known risk factors for venous thrombosis. After stratification for age, sex, FV Leiden and FII A²⁰²¹⁰ mutation, the estimated risk of venous thrombosis among carriers of the Leu34Leu genotype was 1.3 (95% CI: 0.6-2.7). Findings from this statistical analysis showed a significant excess of the occurrence of deep venous thrombosis in carriers of the FV Leiden and of the FII A²⁰²¹⁰ gene mutations (adjusted odds ratios: 3.4 and 2.7, respectively). As expected, carriers of the FV Leiden and of the FII A²⁰²¹⁰ gene mutations were more frequent among patients having deep venous thrombosis, whereas the FXIII Leu34Val gene polymorphism was unrelated to a history of deep venous thrombosis. In the present setting, there is little evidence of an association between the FXIII Leu34Val gene polymorphism and a history of deep venous thrombosis.

PO-240

PSEUDOHOMOZYGOUS RESISTANCE TO ACTIVATED PROTEIN C: CO-SEGREGATION OF FACTOR V CAMBRIDGE AND LEIDEN MUTATIONS

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A poor response to activated protein C (APC) is an independent risk factor for venous thromboembolism, regardless of the FV Leiden mutation. Recently, a mutation (Arg306->Thr) in the FV gene exon 7 (FV Cambridge) has been found in association with APC resistance regardless of the Leiden mutation and the HR2 haplotype. However, conclusive evidence of the relationship between the FV Cambridge mutation and the risk of venous thrombosis is still lacking. We have investigated a 27-year old man with an APC resistance ratio suggestive of a homozygous condition, and his relatives. The index patient had a deep venous thrombosis in one leg after a prolonged immobilisation at the age of 10 years. He has subsequently experienced idiopathic recurrent deep and superficial venous thromboses. His 64-year old father suffered from the age of 40 years from idiopathic recurrent deep and superficial venous thromboses. In the index patient and in his father, deep venous thromboses were confirmed by phlebography or ultrasonography. His 64-year old mother, his sister and brother, aged 34 and 32 years, respectively, have never suffered from a thrombotic event. Low total

and free protein S values in the index patient (60% and 12%, respectively) and in his father (59% and 28%) were recorded, whereas normal levels were detected in samples from the mother (126% and 100%), sister (99% and 82%), and brother (121% and 115%). The patient showed a normalised APC resistance ratio (0.40), performed using FV-depleted plasma, comparable to those observed among FV Leiden homozygotes (0.38-0.43). Father (0.47), mother (0.68) and the sister (0.50) showed values similar to those of FV Leiden heterozygotes (range 0.45-0.76), whereas the brother had a value (1.02) comparable to normal subjects (>0.78). A FV Leiden allele was detected in the patient, his father, and sister. Acquired confounding conditions, such as oral anticoagulation, FVIII levels, lupus anticoagulant, and hormone replacement therapy were excluded. The FV HR2 haplotype was not recognised in any of the family members. The FV Cambridge mutation was detected in the patient and in his mother. Present findings support the hypothesis that the FV Cambridge mutation affects APC sensitivity and accounts for some of the abnormal phenotypes, documenting, for the first time, a pseudo-homozygous APC resistance as a consequence of co-segregation of FV Cambridge and Leiden mutations.

PO-241

PROTEIN S PLASMA LEVELS AND A2148G/C2698A HAPLOTYPES IN PATIENTS WITH PROTEIN S DEFICIENCY

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Background. Protein S (PS) is a vitamin K-dependent plasma glycoprotein (69 kDa) that plays an important regulatory role in the protein-C pathway. Hereditary PS deficiency is associated with familial thrombophilia and accounts for approximately 5% of thromboembolic patients. Diagnosis of PS deficiency is often problematic because of the wide distribution of PS plasma levels in the normal population. PS plasma levels are influenced by sex and age. Recently, two polymorphisms, A2148G in exon 15 and C2698A in the 3' untranslated region, have been suggested to modulate total PS levels in healthy subjects. **Aim of the study.** To evaluate the influence of A2148G and C2698A on PS levels in probands with PS deficiency. **Methods.** Phenotypic data were obtained in different laboratories. Polymorphisms were evaluated using RFLP analysis. **Results.** In the frame of the PROSIT study, A2148G and C2698A polymorphisms were investigated in 100 probands with confirmed PS deficiency. Total and free PS plasma levels were available in 64 and 54 probands, respectively. As reported in table, among haplotypes positive trends of both total and free PS plasma levels were observed. Trends were more suggestive after grouping different haplotypes.

	2148/2698AA/AA	AG/AA	AA/AC	GG/AA	AG/AC	AA/CC	p
Total PS mean (SD)	47.3 (18.7)	61.1 (22.5)	61.1 (19.3)	66.2 (27.1)	68.9 (17.4)	72.0 (17.8)	n. s. 0.055
Free PS mean (SD)	23.2 (12.0)	32.8 (14.1)	35.6 (21.7)	37.9 (34.6)	34.8 (19.5)	46.0 (14.7)	n. s. n. s.

Conclusions. These data suggest a possible influence of A2148G and C2698A polymorphisms on PS plasma levels (mainly total PS levels) in probands with confirmed PS deficiency.

PO-242

EXTRA-HEPATIC PORTAL VEIN THROMBOSIS IN ADULTS: AETIOLOGIC ROLE OF INHERITED AND ACQUIRED THROMBOPHILIC DISORDERS

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Introduction. Extra-hepatic portal vein thrombosis (EHPVT) is a rare disease that accounts for 5-10% of portal hypertensive adult patients. An association with inherited or acquired thrombophilia is described, but since new thrombophilic disorders have been recently recognised, the true prevalence of thrombophilia in these subjects is probably underestimated. **Objective.** To evaluate retrospectively the prevalence of inherited and acquired thrombophilia in adult patients with EHPVT. **Patients and methods.** Fifty-nine patients (35 females, 24 males; mean age 46.9 years, range 16-76) with EHPVT without pre-existing liver disease, were seen at our Institution between 1987 and 1999. A screening for thrombophilia was performed including: antithrombin (AT), protein C (PC), protein S (PS), factor V Leiden, 20210A prothrombin gene mutation, homocysteine (fasting levels and after methionine load), antiphospholipid antibodies and bone marrow biopsy when needed. Inherited deficiency of natural occurring anticoagulants was considered present only when the same defect was also found in one family member. Other acquired risk factors (surgery, abdominal trauma, infections, contraceptive pill use, pregnancy and puerperium) were searched for by an interview. **Results.** Factor V Leiden and 20210A prothrombin gene mutation information was available for 46 and 44 patients respectively. Inherited thrombophilia was found in 10 patients (17.0%): AT deficiency in 1, PC deficiency in 1, factor V Leiden in 1, 20210A prothrombin gene mutation in 2, hyperhomocysteinaemia in 5. In an other 6 cases a deficiency of AT (n=1), PC (n=1), PS (n=3) and AT+PC (n=1) was found but a family study was not performed, so that the possibility of an acquired deficiency of these natural anticoagulants could not be ruled out. Acquired

thrombophilia was diagnosed in 32 patients (54.2%): myeloproliferative disorders in 19 (16 with essential thrombocythaemia and 3 with polycythaemia vera; of these 1 also had 20210A prothrombin gene mutation, 2 antiphospholipid antibodies, 1 hyperhomocysteinaemia), antiphospholipid antibodies in 10, surgery or abdominal trauma in 2, inflammatory bowel disease in 1. EHPVT was idiopathic in only 16 patients (27%). One patient is still under investigation. **Conclusions.** Congenital or acquired thrombophilia was found in 42 out of 59 adult non-cirrhotic patients with EHPVT (71%). This figure is much higher than previously reported and it is likely to be further increased when all patients are screened for factor V Leiden and 20210A prothrombin gene mutation.

PO-243

HERITABILITY OF THE ACTIVATED PROTEIN C RESISTANCE PHENOTYPE

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Background. Resistance to activated protein C (APC) is a risk factor for venous thromboembolism also in absence of the factor V (FV) Leiden mutation, but the influence of genetic factors other than FV Leiden on the plasma response to APC is still unclear. **Aim of the study.** To evaluate the influence of genetic factors on APC resistance within a population-based cohort. **Methods.** We identified 1,519 sib-pair pairs within the subjects enrolled by the Vicenza Thrombophilia and Atherosclerosis (VITA) Project, a large epidemiological investigation on venous thrombophilia. APC resistance was measured as the normalized APC sensitivity ratio (n APC-SR) on an aPTT system, as originally described by Dahlback. Subjects with FV Leiden or a very prolonged (undetermined) aPTT after addition of APC were excluded from the analysis. In heritability was computed as the ratio between additive genetic variance and phenotypic variance, from the slope of regression correlating the parent and sib n APC-SR. **Results.** After adjustment for known influencing factors, a high in heritability coefficient (0.58) was observed. Parental response to APC was the single most important factor in predicting the corresponding phenotype in sibs. In 32 parent-sib pairs in which phenotypic resistance to APC unrelated to FV Leiden was present in both parents and sibs, no additional mutation on the 306-amino acid residue of FV (FV Cambridge and FV Hong Kong) was found. In these 32 parent-sib pairs, FVIII:C and vWf:Ag levels were not significantly increased and there was no excess prevalence of the R2 allele of exon 13 of FV gene. **Conclusion.** This study suggests that the response to APC is significantly influenced by genetic factors also at a population level, but the mechanisms responsible are still undefined.

PO-244**CONGENITAL AGENESIS OF THE INFERIOR VENA CAVA: A RARE CAUSE FOR "IDIOPATHIC" DEEP VEIN THROMBOSIS IN VERY YOUNG PATIENTS**

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Absence of the inferior vena cava (IVC) is an uncommon congenital vascular abnormality, in which a theoretical condition of hypercoagulability exists for a purported venous stasis. IVC is not normally involved by deep venous thrombosis (DVT), because of a compensatory enlarged vena azygos system. Only six cases of DVT in patients with IVC have been published in English literature, without data on the coagulation pattern and on the follow-up after the episodes. We report here three cases of young patients with spontaneous episodes of deep vein thrombosis of the legs, diagnosed with β -mode ultrasound scanning (see table). In all three patients the coagulation pattern (antithrombin III, protein C, protein S, plasminogen, thrombin time, lupus anticoagulant, antiphospholipid antibodies, factor V Leiden (G1691A) mutation, prothrombin gene (G20210A) mutation, methylenetetrahydrofolate reductase gene (C677T) mutation) studied with standard methods was normal. A computed tomography scanning showed an agenesis of the IVC. The patients were fully heparinised, then anticoagulation treatment with warfarin (OAT) was initiated. The patients were followed at our anticoagulation clinic for 12-38 months, without relapses of DVT. In the patients III OAT was withdrawn, without recurrences after 19 months. This experience shows that in very young patients with proximal deep vein thrombosis "idiopathic" (thrombotic episodes occurring spontaneously, without triggering factors such as surgery, pregnancy, oestrogen therapy, immobilisation, cancer, trauma), without defects of coagulation predisposing to thrombophilia, an instrumental approach (CT and/or angiography) may be warranted to make a diagnosis of a possible congenital vascular defect.

cases	I	II	III
sex	male	male	male
age at DVT episodes	20	22	15
veins involved	right femoral-iliac	bilateral femoral	right femoral
trigger factors	no	no	no
coagulation defects	no	no	no
therapy	heparin and OAT	heparin and OAT	heparin and OAT

PO-245**TWO FAMILIES WITH COEXISTENCE OF HOMOZYGOUS F V LEIDEN AND HETEROZYGOUS F II MUTATION**

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Factor V G1691A (F V Leiden) and factor II G20210A (F II) are the most frequent causes of inherited thrombophilia. Both factor V Leiden and F II have a high allelic prevalence in the general population, varying from 2% to 4% and an higher prevalence in thrombophilic patients (15%-20%). Thus, the association of heterozygous defects might be found in several patients. On the other hand, homozygosity for these polymorphisms is very rare (0.01%-0.05%) and only sporadic cases of coexistence of homo- and/or heterozygous defect are reported. The proband of our first family is a young girl (18 years) who developed massive deep vein thrombosis and pulmonary embolism after a short cycle of oestro-progestin therapy (20 days); the proband of the second family is a young girl (20 years) who developed DVT during oestro-progestin therapy. Antithrombin, protein C, protein S and plasminogen were normal; the lupus anticoagulant test was negative and antiphospholipid IgG antibodies were absent. The two patients were found to be homozygous for F V Leiden and heterozygous for F II. The genetic analysis of the first family showed combined heterozygosity for F V and F II in a brother and in the father; F V Leiden heterozygous mutation in the maternal and paternal grandmothers, mother and sister; F II homozygosity in a maternal cousin; F II heterozygosity in a maternal cousin, and in three maternal uncles. Interestingly, deep vein thrombosis was observed only in the proband, although several members of the family had used oestroprogestin therapy or had surgery or pregnancies. Thus, combined prothrombotic defects were found in 3/12, and single defects were present in 9/12 (with a homozygosity for F II). In the second family a combined heterozygosity for F V Leiden and F II was found in the father, whereas the mother was heterozygous for F V Leiden. The father suffered from an episode of DVT after surgery. In our families the thrombotic risk was lower than expected, despite the presence of trigger factors. In the two probandi, with homozygosity for F V Leiden mutation and heterozygosity for F II, the thrombotic episode seem to be associated with the use of the oral contraceptive pill.

PO-246**THE 23bp INSERTION IN THE ENDOTHELIAL PROTEIN C RECEPTOR GENE IS VERY RARE IN PATIENTS WITH INHERITED THROMBOPHILIA**

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Recently, a 23 bp insertion in exon 3 of the endothelial protein C receptor (EPCR) gene has been demonstrated to be a possible risk factor for venous thrombosis. The insertion has also been detected in association with inherited thrombophilic abnormalities (FV Leiden and protein S deficiency). We screened for the abnormality 44 families with inherited thrombophilia (19 with FV Leiden, 5 with the

G20210A prothrombin mutation, 1 family with combined abnormality, 1 with protein C deficiency, 1 with protein C and FV Leiden, 1 with protein S and FV Leiden, 5 with G20210A mutation and protein S deficiency and 11 with protein S deficiency) for a total of 144 affected members and 84 normal relatives. Furthermore, 52 consecutive subjects with thrombosis occurring <40 years of age or with familial thrombophilia (presence of other symptomatic family members regardless of age of onset of thrombosis) were also studied. The EPCR fragment was amplified by PCR, along with the PAI-1 gene used as control for proper amplification. A positive control was always run in every set of experiments. None of the investigated subjects showed the presence of the insertion. In conclusion, the 23 bp insertion in exon 3 of EPCR gene is very rare in families with inherited clinical and laboratory thrombophilia, thus suggesting a causative role *per se* of the mutation rather than it representing only an additive factor in the presence of other laboratory thrombophilic abnormalities.

PO-247

THE A20210 ALLELE IN THE PROTHROMBIN GENE ENHANCES THE RISK OF VENOUS THROMBOSIS IN CARRIERS OF INHERITED PROTEIN S DEFICIENCY

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Sixteen families with inherited protein S deficiency and venous thromboembolism (VT) were screened for the presence of FV Leiden mutation and for the G20210A allele in the prothrombin gene. While FV Leiden was not detected in any of the families, in five families protein S deficiency and prothrombin mutation were present. To assess the risk of VT in carriers of the combined defects, a total of 92 members of the 16 families, including *propositi*, were examined. Thirty subjects were normal, 40 showed protein S deficiency, 10 the prothrombin mutation and 12 both the abnormalities. When index cases were excluded, a history of thrombosis was present in 40.7% of protein S deficient patients, 75% of patients with combined abnormalities, one out the 10 (10%) with the prothrombin mutation and only one (3.3%) of the normal subjects. Relatives with combined defects showed the highest incidence rate of VT in comparison to normal relatives (rate ratio=32.4), those with PS deficiency an intermediate degree (rate ratio=15.7) and G20210A relatives the lowest (rate ratio=3.4). Relatives with combined defects had an increased risk of VT in comparison to relatives with protein S deficiency (IRR 2.1, 95% CI 0.7 - 5.41; $p=0.1$). In conclusion, the presence of the prothrombin mutation seems to increase the risk of VT carriers of protein S deficiency, although additional families are required to estimate the magnitude of the risk fully.

PO-248

THROMBOPHILIA SCREENING PROGRAMME IN CHILDREN WITH ACQUIRED OR CONGENITAL RISK FACTORS FOR THROMBOSIS

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In recent years it has become apparent that childhood thromboembolic disorders present a serious and increasing problem and guidelines for diagnosis, prevention, and treatment are urgently needed. Large, prospective and case control studies should be performed in order to estimate the real incidence of thrombotic event in various cohorts of patients with at least one known risk factor, and to develop specialised programmes of prevention, treatment and counselling. In the present ongoing prospective study we are evaluating the presence of a genetic or acquired thrombophilic state in selected paediatric groups potentially at risk of thrombosis; the accrual and follow up of patients, admitted since January 1999 to the various units of two Children Hospitals in Piedmont, is still continuing. The cohorts of patients admitted to the study were selected according to the following conditions: 1) long term central venous lines 2) chemotherapy for leukaemia 3) stem cell or organ transplantation 4) cyclosporin treatment 5) acute and chronic autoimmune diseases, including Kawasaki's disease, diabetes, inflammatory bowel disease and APLA syndrome 6) infections such as sepsis, varicella, and HIV 7) metabolic diseases such as homocystinuria and carbohydrate glycosylation defect 8) cardiovascular disease, and related surgical procedures 9) nephrotic syndrome and haemodialysis 10) neurological disorders such as TIA, migrain with aura and partial seizures with post-critical paresis 11) haemoglobinopathies, mainly sickle cell disease 12) surgery and trauma in post-pubertal patients 13) newborns with positive family history and/or outside trigger or ischaemic damage *in utero*. The thrombophilia profile includes accurate family history for thrombosis, standard coagulation tests and measurement of fibrinogen, factors VII, VIII, vWF, XII, proteins C and S, ATIII, APC resistance, assays for prothrombin gene and MTHFR, plasminogen, D-dimer, anti-phospholipid and cardiolipin antibodies, complete lipid profile, dosage of Ip(a), folate and B12 levels, homocysteinaemia, platelet function analysis, storage of DNA and frozen plasma for further assessment and future studies. Analysis of preliminary data is pending (occurrence of thrombotic events in cohort groups 1, 2, 3, 8, 11 and association with persistent antiphospholipid antibodies) and guidelines for a prevention programme are in preparation.

POSTERS

Clinical aspects of inherited coagulation disorders**PO-249****EUROPEAN BATCH RELEASE OF CLOTTING FACTORS CONCENTRATES: ITALIAN EXPERIENCE FROM 1996 TO 1999**

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The council Directive 89/381/EEC was made mandatory in Italy in 1996. Since then all blood products to be put onto the Italian market must be batch released by a National Competent Authority (NCA) within the European Union. The Istituto Superiore di Sanità (ISS) is the Italian NCA. According to the notes of the Commission of the European Communities III/3008/93 a batch is released both on the basis of the assessment of the batch documentation and testing of samples submitted by the manufacturers. The tests used for clotting factors are those indicated in phase 1 of the relevant Note for Guidance - potency, solubility (FVIII/FIX/PCC) and thrombogenicity *in vitro* (PCC) - all tests are performed according to the relevant monograph of the European Pharmacopoeia (E.P.). From 1996 to 1999 a total of 328 batches of clotting factors concentrates were submitted to the Italian NCA for batch testing. About 79% were FVIII with a declared potency (label value) of 200 I.U. (0.6%), 250 I.U. (1.6%), 500 I.U. (24.4%) and 1000 I.U. (52%); about 21% were FIX/PCC with a declared potency of 500 I.U. (14.6%) and 1000 I.U. (6.8%). The biological activity was evaluated taking into account the label value. The percentage deviation rate of ISS estimated potency with respect to that declared by the company, was calculated and classified according to four different classes: <80% (11.7%), 80-99.9% (65.4%), 100-120% (21%), >120% (1.6%). Threehundred and fifteen batches were released, 9 were withdrawn by the company, 4 were not released by the ISS because of non-compliance with the E.P. requirements; three batches had a lower potency level (67%, 69.6% and 67%), one higher (143%) than that stated by the E.P. requirements. The analysis of the overall results demonstrated a general overestimate by the manufacturers. However the coefficient of correlation between the potency determined by the ISS and nominal potency assigned by the company on each batch was 0.903.

PO-250**FACTOR VIII INHIBITOR ANTIBODIES IN HAEMOPHILIA A PATIENTS TREATED WITH RECOMBINANT FVIII (RFVIII): A SINGLE INSTITUTION EXPERIENCE**

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The development of factor VIII (FVIII) inhibitor antibodies (IA) is one of the most serious complications of haemophilia A replacement therapy. In most cases IA develop at an early age and after relatively few days of exposure; the complication is uncommon in moderate and mild haemophilia. There are still large differences in the reported incidence of FVIII IA in haemophilia A patients; studies concerning the use of recombinant factor VIII (RFVIII) in previously untreated patients has given more clear information on this complication. From April 1995 to November 1999 twenty haemophilia A patients (7 severe, 6 moderate, 7 mild unresponsive to DDAVP) were treated with RFVIII concentrates (Kogenate, Bayer and Recombinate, Baxter). All the patients were HBV, HCV and HIV seronegative. Eleven patients had been previously treated with plasma derived FVIII concentrates of intermediate or high purity. The inhibitor testing with Bethesda assay was performed 2 and 4 weeks after the first treatment with RFVIII and then every 3 months. The median age at the time of the first treatment with RFVIII was 6 years (range 1 month-22 years). Over 4.7 years 3 patients with severe haemophilia A (2 previously treated with plasma derived FVIII) developed FVIII IA at a median age of 6 years and after 5-38 days of exposure. Two patients had a low Bethesda titre (1.5 and 2 Bethesda Units-B.U.) and FVIII IA were transient, with spontaneous disappearance after 9 and 11 months. One patient had a maximum titre of 7 B.U. and underwent an immunotolerance programme; this patient obtained tolerance to RFVIII. The cumulative FVIII IA frequency is 23% in our severely and moderately affected patients treated with RFVIII and compare with the reported frequency in some large studies. The RFVIII does not seem to increase the risk of FVIII IA development significantly. In our experience FVIII IA were more frequently of low titre and transient.

PO-251**SUCCESSFUL CORONARY ARTERY BYPASS GRAFT SURGERY WITHOUT EXTRACORPOREAL CIRCULATION IN A PATIENT WITH TYPE 2 A VON WILLEBRAND'S DISEASE (VWD)**

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Introduction. Myocardial revascularisation by coronary artery bypass graft surgery (CABGS) without

extracorporeal circulation (ECC) has recently played an important role in cardiac surgery. Surgeons usually perform CABGS without ECC in high-risk patients such as those with left ventricle dysfunction, renal failure, respiratory problems, advanced age, cerebrovascular accidents and other systemic diseases. Patients with severe congenital bleeding disorders such as type 2 A von Willebrand's disease (vWD) may be also considered at high risk. *Aims of the study.* To evaluate whether CABGS without ECC could improve myocardial function with a reduced risk of bleeding in a vWD patient. *Case report and methods.* The patient is a 72 year old woman with type 2 A (V902E) vWD characterised by a life-long history of mucosal bleeding, bleeding time >30 min, vWF:Ag 40 U/dL, vWF:RCo <6 U/dL and FVIII:C 70 U/dL. She was admitted to hospital with stable effort angina. The coronary angiogram revealed triple-vessel coronary artery disease (CAD) with stenosis of the left anterior descending (LAD, 90%), of the proximal circumflex (CFX, 60%) and of posterior descending (D1, 90%) arteries. CABGS without ECC was carried out on the anterior cardiac wall only (LAN and D1). The patient was given 40 U/Kg of FVIII/vWF concentrate (Haemate-P) before surgery: single daily infusions were then given according to FVIII:C measurements to keep FVIII:C plasma levels >100 and <250 U/dL until day 8. The anastomoses with saphenous vein graft were performed according to standard methods. *Results.* No excessive bleeding occurred during surgery and the postoperative course was uneventful. The patient was discharged from the hospital on day 8 without aspirin because of the prolonged bleeding time. Ten months after surgery she is asymptomatic and the control angiogram revealed patent anastomoses of the sequential saphenous vein graft. *Conclusions.* CABGS without ECC is an effective and safe therapy in vWD patients who may develop CAD.

PO-252

THE COST OF CARE AND QUALITY OF LIFE OF SUBJECTS WITH SEVERE HAEMOPHILIA: THE COCIS STUDY

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Objective. The objective of COCIS (Cost Of Care Inhibitors Study) is to evaluate the economic impact and quality of life of adult patients with haemophilia and inhibitors in Italy. *Methods.* The COCIS study is a longitudinal cost-of-care study. Haemophilia A patients, high and low responders, aged 14-65 years, were sequentially enrolled during 1998 and 1999 in 10 centres in Italy (Bari, Catania, Milan [2], Florence, Naples, Castelfranco V, Parma, Pavia, Perugia, Rome). Information on demography, co-morbidity (HIV, HBV, HCV), diagnostic and laboratory examinations, hospitalisations, drug and medical therapies, physicians' visits and patients' quality of life was collected during the baseline visit. Health care provided to patients (clinical and laboratory examina-

tions, drugs etc.) was quantified in the perspective of the Italian national health service (NHS), by means of tariffs. Quality of life was investigated using several instruments, including the EuroQoL-5D. We report on health care costs and production losses during the six months before the enrolment and on EQ-5D at the time of enrolment. All costs are expressed in Italian Lire 1999. Production losses are expressed in physical units, i.e. working and school days lost. *Results.* Forty-six subjects were enrolled; of these 44 patients (age 36.8±11.9 yrs) had complete information and were assessed for the purpose of this analysis. The total six months medical cost of caring for the 44 subjects was 6.47 billion ITL (3.3 millions US\$), corresponding to 147.2 million ITL per patient (75.930 US\$). In our sample, drugs accounted for the largest part of costs (>99%, 146.4 millions ITL per patient), followed by hospitalisation (0.4%, 0.5 million ITL per patient). There were 201 hospital days, including 56 days in ICU. As to indirect costs, there were 766 working or school days lost for the 44 patients over 6 months (17.4 per patient) and 109 days were lost by caregivers (mean 2.5). The distribution of both direct and indirect costs is highly skewed, e.g. the vast majority of costs generated by three patients who underwent surgical arthroplasty (2.5 billion ITL, almost 1.3 million US\$). Subjects rated their quality of life at a level of 60.4 (+22.5) using the 0-100 VAS of the EQ-5D instrument. *Discussion.* Haemophilia with inhibitors is a very expensive condition. Drugs are the major component of costs, especially in surgical patients. Acceptable levels of quality of life are achievable. Treatment strategies capable of preventing the need for surgery are also likely to dramatically affect costs.

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DISSEMINATED INTRAVASCULAR COAGULATION ASSOCIATED WITH SEPSIS IN NEWBORNS

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Although disseminated intravascular coagulation (DIC) is an "intermediary mechanism of disease" that has been intensively studied during the last three decades, several aspects relating to the clinical problem of DIC are still under debate. It is agreed that DIC is an acquired disorder in which the hemostatic system (platelets, plasma coagulation system, fibrinolysis, endothelial cells) is activated to the conversion of fibrinogen to fibrin. Patients suffering from sepsis show a lot of changes in the coagulation-fibrinolysis balance of endothelial cells and mononuclear phagocytes. The role of coagulation inhibitors in the initiation of clinically relevant thrombotic disorders has been intensively explored within recent years. We aimed to investigate coagulation balance

at a very young age. So we studied 15 neonates, 11 were pre-term (27-36 weeks of gestation) and 4 full-term. All subjects enrolled into the study suffered from DIC as a secondary response to sepsis. The treatment protocol was antibiotics, heparin, antithrombin III (AT III), fresh frozen plasma and platelets. The neonates were monitored by the following investigations: white and red cell counts, prothrombin time, activated partial thromboplastin time, fibrinogen, AT III, protein C, plasminogen, D-dimer(s). All these tests were performed at time 0, after 24 hours and at resolution of DIC, from 2 to 6 days later. All the subjects have overcome the critical phase. Our findings show that AT III may be considered not only a powerful inhibitor of coagulation system, but also a molecule with high antiinflammatory properties. In conclusion, the authors feel that AT III has therapeutic relevance in the early evaluation of the success of any urgent intervention.

PO-254
COLLAGEN BINDING ACTIVITY OF VON WILLEBRAND FACTOR IN THE DIAGNOSIS OF VON WILLEBRAND'S DISEASE

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The capacity of von Willebrand factor (VWF) to bind platelet GPIb and promote platelet aggregation is currently evaluated *in vitro* by the ristocetin cofactor activity (VWF:RCo) assay. One of the peculiarities of VWF:RCo is its sensitivity to the presence of large, haemostatically more efficient, VWF multimers. Even though it was the first, and most long performed test, it remains cumbersome and not always well standardised. In this work we investigated whether collagen binding activity of VWF (VWF:CBA) might give information on the adhesive/aggregating activities of VWF similar to those offered by VWF:RCo, considering that VWF:CBA is also sensitive to large VWF multimers. To this purpose we studied 10 patients with type 2A, and 12 patients with type 2B VWD; all were characterised genetically. In addition, 30 patients with type 1 VWD with decreased VWF platelet content were studied. In types 2A and 2B VWD, both VWF:CBA and VWF:RCo were decreased, and more than VWF antigen; however, the decrease in VWF:CBA was more consistent than that in VWF:RCo, both when results were expressed as an absolute value or as a ratio, which normalised VWF:CBA or VWF:RCo with VWF antigen. In contrast, in type 1 VWD, VWF:CBA was decreased to a similar degree as VWF:RCo, and the ratios were always normal, as in normal subjects. Within our patient group, we observed no false negatives and no false positives, since none of the type 1 VWD patients had VWF:CBA more decreased than VWF:RCo, as predicted by the homogeneous decrease in large multimers. These findings indicate that VWF:CBA is more

capable of detecting the absence of large and intermediate VWF multimers than VWF:RCo. This conclusion is also confirmed by its behavior following DDAVP infusion; in type 2A VWD, despite the persistent decrease in the large multimers, VWF:RCo was normalised while VWF:CBA was still decreased. Hence, VWF:CBA, expressed both as an absolute value and as a ratio is able to discriminate the absence of large multimers better than VWF:RCo. It therefore appears useful in the diagnosis of both VWD variants, in addition to the quantitative forms. Due to the difficulty in performing VWF:RCo and its low reproducibility, we suggest adding VWF:CBA to the panel of tests employed in the diagnosis of VWD; if necessary, it may substitute VWF:RCo.

PO-255
VON WILLEBRAND FACTOR COLLAGEN BINDING ASSAY IN THE DIAGNOSIS OF VON WILLEBRAND'S DISEASE

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The von Willebrand factor collagen binding assay (vWFCBA) is based on the physiological property of von Willebrand factor (vWF) and, in particular of high molecular weight multimers, to bind to collagen. To evaluate the ability of vWFCBA to detect von Willebrand's diseases (vWD) and to identify qualitative abnormalities (i.e. diagnosing, typing and subtyping vWD), we carried out the vWFCBA in vWD patients (pts). Results were compared to those obtained with vWFAg, vWFRCoF, multimeric analysis. The sensitivity of the test was calculated in 20 vWD pts diagnosed and followed in 1 centre. The reference interval was determined in 40 normal subjects (vWD0). The test was carried out in 25 additional vWD patients (15 vWD1, 6 vWD2, 4 vWD3). The ratios vWFAg/vWFRCoF and vWFAg/vWFCBA were calculated in order to discriminate quantitative from qualitative forms. vWFAg, vWFRCoF, vWF multimer analysis carried out by standard procedures, vWFCBA (ELISA) as described by Favaloro (Blood Coag Fibr 2:285-91, 1991).

Results.

	mean	min-max
vWD0 (40)	0.97	0.45-1.48*
vWD1 (20)	0.31	0.5-0.62*
vWD2A (4); 2B (1)		0.02-0.13
vWD3 (2)		0.02-0.03

vWFCBA (U/ml) * mean \pm 2D

The sensitivity of the vWFCBA is 84%. The overlap of the vWFCBA values between vWD1 and vWD2 does not allow to differentiate the two forms of the disease.

Cut-off values for type classification

<1.36	vWFAg/vWFRCof	>1.66
<1.27	vWFAg/vWFCBA	>1.57
vWD1		vWD2

vWFAg/vWFRCof and vWFAg/vWFCBA in vWD diagnosis

vWD type attribution

	pts	vWFAg/vWFCBA	vWFAg/vWFRCof
vWD1	15	11	9
		borderline 4	misclassified 6
vWD2	6	6	5
			misclassified 1

vWFAg/vWFRCof and vWFAg/vWFCBA in vWD diagnosis

Comments. The vWFCBA is a simple, reproducible and sensitive test. The vWFAg/vWFCBA ratio could help to discriminate between vWD1 and vWD2.

PO-256**SUCCESSFUL TYMPANO JUGULAR GLOMUS TUMOUR REMOVAL BY THE OCCIPITAL TEMPORAL APPROACH IN A PATIENT WITH HEMOPHILIA**Tagliaferri A,* Gandolfi A,^o Pattacini C,* Quintavalla R,* Santagostino E,[#] Manotti C*

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Introduction. The removal of tympano jugular glomus tumors must be achieved with minimal bleeding, particularly if carried by enlarged occipital temporal approach for tumor excision and microsurgical dissection of facial nerve. *Case report.* The patient was a 27-year old man with moderate haemophilia A (FVIII:C 2 U/dL). He underwent the otoneurosurgical procedure to remove a tympano-jugular glomus tumor raised at the level of posterior foramen lacerum into the middle ear, invading the petrous pyramid and the bony canal of petrous carotid artery. The day before surgery arterial embolisation of tumour arterial vessels was carried out. The patient was given 80 U/kg of recombinant FVIII (Kogenate, Bayer) before surgery; FVIII:C rose to 174 U/dL after infusion. In the post-operative period he received 25 U/Kg of FVIII every 12 h, until day 7, to keep plasma levels of FVIII:C between 90 - 110 U/dL; from day 8 to 10, 15 U/Kg FVIII were given and FVIII:C was maintained between 50-60 U/dL. *Results.* This kind of treatment allowed an operation of several hours, i.e., a major otoneurosurgical procedure at the skull base, with no particular problem of bleeding during tumour enucleation and facial nerve dissection. During the final steps of the procedure, when the blood pressure is usually raised by the anaesthesiologist, no haemorrhagic tendency was noted and wound closure was performed uneventfully. At discharge from

the ward and during removal of wound stitches, the patient had no problem of bleeding from the occipital temporal wound. *Conclusions.* Substitutive treatment with recombinant FVIII is feasible for patients with moderate haemophilia also for major otoneurosurgical operations. These, in fact, require handling of major arterial vessels and venous sinuses, during which it is particularly important that intratumoural small arterial feeders are kept under control, to avoid diffuse bleeding, difficult to control using haemostatic surgical procedures with the bipolar forceps.

PO-257**SAFETY AND EFFICACY OF CELECOXIB, A COX-2 INHIBITOR, IN HAEMOPHILIA: PRELIMINARY RESULTS**

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Many currently used non-steroidal anti-inflammatory drugs (NSAIDs) are mixed inhibitors of both constitutive (COX-1) and inducible (COX-2) forms of cyclo-oxygenase. Inhibition of COX-1 is thought to result in adverse effects on the upper gastrointestinal tract, kidney and platelets. Celecoxib is a new anti-inflammatory and analgesic agent that selectively inhibits the isoform COX-2. Clinical trials performed in osteoarthritis and rheumatoid arthritis showed that Celecoxib had no effect on platelet aggregation, bleeding time, prothrombin time (PT) or activated partial thromboplastin time (aPTT). No studies have been performed in patients with disorders of coagulation, particularly in patients with inherited disorders. To investigate the efficacy and safety of this new drug in haemophiliacs, six patients affected by chronic and painful synovitis at target joints received celecoxib 100 mg twice daily for a week. Assessment included clinical scores, liver and kidney function tests, PT, aPTT, platelet count, delta granules (nucleotides) content, platelet aggregation in response to collagen, arachidonic acid, ADP, adrenaline and ristocetin. All parameters were determined before, 4 and 7 days after the first dose, and again on day 14, after a 7 day washout period. No alterations in liver and kidney function tests were detected. Celecoxib succeeded in reducing pain as much as other currently used NSAIDs. No significant trend was observed in clotting test, platelet count and aggregation tests, whereas a significant parallel decrease of nucleotides content was observed during treatment, followed by a reverse tendency to basal levels after the washout period. Preliminary results about the effects of celecoxib administration in haemophiliacs, show its efficacy in improvement of pain from arthropathy. As far as the haemostatic mechanism is concerned, the therapeutic doses of this drug can be considered safe. No biochemical effects on platelet aggregation or basal clotting parameter modifications were observed. We are going to study other haemophilia patients to confirm these data and to explain the nucleotide modifications.

PO-258**EFFECTS OF rFVIIa ADMINISTRATION ON PLATELET FUNCTION IN ASYMPTOMATIC HAEMOPHILIA A PATIENTS WITH FACTOR VIII INHIBITORS**

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Recombinant activated factor VII (rFVIIa) is widely used for treatment of haemorrhagic episodes in haemophilia A patients with FVIII inhibitors. rFVIIa does not have protease activity by itself and therefore cannot induce systemic activation of coagulation unlike aPCCs or PCC. Since FVIIa is not neutralised in the blood stream by ATIII, infused rFVIIa can reach the sites of injury directly. Forming a complex with locally exposed tissue factor (TF), it is able to promote Factor X activation on the surface of endothelial cells and/or of activated platelets. This effect does not depend on FVIII and FIX: in this way, the inactivating inhibitors can be bypassed. With the purpose of evaluating the potential thrombotic risk of rFVIIa, we investigated platelet function during the single dose pharmacokinetics (90-120mg/Kg) of rFVIIa in three haemophilia A patients with FVIII inhibitors. Blood samples were collected before and at 5, 15, 30 minutes after the end of the infusion. FVII:C and FVIIa (one-stage clotting assay) and GMP-140 (P-selectin, CD62), GP53 (CD63), ADP (quinacrine uptake) and mRNA content (reticulated platelets) were determined by flow cytometry. The expression on platelet surface of GMP-140 and GP53 is considered secondary to the activation process. ADP content of activated platelets decreases because the dense bodies undergo release reaction. On the other hand, mRNA may increase due to platelet pool renewal. Statistical analysis of the results (trend test) did not show any significant change of platelet function parameters before and after the rFVIIa infusion, in all three patients. These results in our patients seem to show that rFVIIa, at least at the recommended dosage for kinetics, is not able to induce platelet degranulation and activation. Probably, only when a suitable local TF is available with consequent thrombin generation, can platelets undergo a release reaction and make phospholipids available on the platelet surface.

POSTERS
Platelets III**PO-259****EVIDENCE THAT INDEPENDENT ACTIVATION OF PROTEIN KINASE C OR PHOSPHOLIPASE C CAN INDUCE PLATELET AGGREGATION PROVIDED A GI PROTEIN-COUPLED RECEPTOR IS ACTIVATED**

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Concomitant activation of two different G-protein-coupled receptors, one coupled to the activation of Gq, responsible for the activation of phospholipase (PLC), and the other coupled to Gi, that inhibits adenylyl cyclase is essential for platelet aggregation. The aim of the present study was thus to identify which biochemical pathway downstream of Gq protein activation is necessary to Gi in order to induce platelet aggregation. For this purpose we studied aggregation in a platelet suspension treated both with aspirin, to eliminate the formation of TxA2 which activates Gq, and with the ADP scavenger system creatine phosphate/creatine kinase (CP/CPK), to eliminate ADP release, activating both Gq and Gi proteins, in response to adrenaline, used as a Gi activator, and to the snake venom toxin convulxin, a PLCg2, but not Gq-protein, activator, and to phorbol myristate acetate (PMA), a protein kinase C (PKC) activator. The results obtained showed that platelet aggregation in response to the snake venom toxin convulxin or PMA occurred provided that adrenaline epinephrine was added concomitantly. A PKC inhibitor, Ro 31-8220, did not suppress platelet aggregation in response to convulxin plus ADP or adrenaline; this strongly suggests that PLC and PKC are both involved in platelet aggregation. Neither pathway involved is dependent on enhancement of the calcium concentration, as platelet treatment with the cytosolic calcium chelator BAPTA did not inhibit the aggregometric response to the combined stimulation by convulxin or PMA plus ADP or epinephrine. In conclusion, we demonstrated that a direct Gq activation is not required for platelet aggregation and that the activation of an enzyme downstream of Gq, such as phospholipase C or protein-kinase C, is sufficient for such process provided a Gi coupled receptor is activated.

PO-260**EVIDENCE FOR PLATELET α IIb β 3 EXPOSURE IN cAMP ELEVATING CONDITIONS**

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Cyclic nucleotides, such as cAMP, are known to inhibit the multistep cascade that results in platelet aggregation. In the present study we provide evidence that it is possible to bypass the cAMP inhibitory effect elicited by the prostacyclin (PGI₂) stable analogue iloprost on fibrinogen binding site exposure induced by the thromboxane A₂ synthetic analogue U46619, the snake venom toxin convulxin, or by the direct PKC activator oleoyl acetyl glycerol (OAG), by activating a Gi-coupled receptor or by inducing cytosolic calcium influx. Iloprost (3 μM) treatment, which produced 1172.5±121.1 pmol/10⁹ cells cyclic AMP, was able to cause a total inhibition only of U46619 (1 μM)-induced aggregation (absence vs. 80.7±21.2% untreated samples) and strongly inhibited the response to convulxin (5 ng/mL) (18.5±5.1% vs. 78.7±15.5%) or OAG (40 μM) (32.4±16.9% vs. 84.8±20.3%). A complete inhibition of the convulxin- and OAG-induced platelet aggregation was achieved by the addition of small concentrations of an ADP scavenger system, CP/CPK (4 μM and 10 U/mL respectively). This might be consequent to the small amount of ADP that both convulxin and OAG are able to release. Iloprost-induced inhibition of platelet aggregation was overcome by adding a Gi protein-coupled receptor activator, such as adrenaline (5 mM) or a cytosolic calcium ionophore, such as ionomycin (2 μM). Such aggregation turned out to be dependent on PKC activation, as platelet incubation with a PKC inhibitor, Ro 31-8220 (10 μM for 3 min at 37°C), did not allow platelet aggregation to be restored. The aggregometric studies found further confirmation in fibrinogen binding. In fact, the results show that the combination of U46619, convulxin or OAG with epinephrine or ionomycin in iloprost-treated platelets induced the same rate of fibrinogen binding as compared to untreated samples stimulated with the agonists alone. From our data we can conclude that in cAMP elevating conditions it is possible to obtain αIIbβ3 exposure only by the simultaneous activation of PKC and a Gi-coupled receptor or, more efficiently, inducing calcium influx in the presence of an activated PKC. These results strongly suggest that cAMP inhibitory action is not exerted at the level of platelet cytosolic domain of the integrin αIIbβ3.

PO-261

WITHDRAWN FROM PRESENTATION

PO-262

ABNORMAL VENTRICULAR LATE POTENTIALS AND *IN VIVO* PLATELET ACTIVATION IN MYOTONIC DYSTROPHY

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Myotonic dystrophy (MD) is the commonest muscular dystrophy of adult life. It is an inherited disease due to an abnormal expansion of CTG trinucleotide repeat at 19q13.3 of myotonin protein kinase gene (DMK). MD protein kinase is involved in the modulation of calcium homeostasis in muscle cells. This defect in calcium metabolism suggested that it may be possible to use blood platelets as an experimental model for this disorder. Indeed, increased *in vitro* platelet aggregation and elevated levels of beta-thromboglobulin have been reported to occur in MD. Recently, it has been suggested that soluble (s) P-selectin can be used as a marker of *in vivo* platelet activation. In this study, sP-selectin was determined in 41 MD patients (22 men and 19 women; mean age 42±14 yr.) and 41 age- and sex-matched healthy subjects (HS) (22 men and 19 women; mean age 47±11 yr.). The severity of skeletal muscle involvement was scored as mild (60%), moderate (20%) and severe (20%). On the basis of CTG trinucleotide repeat expansion, patients were classified as E1 (0-500 CTG repeats, 55%), E2 (501-1000 CTG repeats, 39%) and E3 (>1000 CTG repeats, 6%). Abnormal ventricular late potentials (LP), which represent a substrate for malignant re-entrant ventricular arrhythmias, were observed in approximately 50% of MD patients. The results obtained demonstrated that plasma sP-selectin levels were significantly higher in MD patients than in controls (75±24 vs 54±18 ng/mL, p<0.0001). Moreover, 33 of 41 patients (80%) had sP-selectin levels above the median of HS (49.4 ng/mL). Of interest, sP-selectin levels were higher in patients with an abnormal LP than in those with a normal LP (80±26 vs. 63±18 ng/mL, p<0.05), whereas no correlation was found with either the severity grade or E status. We may, therefore, conclude that *in vivo* platelet activation may occur in MD patients, as reflected by elevated sP-selectin levels. Patients with abnormal ventricular late potentials are at high risk of ventricular arrhythmia and sudden death. Here we demonstrated that this subgroup of patients also have increased sP-selectin levels. Whether elevated sP-selectin levels are a consequence of ventricular arrhythmias or not is still under evaluation. Nevertheless, we might propose its potential use as an adjunctive tool in the prognostic scoring of MD patients.

PO-263**REGULATION OF L-ARGININE UPTAKE BY Ca^{2+} IN HUMAN PLATELETS**

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Nitric oxide (NO) exerts various action on cell activities, including an inhibitory effect on platelet activation. The regulation of platelet function is crucial to prevent hyperaggregation, thrombus formation and stroke and it seems to be due partially to both endothelium - and endogenously - derived NO. This last contribution could be due to the activation of the Ca^{2+} - dependent NOS constitutive isoform that was purified and characterised in platelets. Nevertheless, despite the clear anti-aggregating effect of NO, the effective role of the endogenous enzyme is still unclear. The presence of a Ca^{2+} - dependent isoform is in contrast with the virtual absence of endogenous NO production as a consequence of specific Ca^{2+} mobilising stimuli. Thus a complicated problem is the regulation of the L-arginine-NO pathway inside platelets. L-arginine uptake in resting and stimulated platelets was studied. The role of Ca^{2+} in the control of the transport was also evaluated. L-arginine uptake studies carried out in resting platelets allowed us to define kinetic parameters: Km value was 15.4 ± 2.5 mM and Vmax corresponded to 94.5 ± 3.8 pmol / 2.0×10^8 plts / min (n=5). Moreover L-arginine uptake and Ca^{2+} changes in unstirred platelets activated by collagen, thrombin and Ca^{2+} ionophore A23187 were measured. Collagen was ineffective. Thrombins lightly reduced L-arginine uptake at short incubation times (1-15 min), and slowed down amino acid intracellular concentration after longer times (30-60 min). A23187 decreased the L-arginine uptake in a dose-dependent manner, producing maximal inhibition at 5 μ M. The effect of thrombin and A23187 was reversed by EGTA. In Fura 2-loaded platelets collagen did not change Ca^{2+} basal level, thrombin induced a small Ca^{2+} rise and A23187 dose-dependently increased cytosolic Ca^{2+} , eliciting the highest rise at 5 mM. It is likely that L-arginine uptake is inversely modulated by Ca^{2+} and is inhibited during platelet stimulation with agonists which induce cytosolic Ca^{2+} elevation. In conclusion, our data strengthen the hypothesis of a very refined control system of the L-arginine/NO pathway in human platelets, in which $[Ca^{2+}]_i$ is at the same time the activator of NOS and the inhibitor of L-arginine uptake.

PO-264**PATIENTS WITH CARDIAC SYNDROME X HAVE ABNORMAL PLATELET NA/H EXCHANGER ACTIVITY AND INCREASED URINARY THROMBOXANE METABOLITE EXCRETION**De Candia E,* Lanza G,^o Romagnoli E,^o Ciabattoni G,# De Cristofaro R,* Sestito A,^o Landolfi R**Department Internal Medicine, ^oDepartment of Cardiology, #Department of Pharmacology, Catholic University School of Medicine, Rome, Italy

Cardiac syndrome X (SX) is characterised by effort angina and coronary angiography negative for atherosclerotic plaques. Although a microvascular dysfunction has been suggested to cause this syndrome, little is known about the pathophysiologic mechanisms of the disease. An increased Na^+/Li^+ exchanger activity in red blood cells from SX patients has recently been reported. We studied the platelet Na^+/H^+ exchanger (NHE) activity and the urinary excretion of thromboxane (TXB2) metabolites in these patients in order to evaluate whether the NHE is altered in cells other than red blood cells and also to evaluate whether platelet activation may have a role in this disease. The platelet NHE activity was evaluated by inducing artificial acidification of BCECF-loaded platelet cytoplasm using increasing concentrations (17 to 60 mM) of Na propionate with or without stimulus with 1 nM α -thrombin. The velocity of pH recovery after stimulus was followed spectrofluorometrically and expressed as Δ pH/min. Seventeen SX patients and 17 age and sex-matched controls were analysed.

Na propionate (mM)	SX patients(Δ pH/min)	controls (Δ pH/min)	p
17	0.0255 \pm 0.0091	0.0174 \pm 0.0067	0.0103
26	0.0472 \pm 0.0168	0.0298 \pm 0.01	0.0015
40	0.0785 \pm 0.0359	0.0511 \pm 0.0185	0.0099
60	0.1123 \pm 0.0422	0.0795 \pm 0.0255	0.0104

Na propionate (mM)+ 1 nM α -thrombin	SX patients(Δ pH/min)	controls (Δ pH/min)	p
17	0.1727 \pm 0.0696	0.22 \pm 0.1233	0.1707
26	0.2154 \pm 0.0884	0.2725 \pm 0.1234	0.1245
40	0.2666 \pm 0.0994	0.3512 \pm 0.1480	0.0555
60	0.3147 \pm 0.1265	0.3978 \pm 0.1805	0.1313

The velocity of pH recovery after acidification alone was significantly increased in SX pts, whereas it was generally reduced in respect to controls when stimulus with α -thrombin was added to acidification. Increased 11-dehydro-TXB2 excretion was found in 7 out of 13 patients. Altogether these results indicated that in SX the NHE activity is abnormally regulated in different cells and that platelet function seems to be altered toward an "activated state".

PO-265**EFFECT OF FIBRINOGEN CONCENTRATION ON THE ANTIPLATELET EFFECT OF TYROFIBAN**Rocca B,* Renda G,^o De Cristofaro R,* Landolfi R**Haemostasis Research Centre, Department of Internal Medicine and ^oDepartment of Cardiology, Catholic University School of Medicine, Rome, Italy

Anti-IIb-IIIa agents are effective in reducing vascular complications after high risk coronary angioplasty and may, in the future, be extensively used in the treatment of patients with acute coronary syndromes. These agents inhibit the final step of the aggregation phenomenon i.e. platelet glycoprotein IIb-IIIa interaction with adhesive proteins. The mon-

oclonal antibody abciximab acts through binding site obliteration while the effect of RGDS analogues is mediated by platelet receptor occupancy. It has been shown that the antiplatelet effect of abciximab is influenced by platelet count while no information is available on possible variations of the effect of RGDS analogues. In this study we evaluated the effect of fibrinogen and von Willebrand factor concentration on the antiplatelet effect of the RGDS-like compound tyrofiban. Platelet aggregation was evaluated by Born's method using both platelet rich plasma and get-filtered platelets. Platelet function was also measured in citrated whole blood samples by a platelet function analyser (PFA) (DADE) allowing measurement of the closure time of ADP/collagen coated cartridges exposed to flowing blood. Whole blood, PRP and gel filtered platelet samples were incubated *in vitro* with a wide range of tyrofiban concentrations before being tested. PFA data allowed calculation of the tyrofiban dose doubling the basal closure time ($2T_0$). Aggregation velocities of PRP and gel filtered platelet samples exposed to 10 μ M ADP and various tyrofiban concentrations allowed the tyrofiban inhibitory effect, expressed as IC_{50} , to be calculated. In whole blood samples the $2T_0$ value was positively correlated with plasma fibrinogen concentration (concentration range 138-655 mg%, $r=0.85$, $n=11$, $p<0.05$). The effect of fibrinogen concentration on the tyrofiban effect was also demonstrated in aggregation experiments performed by Born's method. The IC_{50} values were, in fact, positively correlated with fibrinogen concentration both in PRP samples ($r=0.89$, $n=9$) and in aggregation experiments performed using gel filtered platelets and different concentrations of purified fibrinogen ($r=0.98$). No relationship was found between $2T_0$ or IC_{50} values and other variables such as platelet count, von Willebrand factor and ristocetin cofactor. In summary fibrinogen but not von Willebrand concentration influences the antiplatelet effect of tyrofiban *in vitro*. This is probably occurs also *in vivo*, and calls further attention towards the need for laboratory monitoring of anti-IIb-IIIa strategies.

PO-266

USEFULNESS OF A MODIFIED ANTIGEN CAPTURE ELISA (MACE) IN THE DIAGNOSIS OF IMMUNE THROMBOCYTOPENIA

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Background. The search for a useful laboratory diagnostic assay for platelet autoantibodies has been long and difficult, and so the diagnosis of immune thrombocytopenia (ITP) has been a clinical one. Measurement of platelet associated IgG (PAIgG) has been disappointing because the amount of PAIgG is affected by plasma IgG levels, age of platelets, presence of circulating immune complexes and platelet activation. The availability of monoclonal antibodies against

human glycoproteins has opened up the possibility of measuring specific autoantibodies that bind directly to surface platelet glycoproteins and platelet antigen capture techniques have been developed. The aim of this study was to assess the value of modified antigen capture ELISA (MACE) testing in the diagnosis of ITP. **Patients and Methods.** PAIgG and platelet specific autoantibodies were measured in 51 patients with immune thrombocytopenia (38 patients with clinically diagnosed ITP and 13 affected by thrombocytopenia secondary to other immune diseases) and 26 patients with thrombocytopenia secondary to a decreased bone marrow production (NITP). PAIgG was measured by immunofluorescent flow cytometry whereas specific platelet-associated autoantibodies (antibodies against GP IIb/IIIa, Ib/IX, Ia/IIa) were measured by a modified antigen capture ELISA (GTI-MACE AUTO, USA). **Results.** The mean platelet counts of the ITP group ($44.1 \pm 28.1 \times 10^9/L$; $M \pm SD$) and NITP ($42.9 \pm 10^9/L$) were not significantly different. PAIgG was elevated in 28/51 patients with ITP (55%) and 8/26 patients with NITP (31%). Using MACE, 26/51 patients with ITP (51%) and 3/26 patients with NITP (11%) had specific platelet-associated autoantibodies. The degree of concordance (positivity and negativity) between PAIgG and MACE was 68%, that of discordance 32% ($\chi^2=7.844$, $p<0.006$). The sensitivity of the PAIgG assay for ITP was 54%, the specificity 69%, the positive predictive value 77% and the negative predictive value 43%. For the MACE assay, the sensitivity was 50%, the specificity 88%, the positive predictive value 89% and the negative predictive value 47%. **Conclusions.** We found a good concordance between PAIgG and MACE assays. Both PAIgG and MACE had a low sensitivity, but MACE had a greater specificity and positive predictive value than PAIgG for the diagnosis of ITP.

PO-267

SERINE BASE EXCHANGE ENZYME IN COMMERCIALY AVAILABLE PORCINE LYOPHILISED PLATELETS: DIFFERENT EFFECT OF UNFRACTIONATED AND LOW MOLECULAR WEIGHT HEPARINS

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Background. Phosphatidylserine (PS) is a membrane phospholipid required for the activity of all the isoforms of PKC, and its content in the plasma membrane of platelets is critical for the regulation of platelet dependent haemostatic mechanisms. PS is not synthesised *de novo* in human tissues, but is produced from phosphatidylcholine and phosphatidylethanolamine by base exchange. The use of commercially available lyophilized platelets may represent a suitable model for the study of serine base exchange enzyme (SBEE), avoiding the procedures for platelet preparation and the effect of inter-individual variability. **Aim of the study.** A) to analyse the

composition on phospholipids of lyophilised porcine platelets b) to verify the existence of base exchange reactions and their properties and c) to evaluate the effect of heparin on SBEE activity. *Materials and Methods.* Lyophilised porcine platelets were purchased from BioSmia AS, Agro Bio Systems, Norway. Unfractionated heparin and low molecular weight heparin (LMWH) were Na salts from porcine intestinal mucosa. L(U14C)-serine was from the Radiochemical Centre Amersham, England. Radioactivity was measured with a Packard 1600 CA Tri-Carb (Packard Cambera Company, USA). *Results.* Phospholipid content and composition of lyophilised porcine platelets was similar to that of fresh porcine and human platelets. SBEE activity was shown to have different optimum pH at different Ca^{2+} concentrations. SBEE activity was linear up to 30 min. 2.5 mM AIF4-, a G protein activator, inhibited SBEE activity by 30%. The addition of UFH up to 0.06 mg/mL stimulated PS synthesis in a dose-dependent manner. This effect was maximal at pH 7.4. LMWH only stimulated PS synthesis slightly. The effect of heparin was independent of preincubation, so excluding a G protein mediated mechanism. *Conclusions.* Lyophilised porcine platelets can be utilised as an experimental model for SBEE. LMWH did not significantly modify SBEE activity. The potential role of PS concentration modulation in regulating or triggering platelet aggregation remains to be shown.

PO-268

MEPACRINE-BASED FLOW CYTOMETRIC ASSAY CORRECTLY IDENTIFIED TWO ADDITIONAL NON PUERTO-RICAN CASES OF HERMANSKY-PUDLAK SYNDROME

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The Hermansky-Pudlak syndrome is a rare autosomal recessive disorder, characterised by tyrosinase-positive oculocutaneous albinism, reticuloendothelial deposits of ceroid in macrophages and absence of platelet dense granules. The clinical presentation is associated with a mild to moderate bleeding diathesis, gastrointestinal lesions and occasionally severe lung fibrosis. The disease is present in Puerto-Rico, where it is related to homozygosity for a 16 bp duplication in exon 15 of the gene HPS on chromosome 10q23. Conversely, no genetic defect was identified in the few non-Puerto-Rican cases described in the literature. Diagnosis of platelet dense granule defects requires a combination of studies including luminoaggregometry, conventional platelet aggregation, radioactive serotonin uptake and release, and electron microscopy. Mepacrine, a fluorescent marker, is rapidly taken up and localised in dense granule of platelets because of the high affinity with adenine nucleotides. Hence, after mepacrine labelling, flow cytometry is able to quantify fluorescence in a large

sample of platelets. The probands were female twins with a moderate bleeding diathesis characterised by conventional aggregometry by an isolated deficit of arachidonic acid-induced aggregation. Mepacrine-based flow cytometry showed marked reduction of mean platelet fluorescence in comparison with normal controls, in agreement with a severe deficit of dense granules. However, diagnosis was confirmed by the absence of dense bodies on electron microscopy. These two additional cases document the usefulness of the mepacrine-based flow cytometric assay in the detection of dense granule defects.

PO-269

PLATELET GP IIb-IIIa IN GLANZMANN'S THROMBASTHENIA: FLOW CYTOMETRIC QUANTITATIVE ASSAY

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Glanzmann's thrombasthenia is a rare haemorrhagic disorder due to a structural defect, deficiency or dysfunction of the platelet membrane GP IIb-IIIa complex. This disorder is characterised by a prolonged bleeding time, normal platelet count and absent macroscopic platelet aggregation. The quantitative defects in Glanzmann's disease can be classified into two types: type I platelets contain less than 5%, while type II platelets contain 10-20% of the normal amount of GP IIb-IIIa. By flow cytometry we evaluated GP IIb-IIIa expression (CD41) in a patient, a 1-year old male, with a history of spontaneous lung haemorrhage and subsequent surgical bleeding, and in his family (parents and one sister). The amount of GP IIb-IIIa was evaluated using CD41 and standard microbeads (Quantum Simply Cellular- FCSC) to quantify the antibody binding capacity (ABC). The patient had low expression of GP IIb-IIIa, showing type II Glanzmann's thrombasthenia. The sister had a normal amount of GP IIb-IIIa (comparable with the control). Both the parents had normal platelet function but reduced expression of the glycoprotein, showing the defect to be heterozygous. This assay is useful for accurate evaluation of GP IIb-IIIa expression on platelet surface and for a consequent diagnosis of homozygous or heterozygous carriers of the defect.

PO-270

P-SELECTIN EXPOSURE ON PLATELETS OF PATIENTS WITH ABSENT OR BLOCKED GPIIb-IIIa

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Platelet GP IIb-IIIa antagonist therapy has long been used to prevent ischemic complications of per-

cutaneous coronary interventions. The aim of our study was to measure the exposure of P-selectin (an α -granule membrane protein) on platelet surface in the presence of abciximab (ReoPro), the Fab fragment of a mouse/human chimaeric version of the murine 7E3 antibody. We studied whole blood samples obtained from 10 healthy volunteers before and after *in vitro* treatment with 2.5 mg/mL of abciximab, from 5 patients before and after abciximab therapy and from 3 patients with Glanzmann's thrombasthenia. P-selectin exposure was evaluated by flow cytometry using CD62 (CLB-Amsterdam), in basal conditions and after *in vitro* stimulation by TRAP (thrombin receptor activating peptide) and a stable thromboxane analogue (U46619-Sigma). P-selectin was measured as antibody binding capacity (ABC, absolute quantification of the number of antibodies bound per cell) using Quantum Simply Cellular (FCSC - San Juan, PC USA). Platelets of patients with Glanzmann's thrombasthenia showed more exposure of P-selectin than platelets of healthy subjects or platelets of patients before treatment with abciximab. After abciximab treatment *in vivo* or *in vitro* P-selectin exposure on platelet surfaces was greater in both patients and normal subjects.

PO-271

THE ASSOCIATION OF OCTREOTIDE AND OF AN OESTROGEN-PROGESTERONE COMBINATION IN THE MANAGEMENT OF LONG-LASTING SEVERE GASTROINTESTINAL BLEEDING IN AN OLD PATIENT WITH MULTIPLE MUCOSAL VASCULAR ABNORMALITIES AND GLANZMANN'S THROMBASTHENIA

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A 75-yr old woman with type I Glanzmann's thrombasthenia and a history of recurrent bleeding was admitted because of persistent melaena and severe anaemia, requiring transfusion of >20 packed red cell units (PRCU)/mo. Gastrointestinal endoscopic examination showed multiple small diffuse angiodysplastic lesions. Electrocoagulation of several bleeding lesions and s.c. octreotide administration did not change her transfusion requirements. Nor were i.v. conjugated oestrogens (in combination with octreotide) and selective mesenteric embolisation of relevant jejunal vessels effective. After 8 mo. of continuous transfusions, during a life-threatening bleeding episode, a 3-day i.v. octreotide infusion was given, followed by t.i.d. s.c. administration, and an oestrogen-progesterone combination (0.035 mg ethinyl-estradiol+1 mg norethisterone) was started. This led to a progressive correction of patient's haemoglobin values and to persistent negativity of the stools. No transfusion was required in the following 9 mo. After withdrawing octreotide (the patient did not tolerate

multiple daily injections) and changing the hormone combination (ethinylloestradiol 0.030mg+ norgestrel 0.15 mg; the previous combination not being available in Italy), bleeding again became relevant, with a transfusion requirement >10 PRCU/mo. A new octreotide i.v. treatment and the previous hormone combination were administered, with reduction of bleeding and transfusion requirement. In a patient with Glanzmann's thrombasthenia these data suggest that mucosal vascular abnormalities in the elderly are often a major condition predisposing to bleeding and document reduction/cessation of gastrointestinal bleeding accompanying the use of octreotide and an oestrogen-progesterone combination. Consistent with the higher potency of synthetic oestrogens, only the latter were helpful in this setting and a powerful contribution of a progesterone with androgenic properties may be hypothesised.

PO-272

NEONATAL ALLOIMMUNE THROMBOCYTOPENIA: DO WE NEED A HUMAN PLATELET ALLOANTIGEN (HPA) GENETIC BANK?

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Neonatal alloimmune thrombocytopenia (NAIT) is a rare syndrome, occurring in about 1 out of 5000 newborn infants, as a result of maternal alloimmunisation against a foetal platelet specific antigen for which the mother is antigen-negative. At present, five (1-5) major distinct diallelic (a, b) human platelet alloantigen (HPA) systems have been established. The most common antibodies responsible for NAIT among Caucasians are anti-HPA-1a (anti-PL^{A1}) and anti-HPA-5b (anti-Bra). We report a case of NAIT due to alloimmunisation against HPA-1a, after the third pregnancy of a 31-year old woman whose first pregnancy ended with neonatal to death and whose second pregnancy was complicated by severe neonatal thrombocytopenia. The female infant showed severe thrombocytopenia (platelet count 7.000/mm³ during the first day), APGAR score 9 and 8 after 1 and 5 minutes, respectively, and petechiae immediately after delivery. Anti-HPA-1a antibodies were detected in the maternal serum tested against a panel of platelet antigens. This finding was confirmed by monoclonal antibody immobilisation of platelet antigen (MAIPA) assay. Furthermore, cross matching with maternal serum and paternal platelets was strongly positive. HPA 1 genotyping by restriction fragment length polymorphism (RFLP) analysis showed that her mother and father were homozygotes for HPA-1b and HPA-1a, respectively, and that, as expected, the propositus had HPA-1a/b genotype. In the serum obtained from a blood sample of umbilical vein, anti HPA-1a antibodies were found. Three compatible donors with HPA-1a/b genotype were

chosen from our HPA genetic bank, no HPA-1b/b donor being found in our file. In addition to platelet concentrates from the selected donors, the infant also received a 5-day course of i.v. immunoglobulins (400mg/kg/d) and high-dose prednisone (10 mg/d). At discharge, 18 days later, her platelet count was 22.700/mm³. Our report strongly support the need for HPA genetic banks in clinical conditions such as NAIT, post-transfusion purpura and refractoriness to platelet transfusion, in which quickly available transfusions from compatible donors are the main therapeutic approach.

PO-273

IDIOPATHIC THROMBOCYTOPENIC PURPURA WITH FUNCTIONAL PLATELET ABNORMALITIES IN A PATIENT WITH ANTI-HPA5 ANTIBODIES

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Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterised by increased platelet destruction by antiplatelet autoantibodies. Most patients usually have antibodies against specific platelet membrane glycoproteins (GP), primarily GP IIb/IIIa and/or GP Ib/IX and, less commonly, GP Ia/IIa. We report the case of a 32-year old woman with refractory ITP, unresponsive to corticosteroid treatment and to splenectomy (platelet count 50.000-80.000/mm³ 3 months later). In this patient antiplatelet autoantibodies were persistently positive and bleeding time was prolonged (>15 min). In addition, reduced *in vitro* platelet sensitivity to collagen (AC50 3 mg/mL) was detected, that was only in part corrected by the addition of normal plasma. The elution from platelet surface and characterisation of antiplatelet antibodies by commercial plates coated with specific platelet glycoproteins (HPA-1, HPA-2, HPA-3, HPA-5), allowed identification of IgG antibodies against HPA-5 (GP Ia). This did not show the Br polymorphism, the second most common cause of alloimmune thrombocytopenia, by PCR amplification and RFLP analysis. Cytofluorimetric analysis showed a normal panel of the major surface platelet glycoproteins. HPA-5 (GP Ia) is one of the two chains of the platelet collagen receptor; therefore platelet functional abnormalities in this patient are likely to reflect an interference by anti-HPA5 IgG. Such antibodies, uncommonly found in ITP, are probably responsible for the autoimmune thrombocytopenia.

PO-274

HIGH SHEAR STRESS INDUCES NITRIC OXIDE SYNTHESIS IN HUMAN PLATELETS

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Nitric Oxide (NO) is a powerful vasodilator and a platelet inhibitor produced by endothelial cells. Elevated shear stress, produced by accelerated blood flow at sites of arterial stenosis, is a powerful stimulus for the production of NO by endothelium. Platelets contain constitutive NO synthase and several stimuli (collagen, ADP, thrombin) can activate the metabolic machinery producing NO in platelets. No data, however, are available on the effects of shear stress on platelet NO synthase not it is known whether NO can limit platelet activation induced by high shear. We evaluated the effect of shear stress on platelets and the modulatory role of NO on it by using the filtration method of O'Brien. When human, hirudin-anticoagulated whole blood was preincubated with L-Arginine (600µM), the metabolic substrate of NO-synthase, and then forced through a Pall U100 filter, platelet activation was delayed as compared with control (closure time = 66.7±6.3 sec vs 51.2±4.8 sec [p<0.05], retained platelets 20-40sec.=72.9±6.5% vs 79.2±6.2% [p<0.05]). In contrast, preincubation with L-NMMA (600µM), an antagonist of NO-synthase, enhanced platelet activation (closure time=38.7±4.8 sec vs 51.2±4.8 sec [p<0.05]; retained platelets 20-40sec.=84.7±6% vs 79.2±6.2% [p<0.05]). In order to ascertain that the effects of shear stress in our system were exerted on platelets, we performed a series of experiments with platelet-rich-plasma (270 x 10³plts/µl) anticoagulated with hirudin. Preincubation with L-Arg (30µM) totally abolished platelet activation induced by high shear (closure time=>120 sec vs 55.4±7.5 sec [p<0.001]; retained platelets 20-40 sec= 44.5±20.1% vs 54.4±15%); while L-NMMA (120µM) enhanced it (closure time: 42.5±5 sec vs 55.4±7.5 sec. [p<0.05]; retained platelets 20-40 sec= 73.7±13.8% vs 54.4±15% [p<0.01]). L-NMMA (60µM) co-incubated with L-Arg (30µM) neutralised the inhibitory effect of the latter on platelet activation (closure time = 51.7±5.8 sec vs 55.4±7.5 sec; retained platelets 20-40 sec= 56.1±9.4% vs 54.4±15%), demonstrating that the antiplatelet effect of L-Arg is the consequence of NO production. ODQ (10µM), an inhibitor of soluble guanylate cyclase, the enzyme activated by NO, slightly but significantly potentiated shear stress induced activation (closure time = 41.7±7.6 sec vs 55.4±7.5 sec [p<0.05]; retained platelets 20-40 sec= 66.3±12.3% vs 54.4±15%). In conclusion, shear stress induces the release of NO by human blood platelets; platelet-released NO can limit shear stress-induced platelet activation. These observations may be of relevance for the understanding of the modulation of platelet activation in stenotic arteries and may contribute to explain the enhanced susceptibility to thrombosis in patients with reduced NO-synthase activity (e.g. diabetics).

PO-275**HELLP: NADIRS AND ZENITHS**

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Introduction. About 4-12% of pregnant women with severe pre-eclampsia develop a multisystem syndrome characterized by haemolysis, elevated liver enzymes and low platelets (HELLP). According to the lowest observed perinatal platelet count HELLP is classified into 3 groups: Class 1 <50,000, Class 2 >50,000 <100,000, Class 3 >100,000 <150,000. Increased values of LDH, ALT and AST are intrinsic to the diagnosis of HELLP, whereas the presence of alterations in coagulation parameters, suggesting an acquired coagulopathy, is more debated. The aim of our study was to evaluate the highest (zenith) or the lowest (nadir) observed perinatal values of biochemical and coagulation parameters in relation to the HELLP class. **Materials and Methods.** Between 1991 and 1999 23 women were consecutively admitted to our Institution and diagnosed as having HELLP syndrome. Eleven were class 1 (47.8%), 11 class 2 and 1 class 3 (0.4%). The following parameters were evaluated for each class: LDH, AST, ALT at their zenith; D-dimer at its zenith and ATIII at its nadir. Mann-Whitney U test was used to look for differences between class 1 and class 2 patients. **Results.** LDH and AST zenith values are significantly higher in class 1 patients ($p < 0.05$). ALT values, although higher, do not reach a level of significance. D-dimer zenith values were higher in class 1 ($p < 0.05$) and ATIII nadir values were lower in class 1 patients ($p > 0.05 < 0.1$). **Conclusion.** Our data show that the platelet-nadir-based classification of HELLP syndrome is mirrored, in a highly significant way, by the behaviour of the biochemical parameters on which the diagnosis is based. The differences in the behaviour of D-dimer and ATIII between class 1 and class 2 patients are consistent with the occurrence of an acquired coagulopathy as a part of the HELLP syndrome, at least in class 1 patients.

PO-276**COMPARISON BETWEEN THE UPTAKE AND RELEASE OF SEROTONIN AND A PH-SENSITIVE DYE ACRIDINE ORANGE IN HUMAN PLATELETS**

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It is well known that a relatively high concentration of serotonin (5HT) is contained in the dense granules (d granules) of platelets, transported in from plasma. Its accumulation within the intact cells involves a receptor-mediated transport across the plasma membrane followed by a transport across the granular membrane. Acridine orange, which is a pH-sensitive dye driven inside the intracellular granules by a trans-

membrane proton gradient, has been previously used for monitoring the accumulation and release of various substances in a number of cells including platelets. In the present study the cellular accumulation of the two compounds was followed in parallel, under different experimental conditions which first of all revealed a weak competition between acridine orange and 5HT. The inhibitor of the serotonin receptor carrier imipramine and the inhibitor of the vacuolar H⁺-ATPase bafilomycin reduced the uptake of both substances. Contrariwise changes in the activities of a number of protein kinases (PKs) including PKC, cAMP and cGMP-dependent PKs and tyrosine PKs, decreased the accumulation of serotonin but not that of acridine orange. The inhibitory effect on 5HT uptake appeared only partially dependent on the phosphorylation-induced inactivation of the imipramine-sensitive 5HT carrier. Bafilomycin induced a slow release of both compounds, while alkalisation of the intra-granular compartment by addition of NH₄Cl caused a rapid efflux of acridine orange but not that of serotonin. The rapid cellular release of both compounds that follows platelet activation by thrombin or other agonists was markedly decreased by the activity modulation of a number of PKs including cAMP and cGMP-dependent PKs, PKC and tyrosine PKs. Altogether these results, on the one hand useful for a better comprehension of the mechanism of the 5HT transport, on the other, indicate that caution must be adopted when using acridine orange as a tool for monitoring the cellular transport of the neurotransmitter.

PO-277**OCCURRENCE OF HELLP SYNDROME IN A PATIENT WITH PREVIOUS RECURRENT THROMBOTIC THROMBOCYTOPENIC PURPURA DESPITE ASPIRIN PROPHYLAXIS**

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Introduction. Pregnancy and post-partum are classically associated with an increased risk of thrombotic thrombocytopenic purpura (TTP) and of other disorders in which thrombocytopenia develops (pre-eclampsia, HELLP syndrome, 'incidental thrombocytopenia in pregnancy', obstetrical and medical complications and immune thrombocytopenic purpura). The link between TTP and other conditions of non-immunologic platelet destruction (eclampsia, HELLP) is not understood. The incidence of TTP in this setting is too low to make a prevision of outcome in a single patient and epidemiologic data are lacking. In a published report (Bell WR, NEJM, 1991) on 108 cases of TTP, 9 occurred during pregnancy and 5 of these women had subsequent pregnancies without complications. In contrast other papers reported recurrences of TTP in subsequent pregnancies (Williams Hematology, 5th Edition). To our knowledge there are no data published on TTP risk during pregnancy in patients with previous idiopathic TTP.

Case report. During 1999 a 28-year old woman asked to us to plan a pregnancy in spite of being informed of the risk of platelet complications: in fact in 1990 and 1995 she was cared by our staff for bouts of recurrent idiopathic TTP. The first episode was treated with 13 plasma exchanges and the second with 15 plasma exchanges and a six month course of steroid therapy. When the patient became pregnant, it was decided to give her prophylaxis with aspirin (100 mg/day) starting from 5th month of pregnancy, and continuing for 1 month after the delivery. The pregnancy proceeded without complications to the 36th week, when a classic HELLP syndrome developed. Platelets fell from 250 to 115x10³/μL, AST and ALT peaked at 290 and 415 U/L respectively, bilirubin was 1.5 mg/dL. An emergency Caesarean section was performed and a healthy baby was delivered. The puerperium was uneventful. *Conclusion.* The risk of TTP is increased during pregnancy, but it is not known whether patients with recurrent idiopathic TTP are at increased risk of other thrombocytopenic disorders or whether they should receive some kind of prophylaxis.

PO-278
**ESSENTIAL THROMBOCYTHAEMIA IN YOUNG ADULTS:
 A STUDY OF 68 PATIENTS**

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Although essential thrombocythaemia (ET) is usually primarily considered a disorder of middle age, it has also been observed in children and in young adults. However, the real risk for thrombosis in these patients has not yet been clearly established. We report here 68 consecutive patients (28 males and 40 females, median follow-up 99.14 months) affected by ET diagnosed in agreement with the Polycythemia Vera Study Group criteria, younger than 40 at the time of the diagnosis and followed in our Department between 1980 and 1998. Asymptomatic ET patients were not treated. In contrast, in the patients with associated atherosclerotic risk factors, in those with microvascular disturbances and in those with a previous major arterial thrombosis aspirin (100 mg/day) was started. Only patients with major thrombotic complications and a platelet count over 1000 x 10⁹/L received cytoreductive therapy. Fifteen patients had major thrombosis, 11 of which were the presenting features of ET. No re-thrombosis has been observed. Only one patient with thrombotic complication was under efficient treatment. Atherosclerotic risk factors are more common in patients with major arterial thromboses than in asymptomatic subjects. Thirteen normal babies were delivered out of 16 pregnancies, 6 of the pregnant women were under ASA therapy. Most thromboses in young ET patients occurred at the time of the diagnosis and venous thrombotic events represent one third of total thromboses. Cardiovascular risk factors seem to be concurrent stimuli for arterial thrombosis in ET. The throm-

botic complication rate was 2.6 per 100 patients/year. Aspirin seems to reduce microvascular disturbances, thrombosis and re-thrombosis and possibly reduces obstetrical complications in ET women.

PO-279
**DISORDERS OF PLATELET FUNCTION IN PATIENTS
 AFFECTED BY GAUCHER'S DISEASE**

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Gaucher's disease type I (β -glucocerebrosidase deficiency), the most common lysosomal storage disease, is characterised by accumulation of β -glucocerebroside in the reticuloendothelial system leading to bone marrow infiltration, hepatosplenomegaly and skeletal lesions. Bleeding symptoms are the most common presenting clinical manifestation and are usually attributed to thrombocytopenia. However, even patients affected by Gaucher's disease, (GPTs), with normal platelet count can experience severe haemorrhages. Clotting factors and natural inhibitor deficiencies have been reported in GPTs. Abnormal platelet function has been recently detected in Gaucher's disease, by means of Born's aggregation tests. To characterise the disorders of platelet function better, 4 Gaucher type I patients, receiving enzyme replacement therapy, were studied. Two patients had splenectomised, some years previously. Bleeding time, platelet count, and platelet aggregation were measured by standard procedures. Platelet membrane phenotype (GpIb, GpIIb/IIIa), platelet activation markers (GMP-140, GP53) before and after stimulus (ADP+adrenaline) were studied by flow cytometry. In addition standard coagulation tests (aPTT, PT), coagulation factors (FII, FV, FVII, FVIII, FIX, FX) and coagulation inhibitors (ATIII, PC, PS) were assayed in all GPTs. Bleeding time was prolonged in 4/4 GPTs (range: 9-15 min). Thrombocytopenia was revealed in 1/4 (90x10⁹/L), other GPTs showed a normal platelet range: 150-170x10⁹/L. In 3/4 GPTs, platelet aggregation was reduced in response to collagen, ADP and adrenaline. Expression of GpIb was reduced in 2/4 GPTs, whereas GpIIb/IIIa was normal in all. Basal GMP140 was low in 1/4 GPTs and the activated form was markedly reduced in 2/4; basal and activated Gp53 was decreased in 1/4 subjects. A reduction of prothrombin complex factors, first of all of FV, was observed. The natural inhibitors were in the normal range. Similar patterns have been reported in other studies in GPTs. GPTs showed altered platelet function with lower aggregability and reduced expression of membrane glycoproteins. In conclusion, thrombocytopenia and reduced coagulation factors together with the altered platelet function (decreased expression of GpIb and low levels of activation markers GMP140 and Gp53) could explain the manifestations of serious bleeding frequently observed in Gaucher's disease.

POSTERS
Venous thromboembolism

PO-280

DERMATAN SULPHATE FOR THE PREVENTION OF VENOUS THROMBOEMBOLISM IN ONCOLOGIC SURGERY. A COST-EFFECTIVENESS ANALYSIS

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In a recent clinical trial, dermatan sulphate (DS) was found to be more effective than unfractionated heparin (UFH), but equally safe, for the prevention of deep vein thrombosis (DVT) after major surgery for cancer resection (Di Carlo *V et al. Thromb Haemostasis* 1999; 82: 30-4). On the basis of this study, a cost-effectiveness analysis was performed according to the third payer perspective, i.e. hospitals or the Italian National Health Service. Clinical event rates were extrapolated from the observed venographic DVT rates, using appropriate assumptions from the scientific literature. The economic effects of switching DVT prophylaxis from UFH to DS and the potential lives saved were assessed using a predictive decision model. The per patient cost, including the burden of residual thromboembolic events and bleeding complications, was estimated to be EUR 186 for DS and EUR 218 for UFH. With reference to a potential target population of 60,000 patients/year undergoing surgery for cancer in Italy, the total prophylaxis-associated cost was thousand EUR 11,133 for DS and 13,050 for UFH, while the potential deaths from prophylaxis failure were estimated to be 201 and 387, respectively. This represented a saving of thousand EUR 1,917 and 186 potential lives per year with the DS option. The final costs and effects were mainly sensitive to variations in the rates of DVT and pulmonary embolism (PE), and to the possible need for one extra-day of hospitalisation due to the earlier pre-operative starting of DS prophylaxis. However, DS retained its dominant position across the range of DVT/PE rates explored. Applying one extra day of hospitalisation to all the patients resulted in an additional DS-associated cost of EUR 47 per potential life saved. In conclusion, DS is more cost-effective than UFH for the prevention of postoperative venous thromboembolism in patients with cancer. If the hospital stay needs to be prolonged, then the DS option may involve a small additional cost for its greater preventive efficacy.

PO-281

INCIDENCE OF HEPARIN INDUCED THROMBOCYTOPENIA TYPE II IN MEDICAL PATIENTS TREATED WITH SUBCUTANEOUS CALCIUM HEPARIN. INTERIM ANALYSIS

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Heparin induced thrombocytopenia type II (HIT) is a potentially threatening side effect of heparin therapy. The incidence of this event in medical patients receiving heparin subcutaneously is debated. We therefore prospectively evaluated the frequency of HIT in consecutive medical patients treated with subcutaneous calcium heparin for any indication. Patients with initial abnormal platelet count, (onco)haematologic disease, receiving chemo- or radio-therapy, or presenting sepsis at entry, were excluded. HIT was suspected when Greinacher's score was higher than 2; the immunological pathogenesis and diagnosis of HIT was confirmed by means of an ELISA assay for heparin dependent anti PF4 antibodies. Three hundred patients were included. According to Greinacher's score, 12 patients had a total score higher than 2. The immunological assay was positive for anti PF4 antibodies in one case. No thrombotic events occurred in this patient. The incidence of type II thrombocytopenia was equal to 1/300 (0.3%, 95% confidence interval, 0 to 0.9%). Our results, part of a preliminary and interim analysis, show an incidence of HIT in medical patients treated with subcutaneous calcium heparin not higher than 1%.

PO-282

NOMOGRAMS FOR THE ADMINISTRATION OF UNFRACTIONATED HEPARIN IN THE INITIAL TREATMENT OF ACUTE THROMBOEMBOLISM. AN OVERVIEW

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Despite the availability of low-molecular-weight heparins, unfractionated heparin (UFH) still remains the drug of choice for the initial treatment of acute (venous and arterial) thromboembolism in many countries. When appropriately employed, UFH treatment results in a degree of efficacy and safety that is fully comparable with that obtained with the use of heparin derivatives. The use of nomograms for the intravenous or subcutaneous administration of UFH assures that virtually all patients will promptly achieve adequate levels of anticoagulation, thus decreasing the likelihood of recurrent venous thromboembolism without extra bleeding-risk. We reviewed clinical studies on the implementation and validation of UFH dosing nomograms, and attempted a quantitative analysis of their performance. According to the

results of our analysis, a statistically significantly higher proportion of patients treated on the basis of a nomogram reached a therapeutic anticoagulant level within 24 hours of treatment, as compared to patients treated following the standard practice (odds ratio, 3.6; 95% CI, 2.6 to 4.9). The rate of recurrent thromboembolic events was significantly lower for patients treated according to a nomogram (odds ratio, 0.3; 95% CI, 0.1 to 0.8), while no significant differences in terms of either major or minor bleedings were detected between nomogram patients and controls. When administering UFH to patients with acute thromboembolism, either intravenously or subcutaneously, a reasonable choice should always be a nomogram-based heparin adjustment, preferably according to body-weight.

PO-283

ELDERLY PATIENTS WITH SUSPECTED PULMONARY EMBOLISM

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Background. Pulmonary embolism (PE) is often underdiagnosed in the elderly because of the non-specific clinical presentation. **Purpose.** The aim of this study was to evidence clinical, instrumental and laboratory aspects for diagnosis of suspected PE in elderly patients. **Materials and methods.** Retrospective observational study of five years on 118,69 female and 49 male, hospitalised patients, 65-years old and over (mean age \pm SD 77.7 \pm 7.1 years) who underwent a scintigraphic lung scan for suspected pulmonary embolism. Clinical, instrumental and laboratory findings of seventy-five patients with confirmed pulmonary embolism (CPE) were compared with those of forty-three patients with unconfirmed pulmonary embolism (UCPE). For plasma D-dimer assay we used, from 1994 to 1997, the Accuclot D-dimer semi-quantitative method, Sigma Diagnostics, Germany and, during 1998 and 1999, the IL Test D-dimer quantitative method, ACL Futura, Instrumentation Laboratory, Biokit S.A., Spain. For statistical analysis we used a novel technique based on a permutational procedure that combines marginal tests of each considered variable. A p value below 0,05 was considered significant. **Results.** Dyspnoea, chest pain and all the considered signs were significantly more frequent in the CPE group. Bed rest over four days, venous insufficiency, deep vein thrombosis, obesity and stroke were the risk factors significantly more frequent in CPE group. Sinus tachycardia, incomplete right bundle block, and ST-T alterations were significantly more present in the CPE group. Cardiomegaly was the only significant more frequent chest X-ray finding in the CPE group, while echocardiography showed a significant involvement of the right ventricle in the CPE group. Mean value of plasma D-dimer in the CPE group was 1720.7 ng/mL, mean value of plasma D-dimer in the UCPE group was 787.8 ng/mL ($p < 0.01$). In 14% of CPE patients and 40% of UCPE

patients, D-dimer levels were below 500 ng/mL. Since 1998 we have performed quantitative plasma D-dimer assay in 34 patients, 26 CPE and 8 UCPE patients. Plasma D-dimer values were never below 500 ng/mL in the CPE group and in only 25% of cases of the UCPE group. Arterial blood gas analysis showed more severe hypoxaemia, arterial hyposaturation and higher alveolar-oxygen gradient in the CPE group, but there was not a more frequent respiratory alkalosis in this group. Heart failure, ischaemic heart disease and chronic obstructive pulmonary disease were the more frequent diagnoses in the UCPE group. **Conclusion.** The diagnosis of pulmonary embolism in the elderly may be underestimated because of the atypical manifestations due to comorbidity. A high clinical suspicion remains the first step for reaching a quick diagnosis.

PO-284

HIGH VS LOW FIXED DOSE UNFRACTIONATED HEPARIN IN THE TREATMENT OF ACUTE THROMBOPHLEBITIS OF THE THIGH. INTERIM REPORT OF A PROSPECTIVE RANDOMISED STUDY

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In recent reports, acute thrombophlebitis of the thigh has been shown to be associated with a substantial risk of venous thromboembolic (VTE) complications. Whether therapeutic doses of anticoagulant drugs might yield a favourable benefit-risk ratio is presently unknown. Forty-eight patients with acute thrombophlebitis of the great saphenous vein involving the above knee segment, who were free from thrombosis of the proximal deep vein system (as assessed by compression ultrasonography), were randomised to receive 12500 U or 5000 U of unfractionated heparin subcutaneously twice daily for a month. They had the ultrasound evaluation of the great saphenous vein at presentation, after 3 (\pm 1) days, one week, one and three months. In addition, they were followed clinically for 6 months. Out of the 24 patients who received low dose heparin, 5 (20.8%; 95% CI, 7.1 to 42.2) developed symptomatic or asymptomatic VTE complications as compared to none of the 24 (0%; 95% CI, 0 to 14,2%; $p < 0.05$ by Fisher exact test) who were treated with higher doses. No patient experienced bleeding events in either group. The interim results of this study suggest that in patients with thrombophlebitis of the thigh high fixed doses of unfractionated heparin are more effective than low doses for the prevention of early and late VTE complications and are not associated with an appreciable bleeding risk.

PO-285**EFFECT OF LOW MOLECULAR WEIGHT HEPARIN ON SOME MARKERS OF PLATELET AND THROMBIN ACTIVATION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASES UNRESPONSIVE TO THE STANDARD THERAPY**

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The hypercoagulable state observed in inflammatory bowel disease (IBD) might have a causative role on thromboembolic complications and on the failure of standard therapy with 5-ASA and steroids. Thus heparin treatment has been proposed in refractory patients with active disease with some favourable outcomes. The aim of this study was to evaluate the effect of low molecular weight (LMW) heparin, added to conventional therapy, on some markers of platelet and thrombin activation in relation to the clinical outcomes. *Patients.* Nine patients with active IBD - six with ulcerative colitis (UC) and three with crohn disease (CD) - were treated with sc injections of nardoparine calcium 4000 U b.d. for 60 days. Clinical assessment, performed by the CD activity index (CDAI), was recorded before and at the end of LMW heparin treatment. *Methods.* Markers of platelet activation: CD 62 (GMP-140) and CD63 (GP 53) expression was studied by flow cytometry using specific monoclonal antibodies (Immunotech SA) and calculated as a percentage of positive platelets. Markers of thrombin activation: prothrombin frag 1+2 (F1+2), and thrombin-antithrombin complexes (TAT) were detected by an ELISA technique (DADE-Behring) and antithrombin (AT) by the chromogenic method (DADE-Behring). *Results.* During LMW heparin treatment no patient experienced increased bleeding in the stools and/or reduction of blood Hb levels. At the end of the therapy, five patients with mild or moderate CDAI improved to low CDAI. Of the four patients with a low CDAI, the activity index remained stable in 3, while it worsened to mild in one patient. Before heparin CD62+ platelets were increased in six pts and CD63+ platelets in five patients. During the treatment CD62 and CD63 expression was further enhanced in five patients and reduced in three patients. Before heparin only one patient had enhanced values of both F1+2 and TAT, an other one only high TAT and three patient had low AT plasma levels. LMW heparin treatment was able to lower the enhanced F1+2 and TAT values and to increase the AT plasma levels. *Conclusions.* The efficacy of LMW heparin treatment on clinical outcome of IBD refractory to standard therapy seems to be related in only a few cases to the presence of markers suggestive of increased thrombin generation. Furthermore in some patients heparin seems to enhance the expression of platelet activation markers despite a favourable effect on CDAI.

PO-286**LOW MOLECULAR WEIGHT AND UNFRACTIONATED HEPARIN FOR PREVENTION OF VENOUS THROMBOEMBOLISM IN NEUROSURGERY: A META-ANALYSIS**

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Background. Venous thromboembolism is a common, life-threatening complication in neurosurgery, but prophylaxis with anticoagulant agents has not gained wide acceptance because of concern about intracranial bleeding. We performed a meta-analysis of controlled randomised trials on the efficacy and safety of heparin in the prophylaxis of venous thromboembolism in neurosurgery. *Methods.* Relevant trials evaluating heparin for prophylaxis of venous thromboembolism in neurosurgery were identified by Medline search, scan of meeting abstracts and scrutiny of the references of original papers and reviews. Four controlled randomised studies, of which three with low molecular weight heparin (LMWH), were included in the analysis and four uncontrolled studies were commented on in the paper. The outcome measure (observed minus expected number of events) and its variance were calculated for each single trial and then summed-up. Two-tailed p-values and 95% confidence intervals were calculated. Efficacy was assessed per protocol, safety by "intention to treat" analysis. The homogeneity of the studies was tested with the χ^2 statistic. The results were also expressed as number needed for one extra event. *Results.* One hundred and eighty-seven thromboembolic events were recorded in 827 patients (22.6%). Heparin prophylaxis resulted in a 45% relative risk reduction of venous thromboembolic events (OR 0.48, 95% C.I. 0.35 - 0.66, p=.00001). Nineteen major bleedings were recorded in 1022 patients. None was fatal. Heparin treatment resulted in a 71% relative risk increase of major bleeding (OR 1.72, 95% C.I. 0.69 - 4.27, p=ns). The number needed to treat was 7.7 (venous thromboembolism) and 16 (proximal deep vein thrombosis). The number needed to harm was 102 (115 for LMWH). *Conclusions.* Low molecular weight and unfractionated heparin are shown to be effective for prophylaxis of venous thromboembolism in elective neurosurgery without excessive bleeding risk.

PO-287**PULMONARY THROMBOEMBOLISM IN A 16-YEAR OLD WOMAN DURING INFECTIOUS MONONUCLEOSIS**

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Background. Thromboprophylaxis during acute viral infections is generally not recommended. We present the case of a 16-year old woman who developed massive pulmonary embolism during infectious mononucleosis. **Case report.** The patient was admitted to our hospital because of fever lasting more than a week. She had a history of meno-metrorrhagia for which she had started an oral oestrogen-progesterone pill four months earlier. On the 10th day of hospital stay the patient complained of chest pain. Upon physical examination she showed tachypnoea (32 breaths/min) and tachycardia (100 breaths/min). Angiography-CT scanning and a ventilation-perfusion scan were performed: these showed pulmonary embolism. Leg colour-coded duplex scans ruled out deep vein thrombosis. Heparin therapy was immediately started and the patient was then transferred to the hospital's coronary unit. After 3 days she was switched to oral anticoagulant therapy. Serological results were positive for acute infectious mononucleosis. Screening tests for thrombophilia showed: fasting hyperhomocysteinaemia (56 mmol/L), homozygous deficiency of methylenetetrahydrofolate (MTHFR), low levels of protein S activity (PS%= 26). Antithrombin III, protein C, and protein C resistance were within the normal range and antiphospholipid antibodies (lupus anticoagulant and anti-cardiolipin) were negative. Homozygous deficiency of MTHFR and hyperhomocysteinaemia were also found in her mother. **Conclusion.** Viral infections have been rarely associated with thrombotic events, mainly during varicella zoster virus infections. To our knowledge only one case of pulmonary embolism has been so far reported during Epskin-Barr virus infection, with lupus anticoagulant antibodies. This patient, along with an acute viral disease, on admission had at least two concomitant risk factors: oral oestrogen-progesterone therapy and prolonged bed rest. Due to the young age of this patient the possibility of her being at high to moderate risk for developing thromboembolism was unlikely and therefore not considered. Despite the young age this woman developed a life threatening event due to a common and generally mild disease, infectious mononucleosis. This case points to the need for more extensive use of thromboprophylaxis in "medical" patients. We believe that an acute viral disease can be considered as an event triggering thrombosis.

PO-288

HIGH INCIDENCE OF EARLY THROMBOTIC COMPLICATIONS IN PATIENTS WITH ACUTE LEUKAEMIA

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Background. The incidence of thrombotic complications in patients with acute leukaemia has been investigated mainly in patients with acute lymphoblastic leukaemia, whereas scarce information concerning the occurrence of thrombosis during

acute non-lymphocytic leukemia is available. **Patients and methods.** We investigated 228 patients (M/F 111/117, median age 58 years, range 14-89) consecutively admitted from 1993 to 1998 for acute non-lymphocytic leukaemia (ANLL, 181 cases, 19 M3) and acute lymphoblastic leukaemia (ALL, 47 cases). During a total observation time of 311 patient-years we documented 21 thrombotic events in 18 patients. (M/F 5/13, median age 51 years, range 17-84), accounting for an incidence of 6.7/100 patient-years. Diagnosis was ANLL in 14 cases (5 M3) and ALL in 4; the first event was a DVT of the legs in 11 cases (3 with pulmonary embolism), DVT of the arms in 2, portal vein thrombosis in 1, cerebral vein thrombosis in 1, ischaemic stroke in 3. Five events (28%) were associated with a circumstantial risk factor (central venous catheter, CVC=2, CVC and hormonal treatment =1, erwinase=1, puerperium=1). **Results.** Inherited thrombophilia (G20210A prothrombin) was detected in 3 patients. with DVT (2 with recurrence) out of 12 checked. In 12 thrombotic patients (67%) no inherited or acquired risk factor was identified. The Kaplan-Meier analysis showed an overall probability of thrombosis of 4.4% in patients at the onset of disease and in 8.8% within 5 months from diagnosis of acute leukaemia. Three patients with ANLL had a recurrent DVT 3, 15 and 37 months after the first event. The risk for thrombosis was similar in patients with ALL and ANLL (hazard ratio 1.03, 95% CI 0.3-3.2). In the M3 patients the probability of thrombosis was 10.5% at the onset and 27.7% within 3 months from diagnosis, with a risk higher than in the other patients (log-rank test $p=0.001$, hazard ratio 4.4, 95% CI 2.8-89.4). A leukocyte count higher than $20 \times 10^9/L$ was present in 8 cases (6 ANLL -2 M3-, and 2 ALL); in thrombotic patients the count was higher (median $20.5 \times 10^9/L$) than in ANLL pts. (median 11×10^9) or ALL patients (median 16.5×10^9). The platelet count at the time of the first thrombosis was higher than $85 \times 10^9/L$ in 9 cases; yet in M3 patients. (median $32 \times 10^9/L$) was lower than the count found at the onset of disease in ANLL patients. (median $48 \times 10^9/L$) or ALL patients. (median $53 \times 10^9/L$); the other patients with thrombosis at the time of the event had a median platelet count of $100 \times 10^9/L$. **Conclusions.** Acute leukemia is associated with a thrombotic risk especially at the onset of disease and with a normal platelet count; such risk is particularly high in M3 patients, independently of the platelet count.

PO-289

THE HYPERCOAGULABLE STATE OF BREAST CANCER AND NON-HODGKIN'S LYMPHOMA PATIENTS UNDERGOING TREATMENTS FOR AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Malignancy is associated with increased risk of thrombosis. Chemotherapy and haematopoietic

colony-stimulating factors (CSF) increment this risk. To verify whether and to what extent chemotherapy and CSF affect the laboratory signs of clotting and endothelium activation, we prospectively studied two groups of patients selected for autologous haematopoietic stem cell (HSC) transplantation: 1. patients with stage II breast cancer (BC) (n=19), within 30 days of tumour surgery; and 2. patients with high and intermediate grade non-Hodgkin's lymphoma (NHL) (n=25), 20-40 days after remission induction chemotherapy. Both groups underwent a sequential protocol of high dose cyclophosphamide (CTX) + granulocyte-CSF (G-CSF) to mobilise HSC into peripheral blood, and subsequent transplantation. Plasma markers of hypercoagulation (TAT, F1+2, D-Dimer) and endothelial perturbation (TM, vWF, and t-PA) were measured at the following time intervals: 1. at baseline, before CTX (T0), 2. after CTX, before starting G-CSF (T1), 3. at the end of G-CSF, upon apheresis (T2), 4. before pre-transplant chemotherapy regimen (T3), and 5. at HSC reinfusion (T4). The results show that, at baseline, in NHL patients all plasma parameters, and, in BC patients only hypercoagulation markers, were greater than those of healthy controls ($p < 0.05$). Of interest, all variables of the NHL group were higher than those of the BC group; TM, vWF and t-PA levels being significantly so ($p < 0.01$). At T1, after CTX, in both groups, endothelial markers significantly increased compared to the basal values, while only F1+2 levels augmented, among hypercoagulation markers. Again, the levels of TM, vWF, t-PA of NHL patients were higher than those of BC patients ($p < 0.01$). At T2, at the end of G-CSF treatment, the patients' vWF and D-dimer levels further increased compared to T0 in both groups, all the other parameters remaining unmodified. At T3, all the variables were decreased to baseline in BC patients, whereas all of them, except t-PA, were lower than the basal values in NHL patients. At T4, after the pre-transplant conditioning regimen, in both groups the levels of TAT, F1+2 and D-dimer increased ($p = ns$) compared to T3 values. vWF increased significantly only in BC patients ($p < 0.002$). Two thrombotic events occurred in the NHL group, after T2. In conclusion these data show that, at the start of the study, patients previously treated with chemotherapy, eg. NHL patients, have extensive laboratory haemostatic abnormalities, including endothelial cell activation, compared to surgery-treated BC patients. The combination of high dose CTX with G-CSF significantly worsens the endothelial cell damage. Pre-transplant chemotherapy also influences the hypercoagulable state and vWF levels. None of the pre-treatment plasma marker values was predictive for thrombosis. Prospective large clinical studies are necessary to evaluate the utility of these plasma parameters for predicting the risk of vascular complications in these patients.

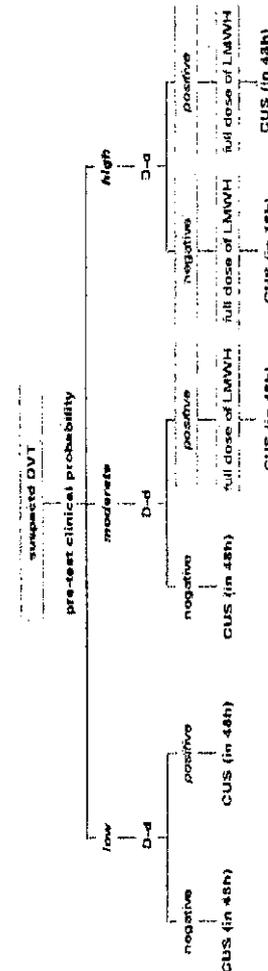
PO-290

A COMBINED APPROACH APPLIED BY EMERGENCY DEPARTMENT PHYSICIANS IN THE INITIAL MANAGEMENT OF ACUTE DEEP VEIN THROMBOSIS

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Background. The management of patients referred to the emergency department (ED) for suspected deep vein thrombosis (DVT) might be problematic since guidelines to assist ED physicians are lacking; difficulties may arise when diagnostic-imaging possibilities are not available. Because venous thromboembolism is a potentially life-threatening disease, practical algorithms should be applied for permitting ED physicians to manage acute DVT correctly and safely. **Objective.** We evaluated a simplified approach based on pre-test clinical probability (PCP) and D-dimer test (semi-quantitative Dimertest® Latex assay, Dade Behring) in 102 patients referred to the ED with suspected acute DVT; compression ultrasonography (CUS) was performed immediately or within 48h (figure):



Results. Forty-two out of 102 (41.1%) patients were classified as having high, 18 (17.6%) moderate and 42 (41.1%) low PCP, respectively. The overall prevalence of DVT was 18.6% (19/102), DVT was confirmed in 14 (31.7%), 3 (16.6%) and 2 (4.7%) patients in the high, moderate and low PCP group respectively. Forty patients (39.2%) were discharged and performed CUS within 48h; according to the algorithm, 10 of them started LMWH (enoxaparine 100 UI anti-FXa/Kg/bid). Among these, 6 patients (4 in the high and 2 in the moderate PCP group) had confirmed DVT; they were enrolled for the "home treatment program". In the remaining patients, CUS refuted the diagnosis and initial LMWH therapy was stopped. Two patients (1.9%) with high PCP were admitted to hospital before CUS results. Sixty patients (58.8%) had CUS on the same day as presentation and were managed according to the algorithm. None of patients had episodes of major bleeding or venous thromboembolism during the initial screening or after a follow-up of 3 months. **Conclusions.** Our combined approach may help ED physicians in the initial management of suspected DVT; it also allows a safe antithrombotic approach to be begun when immediate CUS performance is not possible.

PO-291

MANAGING VENOUS THROMBOEMBOLISM IN THE EMERGENCY DEPARTMENT: THE NEED FOR OBJECTIVE EVALUATION OF PATIENTS CLINICALLY SUSPECTED OF PROXIMAL GREATER SAPHENOUS VEIN (PGSV) THROMBOSIS

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Background. Superficial vein thrombosis (SVT) is rarely associated with deep vein thrombosis (DVT) and usually does not require aggressive antithrombotic treatment. Thrombosis of the proximal greater saphenous vein (PGSV) is potentially the most dangerous among SVTs because of the possible progression of the thrombus to the deep system (almost 10% of the cases) and subsequent risk of pulmonary embolism. PGSV thrombosis might therefore represent a potentially acute vascular disease that requires objective diagnosis and aggressive therapy. Currently, no specific studies have evaluated the usefulness of clinical diagnosis (CD) and D-dimer test in the management of patients clinically suspected of PGSV thrombosis. **Objective.** In this respect, we investigated the accuracy of CD and of a rapid, semi-quantitative D-d test (Dimertest® Latex Assay, Dade Behring) in 43 patients referred to the emergency department for suspected PGSV thrombosis; compression ultrasonography (CUS) was used as reference diagnostic test. **Results.** Thrombosis of PGSV was confirmed in 44.1% of patients (19/43); 3 of them (15.7%) had concomitant involvement of the common femoral vein (CFV). All patients with confirmed PGSV were

treated with LMWH (enoxaparine 100 UI/Kg/bid) for 10 to 15 days and subsequently monitored by CUS; patients with concomitant DVT were applied the protocol for home treatment. The sensitivity, specificity, and the positive and negative predictive values of the CD were 68.4%, 79.1%, 72.2% and 76%. The sensitivity, specificity, and the positive and negative predictive values of D-d were 57.9%, 91.6%, 84.6% and 73.3%. During the LMWH therapy and the following 3 months of follow-up, no cases of SVT progression to the deep system or major bleeding occurred. **Conclusions.** The need for objective tests in the diagnosis of PGSV thrombosis is commonly considered superfluous as superficial phlebitis is currently identified by clinical inspection; nevertheless, the accuracy of the clinical diagnosis has not been validated in appropriate trials. In this study, clinical judgement did not correctly diagnose more than half of patients. We believe that patients suspected of PGSV thrombosis, in whom the concomitant involvement of CVF and/or the risk of extension to the deep venous system are not negligible, deserve more attention. Compression ultrasonography should always be performed at the first visit to confirm the clinical suspicion, as the basis for detecting concomitant involvement of CFV and to monitor possible extension of the thrombus. The clinical usefulness of CD and D-d in this setting is not confirmed.

PO-292

THE ROLE OF A RAPID SEMI-QUANTITATIVE D-DIMER TEST (DIMERTEST®) IN PATIENTS PRESENTING IN EMERGENCY DEPARTMENT FOR SUSPECTED DEEP VEIN THROMBOSIS OF THE LOWER LIMBS

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Background. Patients with suspected deep vein thrombosis (DVT) of the lower limbs frequently present to a hospital emergency department (ED); because of the risk of pulmonary embolism, venous thromboembolism (VTE) represents an acute and potentially life-threatening disease. Signs and symptoms of DVT are non-specific and found in a wide variety of non-thrombotic disorders; objective tests must be performed to correctly identify patients with venous thrombosis (VT). The measurement of D-dimer (DD) is widely claimed to have potential value in excluding the diagnosis; unfortunately, the reference methods, ELISA (enzyme-linked immunosorbent assay), is time consuming, needs specific equipment and is not adapted for use in emergency wards where a rapid results is essential. New rapid methods are available and are consistent with extensive use in emergencies for critically ill patients with suspected VT, but few clinical trials have assessed their performance in this setting. **Objective.** In this respect, we evaluated the overall accuracy of a rapid semi-quantitative D-dimer test (Dimertest®, Latex Assay, Dade

Behring) in 114 patients referred to an ED for suspected DVT of the lower limbs; compression ultrasonography (CUS) was used as the reference diagnostic test. Dimertest® was performed by a technician unaware of CUS results; the test was considered positive at a value >0.2 mg/mL and the results were supplied within 45 min. **Results.** Deep vein thrombosis was confirmed by CUS in 18.4% of patients (21/114). The mean time between the beginning of symptoms and the evaluation at ED was 9.5 days (range 1-45); 19.2% (22/114) of patients were on heparin prophylaxis. When compared to CUS, the sensitivity, specificity, and positive and negative predictive values of the Dimertest® were 94.7%, 70%, 60% and 96.5%. **Conclusions.** Our findings confirm the usefulness of Dimertest® in the management at the ED of patients clinically suspected of DVT; they are also in agreement with our previous results evaluating a different D-dimer test (SimpliRED®) (Siragusa *et al.*, *Thromb Haemost* 1999:2659). However, the test cannot exclude DVT and, in view of extensive use in emergency wards, it could lead to missing some patients; moreover, the poor specificity could also lead to an increasing use of morphological tests to exclude DVT in the numerous D-dimer positive patients detected. In conclusion, Dimertest® should not be used as the only test for safely managing patients clinically suspected of DVT; it might be useful when used in combination with other approaches (pre-test clinical probability and CUS) for simplifying the management of acute DVT in emergency wards.

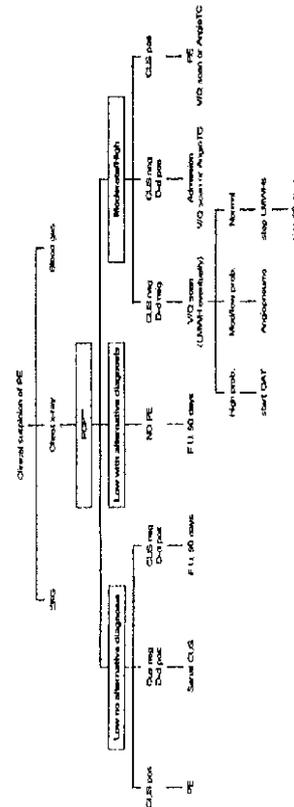
PO-293

A SIMPLIFIED APPROACH FOR THE INITIAL MANAGEMENT OF CLINICALLY SUSPECTED ACUTE PULMONARY EMBOLISM IN EMERGENCY WARDS: AN INTERIM ANALYSIS

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Background. Acute venous thromboembolism (aVTE) is a potentially life-threatening disease that requires prompt diagnosis, rapid and efficacious treatment. Signs and symptoms of haemodynamically stable pulmonary embolism (PE) are non-specific and often confused with other cardiac and respiratory disorders; objective and accurate tests must be performed. Because the diagnosis of PE can require multiple approaches, a practical algorithm should be applied to assist emergency department (ED) physicians. **Objective.** We evaluated a simplified approach based on pre-test clinical probability (PCP), D-dimer (D-d) test (semi-quantitative Dimertest® Latex Assay, Dade Behring) and compression ultrasonography (CUS) in 45 patients referred to the ED for suspected acute PE; patients were managed according to the algorithm



Results. Among the overall population, 22.2% (10/45) and 13.3% (6/45) of patients were classified as having low PCP with or without alternative diagnosis, respectively; 64.4% (29/45) of patients had high/moderate PCP. After a follow-up of 3 months, the prevalence of confirmed PE was 22.2% (10/45); 6 patients, with moderate/high PCP had a positive CUS at the time of the first evaluation. The sensitivity, specificity, positive and negative predictive values of D-d were 90%, 83.3%, 64.2% and 96.1%, respectively. In 16 patients (35.5%) this approach ruled out the diagnosis avoiding inappropriate admission to the hospital; in 6 patients (13.3%) CUS confirmed the diagnosis avoiding further examinations. No cases of recurrent VTE or death occurred. **Conclusions.** Management of patients presenting at the ED for suspected PE might be problematic; our diagnostic approach may help ED physicians to reduce the performance invasive diagnostic tests and inappropriate hospitalisation.

PO-294

FLOW CYTOMETRIC EVALUATION OF PLATELET ACTIVATION IN HEPARIN-INDUCED THROMBOCYTOPENIA

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Heparin-induced thrombocytopenia (HIT) is a known cause of drug induced thrombocytopenia

characterised by platelet activation. It has been demonstrated that patients affected by this immunomediated disease have antibodies (IgG) directed against heparin-platelet factor 4 (PF4) complex. The antibodies and PF4/heparin immunocomplexes bind the surface of platelets and induce platelet activation through the platelet Fc receptor. The aim of this study was to identify HIT and investigate whether different types of heparin may have a different effect on platelet activation. To detect platelet activation we evaluated, by flow cytometry, the expression of P-selectin (CD62, CLB-Amsterdam) on the surface of donor's platelets incubated with plasma derived from HIT patients in the presence of three different types of heparin: unfractionated and two different low-molecular-weight heparins. Donor's platelets showed different levels of CD62 in presence of the three types of heparin demonstrating a different degree of activation. Our results suggest that this functional method, sensitive and specific, may support HIT diagnosis and is useful to verify safety of the therapy.

PO-295 **NEGATIVE PREDICTIVE VALUE OF D-DIMER IN THE DIAGNOSIS OF DEEP VEIN THROMBOSIS**

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We studied 159 (age 24-90 years, mean 63, median 68) consecutive patients presenting at the Emergency Department of Valduce Hospital in Como with clinically suspected deep vein thrombosis (DVT). All patients underwent D-dimer test (Sta Liatest-Roche), colour Doppler (CD) of lower limbs and were categorised as being at low, moderate or high pre-test probability, using the Wells clinical model. Colour Doppler was performed from iliac to calf veins with an abdominal pediatric probe at 5 MHz. D-dimer values ranged from 0.04-17.4 mg/mL. CD was negative in 39 (25.5%) and positive in 120 (75%) patients, of whom 46 (38.3%) had calf DVT. In the CD negative subjects DVT pre-test probability was high in 49%, moderate in 36%, low in 15%. In patients with DVT, pre-test probability was high in 58%, moderate in 25% and low in 17%. A DVT was found in 79% of 89 patients with a high pre-test probability, in 68% of 44 with a moderate probability and in 77% of 26 with a low probability. D-dimer <0.5 was found in 35 patients: 15 were affected with DVT, of which 11 (73.3%) were calf vein thromboses; there were only patients with proximal DVT and D-dimer <0.5 (5.4% of all patients with proximal DVT). Of all these 15 patients, 10 (67%) had a high pre-test probability, 2 (13%) moderate and 3 (20%) low. The D-dimer test in our patients had an overall sensitivity of 87.5%, specificity of 51.3% and negative predictive value of 57.1%. However, if we exclude calf DVT (as CD negative) the negative predictive value increased to 88.6%. Of all patients, 104 had symptoms <10 days from the time of diagnosis: 75 (72%) had DVT of

whom 8 (6 calf DVT) with D-dimer <0.5. Also considering D-dimer test significant only in these patients the negative predictive value was 65%, but if we consider calf veins DVT as CD negative the negative predictive value increased to 91%. In conclusion in our experience the D-dimer test can not exclude CD for the diagnosis of DVT and the association with pre-test probability model does not reach sufficient accuracy. Therefore CD must be recommended for the diagnosis of DVT.

PO-296 **DERMATAN SULPHATE (DS) IN A ALL CHILD WITH HEPARIN-INDUCED THROMBOCYTOPENIA (HIT) AND UPPER LIMB DEEP VEIN THROMBOSIS**

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HIT type II is probably the most common clinically significant allergic side effect to a drug that physicians face today. HIT type II is rare in children and only few case reports have been published. No data are available concerning an anticoagulation strategy alternative to heparin in children with HIT type II. DS, a selective inhibitor of thrombin, has been successfully employed in adults with thromboembolic disease who developed HIT type II. We report the first case of HIT type II treated with DS in a child. To a 6 year-old girl affected by common ALL was inserted a percutaneous subclavian central venous catheter immediately after diagnosis and prior to chemotherapy (AIEOP LAL 95 02). Approximately one month after the insertion of the CVC, the patient presented swollen right upper limb and was admitted to the hospital. Physical examination confirmed a swollen right upper limb and showed a collateral vein circulation. Compressive ultrasonography was performed on the same day and it did show a venous thrombosis of the omegal, axillary subclavian veins of the right arm. Heparin treatment was immediately started by an intravenous infusion (starting with 10.000 U/24h then adjusting the dose to maintain an aPTT to 1.5 times than normal value). Before heparin administration, the platelet count was 151,000. On the fifth day of heparin therapy, platelet count fell to 33,000. Unfractionated heparin was stopped and replaced by the low molecular weight heparin, enoxaparin 2.000 U t.i.d. and CVC was removed. Two days after enoxaparin administration, platelet count was $22 \times 10^9/L$. An aggregation test confirmed platelet reactivity with unfractionated heparin and showed cross-reactivity with three low molecular weight heparins, including enoxaparin but not with DS. DS (Mediolanum Farmaceutici, Milan, Italy) was started immediately as a continuous intravenous infusion of 15 mg/kg/day and was continued for 17 days at the same dose, targeting an aPTT ratio of 1.5. Anticoagulation with DS was very stable and minor dose

adjustments were required. Platelet count began to recover on day 5 and increased up to 181,000 in few days. No bleeding or other side effects were observed. Serial testing with compression ultrasonography reveals the recanalization of the subclavian vein after about one month. In conclusion, in this young patient with HIT type II DS was effective in providing a safe anticoagulation and prompted a recovery of the platelet count. This agent should be considered in patients, including children, with HIT type II and cross-reactivity to low molecular weight heparin.

PO-297

THROMBOLYSIS VERSUS HEPARIN FOR TREATMENT OF PULMONARY EMBOLISM: A CLINICAL OUTCOME-BASED META-ANALYSIS OF CLINICAL TRIALS

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Background. In patients with acute pulmonary embolism, thrombolytic treatment results in a more rapid resolution of pulmonary emboli and hypertension than heparin treatment. However, no advantages have been demonstrated in terms of reduction of adverse clinical outcome events. **Methods.** The aim of this study was to perform a meta-analysis of clinical trials in pulmonary embolism comparing thrombolytic and heparin treatment with respect to the occurrence of adverse clinical outcome events such as death, recurrence of pulmonary embolism and major bleeding. **Findings.** Overall, 524 patients were included in 11 trials. Sixty of 283 (21.2%) patients treated with thrombolytic agents experienced an adverse outcome event compared to 60 of 241 (24.9%) patients treated with heparin (relative risk 0.85, 95% C.I. 0.54 - 1.22). In the thrombolysis group, 12 (4.2%) patients died compared to 18 (7.5%) patients treated with heparin (relative risk 0.57, 95% C.I. 0.27 - 1.16). A recurrence of pulmonary embolism occurred in 14 of 214 (6.6%) analysable patients treated with thrombolysis and in 22 of 201 (10.9%) analysable patients treated with heparin (relative risk 0.60, 95% C.I. 0.29 - 1.15). When death and recurrence were considered together, a relative risk of 0.57 (95% C.I. 0.31 - 0.89, $p=0.015$) was observed. Thirty-four patients of the 256 (13.3%) analysable thrombolysis patients had a major bleeding, compared to 21 of the 228 (9.2%) analysable heparin patients, (relative risk 1.44, 95% C.I. 0.85 - 2.65). **Interpretation.** The meta-analysis of comparative studies in pulmonary embolism showed that death and recurrences are reduced by thrombolysis with respect to heparin. Excessive bleeding is the trade-off for improved efficacy. A comparative clinical outcome trial of thrombolysis and heparin in patients with pulmonary embolism is highly warranted and it should not be further delayed.

PO-298

PREDISCHARGE PLASMA LEVELS OF SOLUBLE FIBRIN POLYMERS (TpPTM) CORRELATE WITH THE DEVELOPMENT OF DEEP VEIN THROMBOSIS (DVT) AFTER ELECTIVE NEUROSURGERY

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Background. Postoperative deep vein thrombosis (DVT) is most often asymptomatic but it can be followed by fatal pulmonary embolism. Non-invasive objective (i.e. ultrasonography) tests have a reduced sensitivity in the diagnosis of post-operative DVT. Blood tests may have a potential role in the screening of asymptomatic DVT and thus in the prevention of pulmonary embolism. **Aim of the study.** To evaluate whether a correlation exists between the pre-discharge levels of soluble fibrin polymers, as determined by an enzyme immunoassay (TpPTM, Thrombus precursor Protein), and the development of DVT after elective neurosurgery. **Patients and methods.** 162 neurosurgery patients were randomized, on the day of surgery to receive at least 7 day of enoxaparin, 40 mg o.i.d., or placebo, as prophylaxis for venous thromboembolism. All patients wore compression stockings. Patients underwent bilateral venography on day 8±1. Blood samples for TpP measurement were withdrawn immediately before venography. TpP is an ELISA assay that adopts a monoclonal antibody against a conformational epitope of soluble fibrin polymeric structure. **Results.** Forty-two patients had a DVT (25.9%) that was proximal in 14 (8.7%) and isolated distal in 28 patients (17.3%). DVT was asymptomatic in all patients. Mean ± SD SFP value in patients with and without DVT was 4.4±1.6 and 2.7±1.1 µg/ml ($p<0.001$). Mean post-operative SFP level in patients with proximal and isolated distal DVT was 5.5±2.8 and 3.8±2.4 µg/ml, respectively ($p=0.04$). Moreover, using the cut-off points of 2 and 4.5 µg/ml, patients were stratified as low (13.7%), intermediate (25.4%) and high (56.7%) risk for DVT. **Conclusions.** Our findings indicate that elective neurosurgery patients with asymptomatic DVT have an increased pre-discharge TpP level than patients without DVT. Pre-discharge assay of soluble fibrin polymers may be helpful in the screening of post-operative DVT. Larger studies are required to define whether the assay of soluble fibrin polymers can be used to make clinical decisions.

PO-299

VENOUS THROMBOEMBOLISM AFTER CAESAREAN SECTION: A PROSPECTIVE COHORT STUDY

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Introduction. Venous thromboembolism (VTE) has been reported to complicate pregnancy and puerperium: the estimated incidence of VTE is between 1-2 each 1000 deliveries in most recent surveys, being higher in older series. This incidence is about 5-10 greater than in healthy, non pregnant, age-matched women. Pregnancy itself is characterised by an hypercoagulable condition, related to increase of some coagulation factors, decrease of natural inhibitors (protein S, and acquired aPC-R) and reduction of fibrinolytic activity. About half of VTE episodes occur after delivery, in the puerperium. Concurrent factors might be a Caesarean section and general anaesthesia. **Aims.** To evaluate the incidence of symptomatic VTE (deep venous thrombosis, pulmonary embolism), within 45±10 days after delivery in a cohort of 1,050 consecutive patients who underwent Caesarean section (both elective and emergency, under regional or general anaesthesia). **Methods.** In the period February- July 1999, in Ospedale S.Anna (a 400 bed Ostetrical-Gynaecological institution with six maternity units and more than 7,000 deliveries/year), 1,050 consecutive patients who underwent Caesarean section were enrolled. Patients were phoned within 2 months and asked a few simple questions regarding their health condition after discharge. In any case of positive or doubtful answer, the patient was called for a complete clinical evaluation and for an objective diagnosis. Diagnoses were made using ultrasonography and lung scanning. **Results.** A total of 1,045 patients answered our phone call (drop-out 0.47%); 31 patients had clinical evaluation for suspected VTE (2.8%); 3 out of 1045 patients had clinical VTE, all in a distal site. No clinical pulmonary embolism was detected. Two out of the 3 patients with VTE were known carriers of a thrombophilic defect, and had already had a previous VTE. **Comment.** Caesarean section carries a low incidence of symptomatic VTE. A major risk factor seems to be to a previous thromboembolic event associated with a thrombophilic defect.

PO-300 AN UPDATE OF VENOUS THROMBOEMBOLIC SIDE EFFECTS FROM THE ITALIAN BREAST CANCER CHEMOPREVENTION TRIAL

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The Italian Breast Cancer Chemoprevention Trial aims to establish the benefit and safety of tamoxifen, 20 mg/day for 5 years, for the primary prevention of breast cancer. Started in 1992, the trial (double-blind randomised controlled vs. placebo) has enrolled 5408 hysterectomised women, 2700 in the tamox-

ifen group vs. 2708 in the placebo group, corresponding to 18375 women/year of treatment (9,031 in tamoxifen vs. 9,344 in placebo) at 31/1/2000. Tamoxifen is associated with an increased risk of venous thromboembolism (VTE) when used as an adjuvant drug in the treatment of breast cancer; for this reason a survey of cases of VTE has been included among the secondary end points of the trial. To date the overall number of venous thromboses is 76; 48 in the tamoxifen group vs. 28 in the placebo group; we have recorded 16 deep vein thrombosis-pulmonary embolism (DVT-PE) (8 vs. 8), 50 cases of superficial phlebitis (35 vs. 15) and 10 "other" thromboses (5 vs. 5), i.e. not described otherwise. The following table summarises the data of incidence of VTE and relative risk at 31/1/2000 compared with those at 31/7/1998:

	Trial		Tamoxifen		Placebo		Relative Risk	
	1998	2000	1998	2000	1998	2000	1998	2000
DVT-PE	0.9	0.8	1	0.9	0.8	0.8	1.25	1.1
Phlebitis	3	2.7	4.5	3.8	1.5	1.6	3*	2.4*
Other	0.6	0.5	0.7	0.5	0.5	0.5	1.4	1
Overall	4.4	4.1	6.1	5.3	2.8	3	2.2*	1.8*

*emphasizes statistical significance for the difference between tamoxifen and placebo

Comments. VTE is still more frequent in the tamoxifen group (RR 1.8, p=0.02, 95% C.I. 1.1-2.95), mainly because of higher incidence of phlebitis (RR 2.4, p=0.005, 95% C.I. 1.3-4.43), while the incidence of DVT-PE in the two groups is similar. Overall incidence of VTE in the trial is about 4/1000, in agreement with previous data. Tamoxifen confirms its association with VTE also when used in a chemopreventive setting; careful screening for a history of previous VTE and for circumstantial and constitutional risk factors seems warranted in women eligible for this treatment. Thus the benefit of tamoxifen as a chemopreventive drug should be evaluated balancing the extent of the expected reduction in breast cancer and coronary artery disease risk factors with the rate of the expected major side effects.

PO-301 LOCO-REGIONAL THROMBOLYSIS IN THE TREATMENT OF CEREBRAL VENOUS AND SINUS THROMBOSIS: REPORT OF TWO CASES

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Although evidence of effectiveness has emerged only from a single case-control study (Einhaupt, Lancet,1991), heparin therapy in the treatment of cerebral venous and sinus thrombosis (CVST) is nowadays considered the best available treatment

(Bousser, Stroke, 1999). Alternatively, loco-regional thrombolysis with urokinase (Horowitz, 1995) or rtPA associated with heparin (Kim, Am J Neuroradiol, 1997; Frey, Stroke, 1999) has been proposed in substitution of the anticoagulant therapy. A more frequent and rapid canalisation seems to occur during the thrombolytic treatment with respect to heparin, although a slight increase of haemorrhagic risk and no significant benefits on the clinical evolution, have been observed. Two CVST cases treated with loco-regional thrombolysis in association with intravenous heparin, are herein described. Patient 1. A 49-year old female, after three days of headache and vomiting, was hospitalised because of coma (Glasgow Coma Score-GCS 5). The CAT scanning documented a hypodense area in the left temporo-parieto-occipital region. The thrombosis of the upper sagittal sinus, straight sinus, transverse sinus and left jugular were revealed by the angiographic study. The patient's critical clinical conditions suggested a loco-regional thrombolysis with urokinase (200,000 IU bolus + 60,000 IU/h for 24 hours) followed by i.v. heparin. The patient conditions rapidly improved without evident residual deficits. Patient 2. A 50-year old female undergoing hormonal replacement oestrogen-progesterone therapy was hospitalised because of coma (GCS 8). Hypodense areas in both thalami were detected by CAT scanning. The angiographic investigation revealed a thrombosis in Rosenthal's vein, Galen's vein and the straight sinus. The patient was treated with i.v. heparin (5,000 IU bolus + 25,000 IU/day), the PTT was maintained twice the normal value. After 8 days of heparin treatment, no clinical improvement was observed. Therefore local thrombolysis was applied (150,000 IU bolus + 65,000 IU/h for 24 hours) in connection with the i.v. heparin supply. The patient's clinical conditions rapidly improved within a few days and no residual deficits remained. In our opinion loco-regional thrombolytic therapy combined with intravenous heparin would represent a useful alternative to the treatment based on just heparin during the following circumstances:

- critical clinical conditions and risk for life;
- ineffectiveness of heparin therapy.

PO-302

NORMALISATION RATES OF REAL TIME B-MODE COMPRESSION ULTRASONOGRAPHY IN PATIENTS WITH A FIRST EPISODE OF ACUTE DEEP VEIN THROMBOSIS

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One hundred and seventy-nine patients with a first episode of symptomatic deep vein thrombosis (DVT) of the lower limbs (38 with cancer) and 104 patients with DVT occurring after hip replacement surgery were serially monitored by real time B-mode compression ultrasonography (C-US) over a period of 12 months (month 1,3,6 and 12). C-US normalisation of popliteal and femoral venous segments was arbi-

trarily assigned with residual thrombus occupying, at maximum compressibility, less than 40% of the vein area calculated in the absence of compression. In patients with no DVT recurrence and/or new thrombosis, C-US normalisation was observed at 12 months in 100% of 99 patients with post-operative DVT, in 59% of 134 cancer-free symptomatic DVT patients and in 23.3% of 30 symptomatic DVT patients with cancer ($p=0.0001$). Predictors of C-US normalisation were evaluated 1, 3, 6 and 12 months after the index DVT. A significant negative effect on the risk of C-US normalisation was observed for increasing age ($p<0.05$). Patients with postoperative DVT ($p\leq 0.017$), with only one venous segment (popliteal or femoral) affected by DVT ($p\leq 0.05$), or free of cancer ($p\leq 0.05$) had a greater risk of C-US normalisation. DVT recurrence and/or new thrombosis was observed in 5 patients with postoperative DVT (4.8%), in 7 cancer-free patients with symptomatic DVT (5.0%) and in 8 patients with cancer (21.1%). Only 4 of these patients had shown normalisation of their index DVT prior to the event. When accounting for events occurring within 3 months from the index DVT, the presence of cancer was the only significant predictor of DVT recurrence and/or new thrombosis (OR=4.90, $p=0.002$). The absence of previous C-US normalization was the only predictor of recurrence and/or new thrombosis occurring after 3 (OR=5.66, $p=0.027$) and 6 months (OR=5.26, $p=0.007$) from the index DVT. Cancer-free patients with no C-US normalization at 3 or 6 months after the index DVT had an 11.6-fold ($p=0.02$) and 11.3-fold ($p=0.002$) greater probability of suffering recurrence and/or new thrombosis than cancer-free patients with C-US normalisation. Absence of C-US normalization appears to be a factor favouring the onset of DVT recurrence and/or new thrombosis in patients with a first episode of DVT. Because the optimal duration of oral anticoagulant treatment in venous thromboembolism is still controversial, C-US normalisation may represent an additional factor affecting the individual tailoring of oral anticoagulant treatment.

PO-303

THE COMBINED USE OF ILOPROST AND DANAPAROID IN THE PERIOPERATIVE MANAGEMENT OF A PATIENT SUBMITTED TO THROMBOENDARTERECTOMY FOR CHRONIC THROMBOEMBOLIC MAJOR VESSEL PULMONARY HYPERTENSION

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Pulmonary thromboendarterectomy (PTE), currently performed on patients with chronic thromboembolic pulmonary hypertension (CTPH), requires heparin as anticoagulant in the extracorporeal circulation system. This represents a major contraindication for patients with heparin-induced thrombocy-

topenia (HIT). RZ, a 70 year old female patient had a recent history of deep vein thrombosis (DVT), pulmonary embolism, and HIT. Her platelet count dropped to $3.0 \times 10^3/\mu\text{L}$ during heparin treatment. Ten months later, the patient was admitted to our institution for dyspnoea at rest. Right heart catheterisation: pulmonary artery pressure (PAP)=69/16/30 mmHg, pulmonary vascular resistances (PVR)=770 dyne/sec/cm⁵, cardiac output (CO)=2.9 L/min; lung scan: absence of perfusion of the whole right lung and ventilation/perfusion mismatch at the left basal segment. Pulmonary angiography demonstrated the presence of thromboembolic material in the proximal arteries, confirming the diagnosis of CTPH and surgical eligibility. On February 10th, 1998 the patient was submitted to PTE. We decided the patient should have surgery under infusion of a prostacyclin analogue (Iloprost). For anticoagulation in the immediate post-operative period, we decided to utilise sodium danaparoid (Orgaran), a mixture of heparan sulphate and dermatan sulfate known to be safe as an anticoagulant in HIT patients. The day of surgery, 3.0 to 48.0 ng/Kg/min of Iloprost were infused at progressively increasing dosage. Once platelet blockade was attained, Iloprost infusion was dropped to 2.0 ng/Kg/min, and surgery was initiated. Iloprost infusion was maintained in the post-operative period, 2.0 ng/Kg/min. Surgery was successful. Large thromboembolic formations were removed from both pulmonary arteries. On post-operative day two, danaparoid administration was initiated, at the dosage of 750 I.U. b.i.d., subcutaneously, with contemporary administration of warfarin, at an initial daily dosage of 2.5 mg. On day four, danaparoid dosage was increased to 1,250 I.U. b.i.d.. Danaparoid administration was continued until INR values reached therapeutic values (2.0-3.0). The post-operative course was uneventful, and characterised by a satisfactory reduction of PVR (363 dyne/sec/cm⁵), and PAP (mean=28 mmHg), with a consistent increase of CO (4.85 L/min). Platelet number remained higher than usually expected for extracorporeal circulation, its nadir being $60.0 \times 10^3/\mu\text{L}$. In a few days the platelet number exceeded $100 \times 10^3/\mu\text{L}$. We propose this bi-phasic, two drugs protocol for the surgical management of patients with HIT, when extracorporeal circulation is indicated.

PO-304

PATIENT EVALUATION, SURGICAL PROCEDURE AND POST-OPERATIVE CARE FOR CHRONIC THROMBOEMBOLIC MAJOR VESSEL PULMONARY HYPERTENSION (CTPH)

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Chronic thromboembolic pulmonary hypertension (CTPH) generally develops after one single, massive episode of pulmonary embolism. It results from obstruction of the major pulmonary arteries by incompletely resolved or organised pulmonary emboli which have become incorporated into the pulmonary artery wall, eventually causing an increase in pulmonary vascular resistances. Frequently, affected individuals do not recall previous thromboembolic episodes, which took place months or years before diagnosis in the absence of classical signs and symptoms. The differential diagnosis with primary pulmonary hypertension is often not easily made. The definitive test needed to define the presence of this syndrome as well as its potential operability, is the pulmonary angiogram. The pulmonary angiographic findings in CTPH are extremely variable. Chronic emboli do not look like acute emboli. The filling defects and cutoffs characteristic of acute embolism are not seen. Pulmonary thromboendarterectomy (PTE) is an effective surgical procedure for CTPH. Careful post-operative management is essential for a successful outcome following PTE. We recently started a programme in Pavia in which members of a multidisciplinary team work in close interaction with the aim of increasing experience in the challenging problems these patients present in the evaluation, surgical, and postoperative phases of their care. The prevalence of antiphospholipid antibody syndrome appears particularly relevant. Overall operative and perioperative mortality among our 45 cases is 8.9%. The immediate and long-term haemodynamic and symptomatic improvement among survivors have been very gratifying. We had a decrease in pulmonary artery pressures and an improvement in cardiac output even in the presence of severe pre-operative right ventricular failure. As a conclusion, we may confirm that the Pavia PTE program is fully operative, the immediate and long-term improvement among patients being highly gratifying, with a highly satisfactory mortality rate.

Pre- and post-operative haemodynamic parameters of our patients submitted to PTE

PVRPRE Dynes x sec/cm ⁵	PVRPOST Dynes x sec/cm ⁵	PAPmPRE mm/Hg	PAPmPOST mm/Hg	COPRE L/min	COPOST L/min
996.5±312.2	432.4±192.5	58.0±12.7	29.55±8.7	3.1±0.9	5.0±1.5

POSTERS

**Biochemistry and genetics
of coagulation factors****PO-305****THE ROLE OF S3 IN SUBSTRATE RECOGNITION BY THE
Na⁺-LINKED ALLOSTERIC FORMS OF HUMAN α -THROMBIN**

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Thrombin catalytic subsites S1-S3 play a key role in controlling thrombin specificity. The enzyme has a marked preference for substrates having Arg in position P1, Pro in position P2, while the presence of acidic/hydrophilic residues in position P3 dramatically reduces the catalytic competence of the enzyme. In addition, under physiological conditions of salts and temperature, the thrombin molecule is in equilibrium between two conformational states, referred to as *slow* and *fast* forms, that is the Na⁺-free and the Na⁺-bound form, respectively. The present study was aimed at investigating the effect of Na⁺ on the thrombin interaction with synthetic substrates mutated in the position P3 alone, and having a constant Pro-Arg sequence in position P2-P1, respectively. The latter is the most represented sequence among the natural thrombin substrates, such as protein C, protease activated receptor 1 and 4 (PAR-1 and 4), factor XIII, thrombin-activable-fibrinolysis-inhibitor (TAFI), coagulation factor V and factor VIII. The effect of Na⁺ was also evaluated for protein C, a synthetic peptide spanning residues P12-P'3 of protein C, the N-terminal region 38-60 of PAR-1, which interacts with both the catalytic site and the thrombin exosite referred to as "Fibrinogen Recognition Site" (FRS), and finally for the N-terminal region 38-45 of PAR-1, which interacts with the catalytic site alone. The difference in the k_{cat}/K_m values between the fast and the slow form of thrombin hydrolysis of P3-mutated substrates ($\Delta\Delta G_{act}$) were analysed as a function of 1) hydrophobicity; 2) accessible surface area, and 3) van der Waals volume of the P3 residue. The $\Delta\Delta G_{act}$ values were always found to be positive, thus meaning that the fast form is more *active* than the slow form. Moreover, similar results were also obtained with both protein C and the synthetic peptide spanning residues P12-P'3 of protein C. The $\Delta\Delta G_{act}$ values linearly correlated with the hydrophobicity of the P3 residue, measured by the partition coefficient octanol/water. Thus, non-polar residues bound to the S3 subsite of thrombin stabilise the fast form of the enzyme, while this linkage is much attenuated by acidic/hydrophilic P3 residues. Furthermore, $\Delta\Delta G_{act}$ values were positively and linearly correlated to both the accessible surface area and the van der Waals volume of the P3 residues. Finally, FRS ligation stabilises the fast form, as shown in experiments using the peptides 38-60 and 38-45 of PAR-1. In conclusion, for substrates bearing a XPR sequence in positions P3-P1: a) the fast form is stabilised when X is repre-

sented by an aromatic/apolar residue, whereas b) this linkage is strongly attenuated when X is a small hydrophilic residue such as D, S, or E. In these cases the fast form may be stabilised *via* the allosteric linkage with FRS ligation.

PO-306**ARG545LEU: NEW CANDIDATE MUTATION IN A FAMILY
WITH TYPE 2 B VON WILLEBRAND'S DISEASE
CHARACTERISED BY ALL SETS OF MULTIMERS IN
PLASMA AND NO THROMBOCYTOPENIA AFTER
DESMOPRESSIN**

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Introduction. Type 2 B von Willebrand's disease (vWD) is due to the increased affinity of an abnormal von Willebrand factor (vWF) for platelets and is characterised by enhanced ristocetin-induced platelet aggregation (RIPA) and absence of large multimers from plasma. A high degree of heterogeneity has been shown among this vWD subtype but usually the highest molecular weight multimers are missing in plasma except for two reported cases previously defined as type 1 vWD "Malmo" and "New York". The candidate mutation for this peculiar type 2 B "type 1 vWD" phenotype already described as Pro503Leu and recombinant vWF Pro503Leu was expressed in mammalian cells (* J.C.I. 91: 2169, 1993). **Patients and results.** We describe here 5 affected members of a new family with enhanced RIPA (0.4 mg/mL), decreased levels of vWF:Ag (40 U/dL), vWF:RCO (43 U/dL), vWF:CBA (22 U/dL) and mildly prolonged bleeding time (11 min) but all sets of multimers in plasma and no thrombocytopenia before or after desmopressin. In the genomic DNA of 4 affected members we found an G→T point mutation at codon 4173 of the vWF gene. This nucleotide transversion predicts the substitution of an arginine by leucine at position 545 of the vWF subunit. This mutation is new and segregates with affected family members; a common polymorphism was excluded after the analysis of 50 normal individuals. We compared the phenotypic data of these new cases (Arg545Leu) with those from 4 vWD cases previously published (*) and from 4 additional different families (Arg545Cys) and results (mean values) are as follows:

Mutation	B.T. (min)	RIPA (mg/ml)	vWF:Ag (U/dL)	vWF:RCO (U/dL)	vWF:CBA (U/dL)	High Multimers in plasma	Platelet N° post-DDAVP
* Pro503Leu 205.000	12	0.4	68	62	n.t.	present	
Arg545Leu	11	0.4	40	43	22	present	218.000
Arg545Cys	11	0.6	44	26	2	absent	56.000

Conclusions. Our data show different vWF phenotypes in plasma of type 2 B cases characterised by distinct mutations at the same codon (Arg545Leu vs

Arg545Cys). This might suggest that a single and specific amino-acid is critical for the modulation of the vWF- GP-Ib [DABF1]-IX receptor interactions in that particular position of the vWF A1 domain.

PO-307

INCREASE OF BRADYKININ IN PLASMA OF PATIENTS UNDERGOING CARDIOPULMONARY BYPASS

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Background. Haemodynamic complications are frequently associated with cardiopulmonary bypass (CPB) and the vasoactive peptide bradykinin (BK), generated by the activation of the contact system, may be a mediator. **Methods.** We studied 21 patients undergoing CPB for cardiac surgery. Intra-arterial blood pressure was monitored and serial venous blood samples were obtained. We measured plasma BK and determined parameters of the contact system (activated factor XII, cleavage of high-molecular-weight-kininogen), of the coagulation system (prothrombin fragment F1+2 and thrombin-antithrombin complexes), of the fibrinolytic system (plasmin-antiplasmin complexes), of the complement system (C3a), and cytokine tumour necrosis factor. **Results.** Mean arterial pressure decreased progressively until the end of CPB (-18 mm Hg, $p < 0.001$) and returned to baseline by the end of surgery. BK, normal in basal conditions (median 1.90 fmol/ml [range 0.36-9.80]), was increased ($p = 0.001$ or less) from 15 minutes after the beginning of CPB (5.71 fmol/mL [0.50-61.92]) to the end of operation (7.07 fmol/mL [0.86-41.86]) with a peak at the end of CPB (9.81 fmol/mL [0.32-90.16]) and was normal at recovery 24 hours later (2.81 fmol/mL [0.40-9.87]). More than a two fold decrease of BK plasma levels was observed across the lung after declamping the aorta and restoring the pulmonary circulation still on CPB. The cleavage of high-molecular-weight-kininogen did not increase throughout the entire procedure. Activated factor XII, thrombin-antithrombin complexes, prothrombin fragment F1+2, plasmin-antiplasmin complexes, C3a and tumour necrosis factor were significantly increased after the beginning of the surgical procedure, further increased during CPB ($p < 0.001$), remained elevated until the end of surgery, but all returned to normal levels within the following 24 hours. Changes in plasma BK were not significantly correlated with any of the measured parameters. **Conclusions.** Our data demonstrate a progressive increase of plasma BK during CPB, at least partially due to a reduced catabolism. The BK increase, which could contribute to induction of hypotension, is not correlated with the activation of contact system, coagulation, fibrinolysis, complement nor cytokines, and might be counteracted by the recently developed BK receptor antagonists.

PO-308

THE ADDITIVE EFFECT OF LOW MOLECULAR WEIGHT HEPARIN AND DERMATAN SULPHATE ON THROMBIN INHIBITION IS THE MECHANISM OF THE ANTICOAGULANT ACTION OF MIXTURES OF GLYCOSAMINOGLYCANS

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Mixtures of glycosaminoglycans (GAGs) such as dermatan sulphate (DS) and low molecular weight heparin (LMWH) are available for clinical use. However the mechanism of their anticoagulant action is not completely elucidated. **Aim of the study.** To evaluate the mechanism of the anticoagulant action of a mixture of glycosaminoglycans (sulodexide, Alfa Wasserman, Bologna, Italy) *in vitro*. **Experimental design:** sulodexide components (DS and LMWH) were separated by means of size-exclusion chromatography. Thrombin clotting time (TCT) using human thrombin and the kinetics of thrombin inhibition were measured in platelet poor plasma (PPP) after the addition of sulodexide and of its components, alone and in combination. Algebraic fractional and isobole graphical methods were used to analyse the interaction between DS and LMWH. **Results.** Sulodexide in the range of 1-4 µg/mL produced a dose-dependent prolongation of TCT. Sulodexide above 4 µg/mL produced a TCT above 260 secs. The LMWH and DS fractions of sulodexide produced a dose-dependent prolongation of TCT, but above 5 µg/mL they both produced an unclottable TCT. Sulodexide and its components alone and in combination (range 1-10 µg/mL) produced a dose-dependent linear increase in the pseudo-first order constant of thrombin inhibition in defibrinated plasma. When the algebraic fractional and the isobole graphical methods were used to analyse the interaction between sulodexide components (DS and LMWH) an additive effect (zero-interaction) was indicated. **Conclusions.** Our data indicate that the components of sulodexide produce a dose-dependent inhibition of thrombin in human plasma through two independent pathways. This effect could be due to the potentiation of heparin cofactor II (HCII) by DS and of antithrombin III by LMWH.

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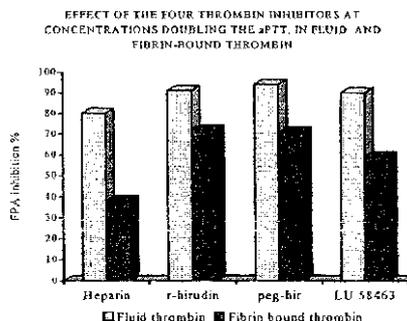
INHIBITION OF FLUID PHASE AND FIBRIN-BOUND THROMBIN: EFFECTS OF HEPARIN, r-HIRUDIN, PEG-rm-HIRUDIN AND A THROMBIN EXOSITE INHIBITOR

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Background. Fibrin-bound thrombin promotes thrombus extension by catalysing the formation of new fibrin. Fibrin-bound thrombin is less efficiently

inhibited by heparin than fluid phase thrombin. *Aim of the study.* The aim of this study was to compare the relative ability of four different antithrombin agents to inhibit fluid-phase and fibrin-bound thrombin. *Methods.* The following agents were tested: heparin, r-hirudin, PEG-rm-hirudin, a poly-ethylene-glycol-coupled derivative of hirudin, and LU 58463, a noncatalytic exosite thrombin-inhibiting decapeptide derived from the C-terminus of hirudin. FPA generation (RIA) was used as an index of thrombin activity. The concentration of the four inhibitors able to achieve a 70% inhibition of FPA a.eneratori in fluid-phase and in clot-bound system were identified. The inhibitory effect on fluid-phase thrombin and fibrin-bound thrombin of the four agents was compared at concentrations doubling the aPTT. *Results.* The ratio of the concentrations producing a 70% inhibition of fluid-phase- and fibrin bound-thrombin were as follows: 100 (heparin) 3 (r-hirudin and PEG-rm-hirudin) and 5 (LU 58463). The concentrations of the four agents doubling the aPTT produced an inhibition of thrombin in fluid phase of $80.0 \pm 18.8\%$ (heparin), $91.0 \pm 7.8\%$ (r-hirudin), $93.7 \pm 5.6\%$ (PEG-rm-hirudin), and $89.5 \pm 7.2\%$ (LU58463). The four inhibitors, at the same concentrations, produced an inhibition of fibrin-bound thrombin of $38.2 \pm 9.3\%$ (heparin, $R=0.48$), $72.6 \pm 18.9\%$ (r-hirudin, $R=0.79$), $71.0 \pm 16.3\%$ (PEG-rm-hirudin, $R=0.76$), $58.9 \pm 9.3\%$ (LU584, $R=0.66$). The ratio of inhibition of clot-bound to fluid-phase thrombin for heparin was significantly lower than the ratio of r-hirudin, PEG-rm-hirudin and LU 58463 ($p < 0.05$). *Conclusions.* The three direct thrombin inhibitors are more effective than heparin in inhibiting fibrin-bound thrombin: they could be more effective than heparin in the prevention of thrombus extension and rethrombosis.



PO-310
AN ADDITIONAL UNIQUE CANDIDATE MUTATION (G2470A; M740I) IN THE ORIGINAL FAMILIES WITH VON WILLEBRAND'S DISEASE TYPE 2 M VICENZA AND THE G3864A (R1205H) MUTATION

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A heterozygous candidate mutation (G3864A; R1205H) in exon 27 of von Willebrand factor (vWF) gene associated with von Willebrand's disease (vWD) type 2 M Vicenza in 7 Italian and 1 German families has been identified (Schneppenheim *et al.*, Thromb Haemostas 2000; 82, 136). Families with the same mutation and similar phenotype have been recently reported (Bodò *et al.*, Blood 1999; abs. 1661). We identified an additional family from Vicenza with a similar phenotype and the R1205H mutation. However, since the family was identified prior to the knowledge of a laboratory pattern compatible with vWD Vicenza (that is, presence of supra-normal plasma vWF multimers), we had screened the entire vWF gene by PCR and CSGE. In addition to the G3864A change in exon 27, we identified a G2470A change in exon 17 predicting M740I amino acid change in the vWF. All the index cases previously reported from the 8 families were screened and only the 3 index cases from Vicenza province showed the presence of the substitution. A total of 12 patients from the three families and seven normal relatives were screened and the two nucleotide changes were identified only in the affected members. The G2470A was not present in 268 normal subjects from the Vicenza province suggesting that also the second change could be a candidate mutation. Expression studies are underway to understand the real impact of the G2470A change in determining the vWD Vicenza phenotype.

PO-311
A NOVEL FAMILY WITH RECESSIVE VON WILLEBRAND'S DISEASE DUE TO COMPOUND HETEROZYGOSITY FOR SPLICING SITE MUTATION IN INTRON 13 AND MISSENSE MUTATION IN EXON 42 OF VON WILLEBRAND FACTOR GENE

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The molecular bases of autosomal recessive von Willebrand's disease (vWD) are heterogeneous. The probands may be homozygotes for null alleles or compound heterozygotes of missense mutations and null alleles, especially in the case in which measurable levels of von Willebrand factor (vWF) are observed. In our group of patients with recessive vWD, a single family showed compound heterozygosity for a missense mutation in exon 42 (G7335T,C2362F) and a splicing site mutation at position 11/24 in the consensus sequence of intron 13 in the propositus (Eikenboom *et al.* Thromb Hemostas 1998, 79, 709). Heterozygous subjects are asymptomatic, as usually reported for the obligatory heterozygotes of type 3 vWD. We screened two additional families subsequently identified as having autosomal recessive vWD for the mutation by PCR amplification and *Eco57I* digestion of the amplified fragments. The propositi, aged 3 and 2 years, were referred for severe epistaxis, requiring substitution treatment. Both had VIII:C 20 U/dL, vWF:Ag 5-6

U/dL and RiCof <3 U/dL. One of the probands resulted to be a compound heterozygote for the C2362F mutation and the splice site mutation in intron 13. In the other, a heterozygous C2362F mutation was detected, whereas the second mutation is still under investigation. Both the mutations appeared to be linked to a single haplotype, defined by 5 genetic markers (VNTR I and II in intron 40, Rsa I in exon 13 and 18 and *Hph* I in exon 28 and *Xmn* I in exon 45), suggesting a founder effect, as already reported for the previous families. A total of 10 sub-

jects were heterozygotes for the C2362F mutation and 5 were heterozygotes for the splice site mutation. None had suffered from significant bleeding, as previously reported for 9 and 5 heterozygotes in the previous families. In conclusion, the C2362F and the splice site mutation in intron 13 are commonly observed in subjects with a history of recessive vWD in the Veneto region, but their role in inducing bleeding is expressed only when in association with another vWF gene mutation.

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