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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. 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 publication[12]]:

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, 1980:363-92.
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, 1974:44-6.
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, 1980:59-89. (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).

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XVII Congress of the Italian Society for the Study on Hemostasis and Thrombosis

Rome, May 9-12, 2002

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XVII Congress of the Italian Society for the Study on Hemostasis and Thrombosis

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Foreword

Dear Colleagues,

I am delighted to welcome you to Rome for the XVII Congress of the Italian Society for the Study on Hemostasis and Thrombosis. During the organization of the congress, the members of the board, the local committee and I drew inspiration from past editions of the congress, following the same design of oral communications and poster sessions between symposia. As far as concerns the oral communications and posters, we have tried to give particular prominence to work carried out by young researchers for whom, also taking into account previous scientific work, we have set aside 10 awards for study projects and 20 prizes for the best oral communications. For the symposia, we have followed a philosophy of embracing other societies with which we have organized joint symposia. Given this new philosophy we have widened the "mailing list", which very probably accounts for the more than 10% increase in abstracts received compared with last year's submissions. I hope that this new direction is merely the first step along a path that will give our society ever greater visibility in the nation and closer interactions with the fields of internal medicine and the many specialties which have considerable and important points of contact with our own.

Since my mandate will come to an end during the congress, I would like to take this occasion to thank the past president, Prof. PierMannuccio Mannucci, and the vice-president, Prof. Maria Benedetta Donati, to whom I give every good wish for the next mandate, and the members of the board for having given me so much professional and personal support.

Despite the intensity of the congress, I hope that will all have some occasion to enjoy the splendor of Rome, whose art, unique in the world, uninterruptedly spans 2500 years of mankind's history.

Francesco Violi, President

Oral Communications
Clinical Aspects of Inherited Coagulopathies

C001

SIX-MONTH VS 12-MONTH SURVEILLANCE OF HEMOPHILIACS FOR EARLY DIAGNOSIS OF HEPATOCELLULAR CARCINOMA

Santagostino E, Rumi MG, Rivi M, Colombo M, Mannucci PM and the Study Group of The Association of Italian Hemophilia Centers

A. Bianchi Bonomi Hemophilia & Thrombosis Center, Internal Medicine Institute, IRCCS Maggiore Hospital and University of Milan, Italy

Aim. To assess whether a surveillance program based on abdominal ultrasound examination (AUS) and α-fetoprotein assay (AFP) at 6-month intervals might improve the early detection of hepatocellular carcinoma (HCC) in hemophiliacs with chronic hepatitis C, in comparison with an annual surveillance schedule. *Methods.* In 1995, 566 hemophiliacs (median age: 39 years, range: 13-88) with detectable serum HCV-RNA were enrolled by 11 Centers. The 6-month surveillance schedule was adopted by 6 Centers that followed-up 208 patients (37%) whereas the other 5 Centers followed the remaining 358 patients with annual surveillance. *Results.* 113 hemophiliacs (20%) had normal ALT levels, 126 (22%) HIV, 30 (5%) HBV, 74 (13%) cirrhosis and 50 (9%) had AFP above the upper-normal limit (7 ng/mL). No differences were found for these features between the 2 groups of patients. During 6-year follow-up, 8 patients (age: 51-69 years) with cirrhosis (1 HIV and 1 HBV), developed HCC. Multiple nodes were detected in 6 (75%), 4 of the 6-month group and 2 of the 12-month group. A single node was detected in 2 patients undergoing annual surveillance. *Conclusions.* 6-month surveillance is not advantageous over 12-month surveillance for the early diagnosis of HCC, because of the multicenter origin of this tumor in HCV-infected hemophiliacs.

C002

IMPACT OF PRENATAL/PERINATAL EVENTS AND EARLY FACTOR VIII REPLACEMENT ON INHIBITOR RISK IN CHILDREN WITH SEVERE HEMOPHILIA

Santagostino E, Muca-Perja M, Gringeri A, Mannucci PM

A. Bianchi Bonomi Hemophilia & Thrombosis Center, Internal Medicine Institute, IRCCS Maggiore Hospital and University of Milan, Italy

To investigate the impact of prenatal/perinatal events and early replacement on inhibitor risk, all children with severe hemophilia A (FVIII<1%) exclusively treated with recombinant FVIII and assessed for inhibitor at least every 3 months were evaluated. Accurate information on prenatal/perinatal events, clinical and infusional history were available in 34 patients (age: 11-116 months). Eighteen patients, 15 treated on demand (83%) and 3 on prophylaxis (17%), developed inhibitors at a median age of 21 months (range: 5-79) after a median of 16 days of exposure (DE, range: 5-86). All patients but two showed inhibitor peaks above 10 BU/mL with a median peak of 53 BU/mL (range: 0.7-10,000). The inhibitor disappeared spontaneously in 1 patient and following immune tolerance induction in 9/12. Inhibitor children were compared with 16 children who did not develop inhibitors after at least 20 DE (2<50, 2<100 and 12>200 DE) on prophylaxis (12 patients, 75%) or on demand treatment. No statistically significant differences were found in the factors listed in the Table below.

	Children with inhibitor (n.18)	Children without inhibitor (n.16)
Family history of hemophilia	5 (28%)	5 (31%)
Family history of inhibitor	2 (11%)	1 (6%)
Median age (months) at 1st FVIII infusion	13 (5 days- 45)	10 (1 day-35)
Surgical procedures	6 (33%)	6 (37%)
Port-a-cath placement	4 (22%)	4 (25%)
Switch of recombinant products	7 (39%)	6 (37%)
Amniocentesis/villocentesis	3 (17%)	2 (13%)
Premature birth	1 (6%)	2 (13%)
Caesarian birth	7 (39%)	7 (44%)
Breast-feeding	14 (78%)	11 (69%)
FVIII infusions associated with infections or vaccinations	3 (17%)	4 (25%)

Three patients who subsequently developed inhibitors (17%, vs 0 non-inhibitor patients) had had their 1st FVIII infusion for surgery. This preliminary observation should be further investigated. Prenatal/perinatal events and early FVIII replacement did not influence the inhibitor risk in our cohort of children with severe hemophilia.

C003

HIGH PURITY FACTOR IX CONCENTRATE CONTINUOUS INFUSION IN PATIENTS WITH HEMOPHILIA B UNDERGOING SURGERY

Tagariello G, Radossi P, De Biasi E, Risato R, Davoli P

Blood Bank, Center For Blood Diseases and Hemophilia Centre, Castelfranco Veneto Hospital, Italy

In hemophiliacs, replacement therapy is traditionally administered by bolus injections (BI). A preliminary condition for continuous infusion (CI) is the safety, stability and sterility of the diluted concentrates. Only sporadic reports exist about FIX CI replacement therapy and, despite the high purity of the concentrates, the risk of thrombotic side effects should be still considered. In this study we evaluated the hemostatic efficacy, the post-operative levels, the safety and the flexibility of a high purity FIX concentrate (Aimafix D.I, Kedrion, Italy). Five hemophilia B patients (4 severe and 1 mild) were enrolled and underwent different types of surgery: 1 total hip replacement, 1 pseudotumor, 2 paraphimosis and 1 circumcision. For all patients therapy started with a bolus injection at the dosage of 50 IU/kg before the intervention. At the end of the operation FIX CI was started immediately at the dosage of 3 IU/kg/h until the seventh day. For patients who needed longer treatment this was continued with a 2 IU/kg/h dosage. A syringe pump (Perfusor Secure FT, 50 mL, Braun, Germany) was used and concentrates were reconstituted once a day according to the instructions of the manufacturer. FIX

levels ranged from 75 IU/mL to 24 IU/mL. All patients were treated with tranexamic acid and FIX:C levels in the sera were monitored daily as were platelet counts, D-dimer and plasma fibrinogen levels. To avoid local phlebitis a 24h saline infusion was used. No major side effects were observed. In conclusion we think that CI with high purity FIX concentrates is safe, flexible and effective in hemophilia B patients undergoing surgery.

C004

HEALTH-RELATED QUALITY OF LIFE IN CHILDREN WITH HEMOPHILIA

Von Mackensen S,* Gringeri A,^ Bullinger M,* for the Haemo-QoL Investigators Group

**Institute for Medical Psychology, University of Munich & Hamburg, Germany, ^Department of Internal Medicine, University of Milan, Italy*

Background. Coagulation abnormalities such as hemophilia have an impact on patients' quality of life, especially in children. In addition every-day life of their families can be influenced as well. For the assessment of health-related Quality of Life (hrQoL) of hemophilic children and adolescents a disease-specific self-report questionnaire (Haemo-QoL) was developed and psychometrically tested in pilot-testing in co-operation with BAYER. *Methods*. In a cross-cultural study 339 children and their parents were assessed in 6 European countries (Germany, Italy, Spain, France, UK, the Netherlands), of whom 70 were Italians. The Haemo-QoL evaluates hrQoL of children and adolescents with three different age group versions. In addition parents were asked about the impact of hemophilia on their family life and concerning their own hrQoL as well as their perception of their children's hrQoL. Medical data were collected by hemophilia treaters from patient files. *Results*: In comparison with other chronic diseases, such as obesity or asthma, hemophiliacs have a higher quality of life in most of the dimensions of the KINDL questionnaire (generic), but a lower QoL in the dimension *school*. In the hemophilia-specific dimension *physical health* they are *afraid of hurting themselves* and they report *pain in joints*. They are bothered mostly by *restrictions in sports and hobbies* and *injections and treatment*. *Conclusions*: The Haemo-QoL is the first valid and reliable disease-specific questionnaire for the assessment of hrQoL in hemophilic children and will be of great utility in hemophilic patients. Since this questionnaire is only available for children such an instrument is required for adults and will be developed for the ESCHQoL-study.

C005

ASSESSMENT OF QUALITY OF LIFE IN ADULT HEMOPHILIACS WITH INHIBITORS

Gringeri A, Mantovani LG, Scalone L, Belisari A, Tinelli M, for the COCIS investigators

Department of Internal Medicine and Centro di Farmacoeconomia, University of Milan and IRCCS Maggiore Hospital, Milan, Italy

Objectives. The aim of the study was to assess the quality of life in hemophilic adults with inhibitors. *Methods*. Evaluation of quality of life was carried out in the frame of the COCIS (Cost Of Care of Inhibitor Study), a longitudinal study conducted in 11 centers in Italy. Hemophilia A patients with inhibitors, aged 14-65 years, were sequentially enrolled and followed-up for 18 months. Information on demographics, co-morbidities, laboratory examinations, hospitalizations, drug therapies, physicians' visits was collected every 6 months. Quality of life (QoL) was investigated using EuroQoL-5D, MOS-SF-36, HUI, QWBS. We report on quality of life as measured with EQ-5D and on the correlation between QoL and orthopedic status (Orthopedic Joint Score, OJS). *Results*. Fifty-two subjects, aged 36.1+12, 48 (92.1%), high responders (>10BU), were enrolled. A large number of subjects reported any (some/moderate or severe) problem in mobility (70%), self-care (35%), usual activities (58%), pain/discomfort (82%) and anxiety/depression (41%) items of EQ-5D. Subjects gave an average value of 65 (+16) in the 0-100 VAS of EQ-5D. OJS and VAS of EQ-5D were inversely correlated (Spearman's rho equal to -0.57, $p<0.001$). *Discussion*. Hemophiliacs with inhibitors show impaired levels of QoL, but similar to those without inhibitors. The most impaired aspects are related with physical functioning, activities and pain as well as with mobility. These observations are confirmed by the correlation between orthopedic status and QoL. For a better understanding of health-related problems of hemophiliacs a disease-specific questionnaire for the assessment of quality of life is urgently needed.

C006

SECONDARY PROPHYLAXIS IN PATIENTS WITH SEVERE HEMOPHILIA A: CLINICAL AND PHARMACOECONOMIC ASPECTS

Coppola A, Cimino E, Madonna P, Tufano A, Garofano T, Cerbone AM, Di Minno G

Centro di Coordinamento Regionale per le Emocoagulopatie, Dipartimento di Medicina Clinica e Sperimentale, Università "Federico II", Naples, Italy

The role of secondary prophylaxis in the management of patients with severe hemophilia A is debated, especially in terms of patients' quality of life and pharmacoeconomic implications. We evaluated clinical course, the use of factor VIII concentrates (FVIII) and its costs in 19 severe hemophiliacs (mean age 29.4±9.4 years, range 17-46) during 3-month treatment on demand or thrice weekly prophylaxis with FVIII (recombinant 10 patients, plasma-derived 9 patients, mean dose 29.3±4.1 U/Kg). As expected, a > 60% reduction of bleeding episodes (especially spontaneous hemorrhages and hemarthroses) during prophylaxis was registered (145 vs. 374, mean rate 2.5±1.7 - range 0.3-6.3 - vs. 6.6±3.4 - range 2-18 - episodes per patient-month). In parallel, an increase of 183,000 U of FVIII was needed (overall 2,046,000 vs 1,836,000 U, mean 107,000 vs. 98,000 U/patient). No patient developed inhibitors over the study period. All but one patient (94.7%) experienced an improved quality of life on the prophylaxis regimen, as judged by a questionnaire concerning efficiency and safety of patients' daily activities and work (or school) days lost. Three patients (15.7%), however, raised questions for multiple venous injections. The overall costs of 3-month treatment were 1,722,389 € (mean 90,652/patient) when patients were on prophylaxis vs. 1,551,655 € (mean 81,666/patient) for treatment on demand. Thus, an overall increase in costs of 170,733 € may prevent 229 bleeding episodes in these patients (755 €/episode). Moreover in 6 patients (31.5%), the clinical benefits during prophylaxis were also associated with reduction of FVIII use and costs. Mean event

rate reduction was 6.9±3.3 (range 4.3-13) per patient-month in this subgroup vs. 2.6±0.8 in the remaining 13 patients. According to these data (to be extended in larger patient populations and evaluation periods), in severe adult hemophiliacs the impact of secondary prophylaxis on costs is well balanced by the higher quality of life.

C007

ASSESSMENT OF THE ECONOMIC IMPACT OF CARING FOR ADULT HEMOPHILIACS AND INHIBITORS

Mantovani LG, Gringeri A, Scalone L, Belisari A, for the COCIS Investigators

Centro di Farmacoeconomia and Dept. of Internal Medicine, University of Milan; IRCCS Maggiore Hospital, Milan, Italy

Objectives: The aim of the study was to evaluate the cost of care of adult Italian hemophiliacs with inhibitors, this condition being one of the most financially and economically challenging of hemophilia treatment. *Methods*: The study, named COCIS (Cost Of Care of Inhibitor Study), is a longitudinal, natural study. Patients with hemophilia A and inhibitors, aged from 14-65 years, were sequentially enrolled in 11 Italian centres and followed-up for 18 months. Information on demographics, co-morbidities, laboratory examinations, hospitalizations, drug therapies, physicians' visits was collected every 6 months. Health care provided to patients was quantified in the perspective of the Italian National Health Service, by means of tariffs. All costs are expressed as costs per person months in € (2001). Production losses are expressed in physical units, i.e. working and school days lost. *Results*. Fifty-five subjects, aged 36.1+12, 48 (92.1%) high responders (>10 BU), were enrolled and followed-up for a global 810 person months. The average cost per person/month was 17,879.2 € per patient/month, so distributed: 8,491.9 for rFVIIa (47.5% of total cost); 3,174.2 for rFVIII (17.5%); 3,077.1 for pdFVIII (17.2%); 2,982.0 for aPCC (16.7%); 153.9 for visits and surgery (0.9%).There were 2.3 working or school days per person/month lost by patients and 0.3 days lost by caregivers. *Discussion*. This study showed that the major component of costs are the clotting factor concentrates. Surgical procedures were carried out only with rFVIIa: this can explain the higher overall cost observed in rFVIIa usage.

C008

MENORRHAGIA AS AN EARLY SYMPTOM OF BLEEDING TENDENCY IN WOMEN WITH CONGENITAL DISORDERS OF HEMOSTASIS

Noris P, Gamba A, Montani N, Bertolino G, Cisternino M,° Sanpaolo P,* Soldavini E, Capezzera M, Gamba G

*Departments of Internal Medicine, °Pediatrics and *Gynecology of the University of Pavia, IRCCS PoliClinico San Matteo, Pavia, Italy*

In contrast to the menorrhagia/metrorrhagia due to the gynecological causes, menorrhagia in congenital bleeding disorders is frequently observed at menarche. Furthermore the clotting test usually performed generally does not diagnose hemophilia carriers, vWd and thrombocytopathies. The aim of this investigation was to research the prevalence of congenital bleeding disorders in women with menorrhagia in relation to the age of onset and to the prevalence of other bleeding manifestations. During a period of 10 months we enrolled 30 women aged from 11 and 52 years. No patient had underlying acquired diseases characterized by diffuse bleeding tendency. The menorrhagia was definied by a pictorial blood assessment chart (PBAC) when a score more than 100 was calculated.[1] A careful medical history including the time of occurrence of menorrhagia and other bleeding manifestations was collected for each woman. Tests performed: platelet count, volume and size, microscopic examination of blood smear, bleeding time (BT), aPTT, PT, F VIII, F IX, FXI, vWF Ricof, vWF Ag, vWF CBA, platelet aggregation to ristocetin, ADP and collagen. Menorrhagia was present: at menarche in 13 patients (43%), during the first year from menarche in 6 patients (20%), before 20 years in 4 patients (13%), from 32 to 45 years in the other 7 women (24%). Other bleeding manifestations were reported in 17/23 teenagers (73%) and in 6/7 adults (85%). PT, aPTT, F VIII, F IX, F XI were in normal ranges in all women. BT was slightly prolonged in 11 patients (37%); 2 of them had menorrhagia at menarche, and moderately low platelet count with increased platelet volumes and sizes. In one of these women a diagnosis of Bernard-Soulier syndrome was made. Abnormal vWF functions were detected in 9 patients (30%) all with menorrhagia at menarcha. In two adolescents with vWd (11 and 13 yrs old) excessive menstrual blood was the first and isolated bleeding symptom. *Conclusions*: In women with menorrhagia other bleeding manifestations are common. Menorrhagia at menarche is frequently an early symptom suggestive of vWd and thrombocytopathies, which should be carefully researched in all young women with menorrhagia.

1. Higham JM, O'Brien PM, Shaw RW. Assessment of menstrual blood loss using a pictorial chart. Br J Obstet Gynaecol 1990; 97:734-9.

Oral Communications
Pathologic Hemostasis in Humans I

C009

HYPERFIBRINOLYSIS PREDICTS POOR OUTCOME IN ESOPHAGEAL VARICEAL BLEEDING IN CIRRHOSIS OF THE LIVER

Bucciarelli P,* Dell'Era A,° Bottasso B,* Bajetta MT,* Primignani M°

**Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, and °Gastroenterology and Endoscopy Service, IRCCS Ospedale Maggiore Policlinico, Milan, Italy*

Background and Aims. Variceal hemorrhage in advanced liver cirrhosis carries a high mortality rate. Since hemostasis and fibrinolysis are impaired in these patients we assessed whether such changes are independently related to the clinical outcome of variceal bleeding. *Methods.* Cirrhotic patients bleeding from esophageal varices (cases) and non-bleeding cirrhotic controls were evaluated. Fibrinogen plasma levels, parameters of activation of coagulation (prothrombin fragment F1+2, thrombin-antithrombin complexes) and fibrinolysis [D-dimer, tissue-type plasminogen activator (t-PA) antigen, plasminogen activator inhibitor type 1 activity] were serially assessed in 43 cases and, at base-line only, in 51 controls. *Results.* All parameters were more impaired in Child-Pugh C patients, either bleeders or controls. Parameters of activation of coagulation and fibrinolysis were more impaired in bleeders, at the time of bleeding, than in controls. In bleeders, t-PA and D-dimer plasma levels were significantly higher in non surviving patients as compared to in survivors (t-PA: 32.0±1.4 ng/mL vs 23.6±1.8 ng/mL, p=0.05; D-dimer: 525.6±3.3 ng/mL vs 172.9±2.7 ng/mL, p=0.05). In survivors these parameters, not significantly different from non-bleeding cirrhotic controls with similar Child-Pugh score, progressively improved with time, whereas they did not change in non-survivors. Independent predictors of mortality were Child-Pugh C class [odds ratio 8.7 (95% CI 2.0-38.4)] and, in Child-Pugh C class, infection [odds ratio 6.9 (95% CI 0.7-71.7)] and hyperfibrinolysis, defined as D-Dimer plasma levels above the 75th percentile of values of Child-Pugh C surviving bleeders [odds ratio 21.0 (95% CI 1.6-271)]. *Conclusions.* Hyperfibrinolysis is an independent predictor of death in patients with advanced liver cirrhosis bleeding from esophageal varices. A clinical trial with antifibrinolytic drugs is warranted in these patients.

C010

IMPAIRED GENERATION OF ACTIVATED THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR IS THE MAIN CAUSE OF PLASMA HYPERFIBRINOLYSIS IN LIVER CIRRHOSIS

Colucci M, Binetti BM, Branca G,* Clerici C,° Semeraro N, Gresele P*

*Dipartimento di Scienze Biomediche, Università di Bari; *Dipartimento di Medicina Interna e Medicina Vascolare; °Istituto di Gastroenterologia, Università di Perugia, Italy*

Hyperfibrinolysis is thought to contribute to bleeding associated with advanced liver cirrhosis. TAFI (thrombin activatable fibrinolysis inhibitor) is a plasma proenzyme synthesized in the liver and is converted by thrombin and plasmin into a carboxypeptidase (TAFIa) that retards fibrinolysis by preventing plasminogen binding to fibrin. Previous studies from our and other laboratories showed that plasma TAFI antigen is markedly reduced in liver cirrhosis. In this study we evaluated the influence of TAFI reduction on *in vitro* fibrinolysis. Fifty-three patients with liver cirrhosis and 43 age- and sex-matched healthy controls were studied. TAFI antigen in patients' plasma was 28.3±12.4% as compared to 86.2±28.4% in controls (p<0.001). The lysis time of diluted plasma clots exposed to 25 ng/mL t-PA was markedly shorter in cirrhotics than in controls (32.3±20.5 vs 49.6±17.4 min, p<0.01). Addition of a specific inhibitor of TAFIa (PTI, 50 μg/mL) reduced the clot lysis time by 42.5±7.3% in controls, by 31.5±6.9% in Child A and B patients and by only 16.7±3.3% in Child C patients (p<0.01), suggesting that TAFI-mediated inhibition of fibrinolysis was less pronounced in cirrhotic plasma. Accordingly, peak TAFIa activity generated in cirrhotic samples during clot lysis amounted to 1/3 of that formed in control plasma. Moreover, the generation of thrombin (the main activator of TAFI), measured by a fibrinogen clotting assay, was also impaired in patients' plasma (< 50% of control). Addition of purified TAFI to cirrhotic plasma prolonged the lysis time and enhanced the response to PTI significantly. Similarly, the addition of thrombomodulin (0.25 μg/mL) normalized both TAFI activation and clot lysis. These data indicate that plasma hyperfibrinolysis in liver cirrhosis is largely due to a defective TAFIa generation resulting from both low TAFI levels and impaired thrombin generation.

C011

MODIFICATIONS OF HEMORHEOLOGIC, FIBRINOLYTIC AND ENDOTHELIAL PROFILE DURING AND AFTER EXERCISE STRESS TESTING

Turchetti V, Boschi L, Bellini MA, Borgogni G, Donati G, Richichi MG, Guerrini M, Forconi S

Institute of Internal Medicine and Geriatrics, University of Siena, Italy

The aim of our study was to evaluate microcirculatory modifications between the study of hemorheologic, fibrinolytic and endothelial parameters in normal and vasculopathic subjects before, during and after exercise stress testing. We studied 10 normal subjects and 10 patients suffering from chronic ischemic vasculopathies with a cycloergometer stress test. In basal conditions (B), at maximum stress (MS) and in recovery phase after 10' and 20' (R1-R2) we evaluated: blood viscosity by a plate-cone rheometer (Carri-Med) cPs at 10s-1, hemocromocytometric examiantion (Coulter Counter), intraerythrocytic calcium (Fura2-AM, Perkin-Elmer Spectrofluorimeter), erythrocytic morphology by Zipursky-Forconi method (EMI); fibrinogen, PT-INR, a-PTT and PAI-1 with a coagulative method (BCT- Beringer), VCAM-1, a molecule of vasocellular adhesivity, (Elisa method), L-arginine and L-citrulline, amino acids of the nitric oxide (NO) pathway, (HPLC method) considering L-citrulline/L-arginine ratio as an index of NO production. Our results demonstrate that in normal subjects we have, at MS, an increase of blood viscosity, intraerythrocytic calcium and hematocrit and a minor increase of plasma fibrinogen with a return to normal values after 20' of recovery. About erythrocytic morphology, we observed no substantial modification in bowl/discocyte ratio during various phases of the stress test: EMI always remained >1; L-citrulline/L-argi-

nine ratio, index of NO production, decreases at MS but increases at R1 and R2 to basal values (B: 0.27±0.09, MS: 0.19±0.08, R1: 0.26±0.08, R2: 0.31±0,1). In patients with vasculopathies we found a significant increase of blood viscosity and total erythrocytic cytosolic calcium which remained elevated at R1 and R2. Discocyte percentages increased during all phases of exercise stress testing with a decrease of EMI from 0.79 to 0.65; L-citrulline/L-arginine ratio does not show significant variations (B: 0.4±0.09, MS: 0.3±0.16, R1: 0.37±0.06, R2: 0.36±0.07). In normal subjects, during the recovery phases, an endothelial response to stress is demonstrated by increases in NO which cause vasodilatation and an improvement of hemorheologic parameters. In vasculopathic patients exercise stress testing does not cause increased NO, which may contribute to understanding hemorheologic impairments.

C012

PREVALENCE OF PORTAL VEIN THROMBOSIS AND ASSOCIATED FACTORS IN CIRRHOTIC PATIENTS: A PROSPECTIVE STUDY

De Santis A, Violi F,* Moscatelli R, Gigliotti F, Piccheri C,* Ferro D,* Fimognari F,* Attili AF

*Dipartimento di Medicina Clinica, Divisione di Gastroenterologia; *Istituto di I Clinica Medica, Università "La Sapienza", Rome, Italy*

Partial vein thrombosis (PVT) is a frequent event that may complicate hepatic cirrhosis. In a previous retrospective study from our Department, PVT prevalence was 10.9%. Starting from March 1st 2000, we have undertaken a prospective study on PVT with the aim to evaluate its prevalence and hemodynamic and biochemical associated factors. *Patients*. We enrolled all cirrhotic patients examined with ultrasound in our department: 154 males (mean age 58±12.6) and 87 females (mean age 65.3±10.2) were submitted to an ultrasound and echocolor Doppler examination of the liver and portal tree. Cirrhosis was viral in 64% of cases, alcoholic in 21.5% and cryptogenetic in 11.2% of cases. A hepatocellular carcinoma (HCC) was present in 16.4% of patients. Prothrombin time (PT), Factor VIII and D-dimers were assessed using commercial kits. *Results*. Partial or total thrombosis was present in one vessel of the portal tree in 27.6% of patients. The prevalence of PVT according to gender was 29.7% in males and 23.8% in females. After exclusion of patients with HCC the prevalence of PVT was 24.6% in males and 24.3% in females. The frequency of the PVT according Child Pugh classification was: 12.1% in class A, 30.4% in class B, 29.7 in class C. PT in patients with PVT was 65.5±17.9% vs 74.9±15.9% in patients without PVT ($t = 2.78$; $p = 0.0062$). The factor VIII in patients with and without PVT was not significantly different: 84.9±53.5% vs 107.1±54.6 %. But stratifying by C-P classes we observed a significant difference between patient with and without PVT in class C: 74±34.5% vs. 145.3±67.1% ($t = 2.5$; $p = 0.027$). The D-dimers in patients with and without PVT were 0.46±0.72 vs. 0.48±1.78 mg/L without significant differences also after stratification according to C-P classification. *Summary*: Our study confirms the high frequency of PVT in cirrhotic patients. The prevalence of PVT seems correlated with C-P class. PT is significantly lower in patients with PVT; also factor VIII is significantly lower in patients with PVT but only in advanced cirrhosis. The D-dimers levels were similar in patients with and without PVT.

C013

SERUM D-DIMER TEST AND ASSESSMENT OF FIBRINOLYTIC CAPACITY BEFORE AND AFTER VENOUS OCCLUSION

Paniccia R, Bandinelli B, Conti AA, Evangelisti L, Gazzini A, Lapini I, Lucarini L, Pepe G, Rossi L, Abbate R, Prisco D

Dipartimento di Area Critica Medico-Chirurgica, Università degli Studi di Firenze, Centro Trombosi, Azienda Ospedaliera Careggi, Florence, Italy

Euglobulin lysis time (ELT) is a global test useful to demonstrate hyperfibrinolysis, but it is less applied in the study of hypofibrinolysis and is time consuming and influenced by technical procedures. Recently, serum D-dimer (s-DD) test after standardized coagulation (4 hr incubation of sample at room temperature - rt) has been described as a global fibrinolytic test related to different fibrinolytic parameters both before and after venous occlusion (VO). Modified standardized coagulation (2 hr incubation at 37°C) has been validated in unstimulated subjects, so allowing a more practical way of performing this test. The aim of this study was to evaluate whether a reduced time of incubation of blood at 37°C from 23 healthy subjects, studied before and after 10 minutes VO, produces s-DD levels comparable with the levels after 4 hrs at rt. Serum was obtained after 1, 2, 3 and 4 hrs of incubation both at rt and at 37°C. The following plasma fibrinolytic parameters were investigated: DD, ELT, t-PA activity (t-PA act) and antigen (t-PA ag) and PAI-1 activity (PAI-1 act). Before VO, s-DD levels after 2 hrs at 37°C were increased ($p<0.001$) with respect to those after 2 hrs at rt and similar to those measured after 4 hrs at rt, so confirming previous results. Following VO, s-DD levels after 2 hrs at 37°C were similar to those after 4 hrs at rt, and remained unchanged after 3 and 4 hrs. Significant correlations were found between s-DD values after 2 hrs at 37°C and ELT ($r=-0.62$, $p<0.01$), t-PA act ($r=0.69$, $p<0.001$), t-PAag ($r=0.52$, $p<0.01$) and PAI-1act ($r=-0.57$, $p<0.01$). The present study demonstrates that, as before VO, also after VO s-DD levels (after 2 hrs of incubation at 37°C) are similar to those measured after incubation for 4 hrs at rt and are correlated with different fibrinolytic parameters. A shorter incubation can facilitate a larger application of this method in general laboratories.

C014

MEPACRINE RELEASE ASSAY: A NEW FUNCTIONAL METHOD FOR THE ASSAY OF HEPARIN-ASSOCIATED ANTIBODIES DURING HEPARIN-INDUCED THROMBOCYTOPENIA

Ramon R, Scandellari R, Zocca N, Carraro G, Fabris F

Dipartimento di Scienze Mediche e Chirurgiche, Università di Padova, Italy

In patients with heparin-induced thrombocytopenia (HIT), antibodies are detectable by antigen and activation assays; the antigen assay is based upon the reactivity of antibodies with PF4/heparin complex while activation assays detect platelet activating properties of antibody in the presence of heparin. The observation that platelets can take up mepacrine into the dense granules, suggested the possibility of developing a non-radioactive functional alternative to the [^{14}C]-serotonin release method. The test samples included plasma from 24 patients with a clin-

ical diagnosis of HIT (true positive) and 24 patients with idiopathic thrombocytopenic purpura (ITP) (true negative). Platelet pool from 3 patients with a history of HIT were washed by differential centrifugation, labeled with 4 μM mepacrine (37°C for 30min), and then resuspended in buffer containing 2 mM Ca (200×10^9 platelets/L). The test mixture consisted of 20 μL of test plasma, 10 μL of buffer solution or heparin (0.3 IU/mL and 100 IU/mL) and 70 μL of mepacrine-labelled platelets. The variation of mepacrine associated fluorescence was detected by flow-cytometry after gentle agitation (1 h at 25 °C) of the mixture. The extent of platelet activation was determined by 5 μM ionophore. A test result was considered positive if there was a decrease of fluorescence (> 10%) at 0.3 IU/mL heparin, which was reversed at 100 IU/mL heparin. Mepacrine release assay (MRA) was compared (χ^2 and Spearman's R tests) with heparin induced platelet activation assay (HIPAA) and polyanion/PF4 ELISA (GTI, WI, USA). The sensitivity and specificity of MRA was 46% and 100% respectively based on clinical diagnosis of HIT, whereas the sensitivity of HIPAA was 62% and that of polyanion/PF4 ELISA was 75%. Considering the immune diagnosis of HIT (presence of Ab-anti polyanion/PF4), the percentage of sensitivity and specificity was, respectively, 61% and 100% for MRA and 78% and 83% for HIPAA. Statistical analysis showed a significant correlation between MRA and HIPAA ($p<0.007$). In conclusion, MRA assay may be a promising functional test in HIT diagnosis.

C015

ISOLATION AND CHARACTERIZATION OF AN ANTI-FACTOR V ANTIBODY CAUSING ACTIVATED PROTEIN C-RESISTANCE FROM A PATIENT WITH SEVERE THROMBOTIC MANIFESTATIONS

Simioni P, Kalafatis M, Tormene D, Beck DO, Luni S, Girolami A

Department of Medical and Surgical Sciences, Second Chair of Internal Medicine, University of Padua, Italy; and Department of Chemistry Cleveland State University, Cleveland, OH, and Department of Molecular Cardiology the Lerner Research Institute, the Cleveland Clinic Foundation, Cleveland, OH, USA

Anti-factor V inhibitory antibodies have always been associated with bleeding disorders and impaired procoagulant activity of factor Va. A 35-year-old woman with a history of severe thrombotic manifestations presented with a markedly reduced activated protein C-sensitivity ratio (APC-SR). DNA sequencing of and around the regions encoding the APC cleavage sites in the factor Va molecule excluded the presence of the factor V Leiden mutation and of other known genetic mutations. No antiphospholipid antibodies were present in the patient's plasma and both PT and aPTT were normal. The total immunoglobulin fraction was isolated from the patient's plasma using protein G-Sepharose and found to induce severe APC-resistance when added to normal plasma and to factor V-deficient plasma supplemented with factor V. Thus, the immunoglobulin fraction interferes with factor Va inactivation by APC. Immunoblotting and immunoprecipitation experiments with the total immunoglobulin fraction purified from the patient's plasma demonstrated that the antibody recognizes factor V, is polyclonal, and has epitopes on both the factor Va heavy and light chains. The inhibitor was isolated by sequential affinity chromatography on protein G-Sepharose and factor V-Sepharose. The isolated immunoglobulin fraction was found to inhibit factor Va inactivation by APC because of impaired cleavage at Arg306 and Arg506 of the heavy chain of the cofactor. Our data provide for the first time the demonstration of an anti-factor V antibody not related to the presence of antiphospholipid antibodies which is responsible for thrombotic rather than hemorrhagic symptoms because of selective interference with factor Va cleavage and inactivation by APC.

C016

MISDIAGNOSIS IN THE CONTEST OF PRENATAL DIAGNOSIS BY DIRECT SEQUENCE ANALYSIS USING POLYMERASE CHAIN REACTIONS PRODUCTS

Peyvandi F,* Garagiola I,* Palla R, Mannucci PM*

**A. Bianchi Bonomi, Hemophilia Centre, IRCCS Maggiore Hospital, Milan; °Fondazione Luigi Villa, Centro Studi di Patologia Molecolare applicata alla Clinica, Milan, Italy*

Molecular diagnostic tests are widely used in clinical medicine and polymerase chain reaction (PCR) - based techniques are of particular interest. However false positive and false negative results can be obtained if phenotype, family history and preclinical aspects are not globally considered. We report a problematic prenatal molecular diagnosis in a family with severe factor VII (FVII) deficiency. The parents were consanguineous and both showed phenotypically a heterozygous state. They have lost two children from severe CNS bleeding. Their third child (the proband) was asymptomatic and phenotypically was heterozygote. Sequence analysis of the FVII gene for the proband was normal and her father, surprisingly, was homozygous for a deletion mutation localized in exon 7 which leads to a stop codon. This molecular result was clearly in contrast with the phenotype. The mother was heterozygous for the same mutation. Since results were inconclusive, the whole genetic study was repeated for the second time. Sequence analysis showed heterozygosity for all three members, compatible with their phenotypes. To confirm our data the analysis was repeated another time. The homozygous state for the proband's father and the heterozygous state for the proband and her mother were found. This result was also confirmed by restriction enzyme analysis. To answer the question of what had lead to such different results, we analyzed the genomic sequence of the region that contains the mutation. The IVS7 of FVII gene is a region rich of repetitive sequences and this leads probably to a difficult primer annealing, causing the possibility of incorrect DNA sequence due to a PCR error product. To confirm this hypothesis we designed two internal primers to exon 7 to eliminate the possibility to have an error during amplification. All members of family were carrying the deletion in the heterozygous state in two different analyses. These data demonstrate that repeat sequences alter routine PCR amplification products and reduce accuracy. This type of error could lead to a wrong molecular diagnosis in the context of prenatal diagnosis.

Oral Communications
Homocysteine I

C017

SICKLE CELL DISEASE, VASCULAR ENDOTHELIUM SUFFERING, HYPERHOMOCYSTEINEMIA AND THROMBOEMBOLIC RISK

Musso R, Cultrera D, Sortino G, Ferlito C, Azzaro MP, Fichera E, Di Francesco E, Giustolisi R

Centro Regionale di Riferimento per l'Emofilia e la Trombosi, Istituto di Ematologia, Università di Catania, Italy

The clinical course of sickle cell disease (SCD) is remarkable for its episodic punctuation with microvascular occlusive events leading to painful crisis. Although the precise pathophysiology of vasocclusion is still understood only incompletely in SCD, several investigations have addressed the possibility that abnormal interactions between sickled erythrocytes (S-RBC), platelets, plasma adhesive proteins and endothelial cells alterations might cause micro- (and macro-) vascular occlusions and multiorgan damage (*Lubin BH NEJM 1997; 27:1623*). Previously, we reported that fibronectin (FN), factor VIII-von Willebrand factor (FVIII-vWF) and thrombospondin (TSP), well known adhesive glycoproteins which regulate the S-RBC adhesion to vascular endothelium (*Kaul DK et al. Proc Natl Acad Sci USA 1989; 86: 3356*), are increased in SCD plasma (*Musso R et al. Blood 1993; 82:472*). In addition, the continuous endothelial cell repair and vascular intima trophism in SCD would strictly depend on the vitaminic *status* of these patients. Therefore, the vitamin B_{12}, pyridoxine and folic acid plasma levels could be implicated in these homeostatic processes if we consider that deficiencies – induced by such vitamins – lead to a homocysteine dangerous plasma increase (*Cattaneo M. Thromb Haemost 1999; 81:165*). In this regard, even a moderate rise in homocysteinemia in SCD patients might further contribute to the vascular occlusions. We firstly report that an abnormal increase of homocysteinemia is present in SCD patients. Fourteen SCD patients (8 females and 6 males, age ranging 24-59 yrs, β^+ thalassemia/S trait, 6 previously splenectomized, without renal or liver dysfunction) both in steady state and during painful episodes (n=21) were studied. Eleven healthy subjects, sex and age comparable, served as controls. FVIII-vWF (ELISA, Diagnostica Stago) and soluble thrombomodulin (sTM), as surrogate markers of vascular disease, were assayed. Thrombin-antithrombin (TAT) complex and prothrombin fragment (F1+2)(ELISA, Behring kit) as indices of thrombin generation *in vivo*, were determined. Plasma D-dimer (ELISA, Behring kit), as indicator of fibrin deposition *in vivo* was evaluated. Citrate plasma homocysteinemia (ELISA, BioRad kit) was measured. As expected, we found an abnormal increase of FVIII-vWF and sTM in conjunction with elevated plasma levels of the indices of thrombin activation in SCD patients both in steady state and during painful crisis respect to the control ones. Interestingly, a moderate significant ($p<0.001$) rise of plasma homocysteinemia was noted in all patients respect to healthy controls (*see Table below*).

	FVIII-vWF (%)		sTM(ng/mL)		Prothrombin F1+2 (nM/L)	
	Ss	Pc	Ss	Pc	Ss	Pc
Patients (n=14)	61±23	232±28	59±16	73±21	4.78±2.02	6.91±3.5
Controls (n=13)	95±9		26± 11		0.93± 0.06	

	TAT (mg/L)		D-dimer (µg/mL)		Homocysteinemia (µmol/L)	
	Ss	Pc	Ss	Pc	Ss	Pc
Patients (n=14)	7.71±2.6	9.8±4.1	2.34±0.92	4.9±1.83	15.3±2.9	16.1±4.7
Controls (n=13)	3.03±1.82		0.26±0.07		9.1±2.3	

Ss: steady state; Pc: painful crisis; * $p<0.001$ vs controls; **$p<0.001$ vs baseline.

From our observation we confirm that in SCD patients a chronic endothelial damage is present also in steady state associated with continuous plasma thrombin generation. The moderate increase of homocysteinemia might work in the same direction by contributing to the impaired microcirculatory reperfusion with district *no-reflow* phenomena and thromboembolic complications often seen in SCD. Therefore, the preventive supplementation by pyridoxine plus folic acid and vitamin B_{12} should be warranted in SCD patients.

C018

FASTING AND POSTMETHIONINE LOAD HYPERHOMOCYSTEINEMIA IN CENTRAL RETINAL VEIN OCCLUSION

Mazzola G,* Lattanzio R,° Maestranzi G,° Tavola A,° Brancato R,° D'Angelo A*

**Coagulation Service and Thrombosis Research Unit and °Dept. of Ophthalmology and Visual Sciences, IRCCS H S. Raffaele, Milan, Italy*

To evaluate the prevalence and potential implication of hyperhomocysteinemia (HHcy) in cetnral retinal vein occlusion (CRVO), we evaluated 139 consecutive patients (83 men and 56 women; median age: 58 yrs) with CRVO referred to our Institution over a period of 2 yrs. Sixteen patients (11.5%) had suffered recurrent CRVO. At the time of blood sampling, 53 patients (38.1%) were 50 yrs or younger. HHcy was determined by fasting and post-methionine load (δ PML, increment in tHcy over fasting levels 8 hrs after oral intake of D-L methionine, 0.1 g/kg b.w.-). Total plasma homocysteine (tHcy) measurements were established according to gender-specific 95th percentiles of the tHcy distribution in a reference population of 103 apparently healthy controls (59 men and 44 women, median age: 35 yrs) recruited during the time-frame of the study. Additional risk factors for CRVO were evaluated. *Results*: Mantel-Haenszel odds ratios (adjusted for gender) for HHcy in younger CRVO patients were 2.77 ($p = 0.055$) in the fasting state, 3.07 ($p= 0.03$) after methionine load and 3.59 ($p=0.003$) for the sum of fasting and PML hyperhomocysteinemia (fasting HHcy + PML HHcy not associated with fasting HHcy). In elderly CRVO patients, corresponding odds ratios were 3.87 ($p = 0.003$), 3.74 ($p = 0.01$) and 4.91 ($p = 0.00006$). Among potential risk factors for CRVO, hypertension, diabetes, hypercholesterolemia, personal history of vascular disease and glaucoma were all more prevalent in elderly patients, while smoking, pregnancy and contraceptive drug assumption were more prevalent in younger CRVO patients.

However, in a generalized linear model there was no statistically significant dependence of tHcy levels on these additional risk factors. APC-resistance was found in 5 patients and in 2 controls (OR = 2.2, ns). Our data support the hypothesis that HHcy is an independent risk factor for CRVO, and that PML tHcy determinations may increase the number of HHcy patients identified. In view of the relevant incidence of recurrent events, a trial is warranted evaluating the impact of multivitamin treatment in HHcy patients with a first episode of CRVO.

C019

HYPERHOMOCYSTEINEMIA PREDICTS PROGRESSION AND SEVERITY OF PERIPHERAL ARTERIAL DISEASE IN TYPE 2 DIABETIC PATIENTS

Ciccarone E,*° Di Castelnuovo A,* Vischetti M,* Assanelli D,@ Archetti S,^ Brentana L,@ Bani P,^ Ruggeri G,^ Salcuni N,# Capani F,° Donati MB,* Iacoviello L* on behalf of GENDIABE investigators

**"Angela Valenti" Laboratory of Genetic and Environmental Risk Factors for Thrombotic Disease, Department of Vascular Medicine and Pharmacology, Consorzio Mario Negri Sud, Santa Maria Imbaro; °Department of Medicine and Ageing and #Institute of Radiology University of Chieti, Chieti and @Chair of Cardiology, University of Brescia and 53th Laboratory, Spedali Civili, Brescia, Italy*

Mild hyperhomocysteinemia is an independent risk factor for cardiovascular disease. We aimed at investigating whether homocysteine levels are associated with the presence of peripheral arterial disease (PAD), its progression (indicated by the increasing number of affected vessels) and severity (indicated by the type of vessel involved:) in patients with type 2 diabetes and whether this relation is modulated by the (C677→T) polymorphism in the MTHFR gene. From a cohort of 944 patients with type 2 diabetes, 144 patients with PAD were identified, (diagnosis confirmed by color-duplex ultrasonography) and matched for age and sex with 288 control patients with type 2 diabetes without macrovascular complications. PAD-patients were subdivided according to the progression of the disease in: 1) only diffuse calcification of all districts without stenosis or occlusions, 2) one to two, 3) more than three stenotic or occluded arteries. Moreover, patients with stenosis or occlusions of any extent were subdivided into having small vessel-SVD and large vessel disease-LVD. Homocysteine was measured in 132 PAD- and 206 control-patients. There were no differences in homocysteine levels between diabetic patients with and without PAD (11.6±5.4 μmol/L vs 11.6±6.2 μmol/L). However, within PAD patients, homocysteine levels were significantly associated with the progression of PAD ($p<0.05$) and with the presence of LVD ($p=0.02$, analysis for trend). In a multivariate analysis in control patients, female gender, history of hypertension, high BMI, low levels of vitamin B12 and folic acid were significantly associated with elevated homocysteine levels; in PAD-patients hyperhomocysteinemia was only associated with folic acid. MTHFR gene polymorphism was not associated with homocysteine levels, nor with the risk of PAD. In conclusion, hyperhomocysteinemia was found to be associated with the severity and progression of PAD.

C020

HIGH PREVALENCE OF HYPERHOMOCYSTEINEMIA IN ATRIAL FIBRILLATION PATIENTS WITH HISTORY OF TRANSIENT ISCHEMIC ATTACKS/STROKE

Poli D, Antonucci E, Cecchi E, Morettini A,^ Nozzoli C,* Alterini B,# Perfetto F,^^ Mugnaini C,** Fedi S, Gensini GF, Prisco D

*Dipartimento di Area Critica Medico Chirurgica, Università degli Studi di Firenze, Centro di Riferimento Regionale per la Trombosi, ^Medicina Generale 1°; *Medicina Generale 2°; #Medicina Generale 4,°Azienda Ospedaliera Careggi; ^^Dip.to Medicina Interna U.O. Patologia Medica 1°Università degli Studi di Firenze; **Medicina Generale 2° Ospedale Santa Maria Annunziata, Florence, Italy*

Atrial fibrillation (AF) is an important risk factor for ischemic stroke. Moderate hyperhomocysteinemia is a mild risk factor for arterial and possibly venous thrombosis. The aim of this study was to measure homocysteine in AF patients with and without a history of cerebral embolism to investigate whether hyperhomocysteinemia can play a role in the occurrence of TIA and stroke in AF. Ninety-eight consecutive patients (42 females, 56 males, mean age 73.3±8.4) with AF who had had TIA (39 patients) or stroke (57 patients) were studied (Group 1). Eighty-two patients (27 females, 55 males, mean age 72.6±8.4) with AF and without a history of cerebral embolism served as controls (Group 2). Plasma homocysteine was measured by an automated fluorescence polarization immunoassay (FPIA) using a commercial kit (IMX system, ABBOT Diagnostics, Oslo, Norway). Normal values of our laboratory were: <19 μm/L in males and <13 μm/L in females. No difference was found with regard to mean homocysteine plasma levels between the two groups (16.4±7.4 and 15.1±6.0 respectively). However, we observed a trend to higher prevalence of moderate hyperhomocysteinemia in AF patients who experienced TIA or stroke in comparison to those without history of cerebral embolism (42% vs 30%). In conclusion this study indicates that: 1) there is a high prevalence of moderate hyperhomocysteinemia in AF patients; 2) this prevalence tends to be higher in those patients with a history of cerebral embolism. Further studies are needed to confirm these data in a large number of patients.

C021

HIGH PLASMA HOMOCYSTEINE CONCENTRATIONS IN CEREBROVASCULAR PATIENTS WITH FABRY'S DISEASE

Fedi S, Buechner S,* Gensini F,** Rizzuti G, Martinelli F,* Bandinelli R,° Coppo M, Arnetoli G,* Abbate R, Borsini W*

*Dipartimento Area Critica Medico-Chirurgica, *Dipartimento di Scienze Neurologiche e Psichiatriche; **Dipartimento di Fisiopatologia Clinica, Sezione di Genetica Medica; °Laboratorio Analisi Careggi; Università di Firenze; Centro Trombosi, A.O. Careggi, Florence, Italy.; Gruppo per lo Studio della Malattia di Fabry*

Fabry's disease (FD) is a rare X-linked recessive lysosomal storage disease secondary to deficiency of α-galactosidase A with resulting glycolipid accumulation, particularly globotriaosylceramide (Gb3) in numerous cell types including vascular endothelial cells. Affected patients have microvascular disease of the kidneys, heart, and brain. Cerebrovascular ischemia in FD has been largely attributed to the progressive deposition of Gb3 in

vascular endothelium, giving rise to thromboembolic events. The aim of this study was to evaluate the role of prothrombotic risk factors in the clinical presentation. We evaluated conventional risk factors for cerebrovascular disease and homocysteine, vitamin B6 and B12, folic acid, lipoprotein (a) and C677T MTHFR mutation in 9 patients with Fabry's disease (4 homozygous and 5 heterozygous). Four out of nine had recurrent strokes or Parkinsonism with multinfartual encephalopathy. The patients were normotensive, normocholesterolemic, non-diabetic and with normal Lp(a) values. In the 4 patients with cerebrovascular disease we observed hyperhomocysteinemia, low levels of folate, vitamin B6 and B 12, and C677T MTHFR mutation.

Pt, sex, family	Age yrs	Clinic manifestations	Hcy µmoli/L (NV: M <19, F <13)	FA ng/mL (NV: 3-17)	V. B12 pg/mL (NV: 180-970)	V. B6 pg/mL (NV: 23-35)	Mutation MTHFR C677T	Lp(a) mg/L
VE, M, 1	44	Stroke Renal Tx A*	22.0	3.5	303	8.6	Heterozygous	150
CG, F, 2	53	Stroke Parkinson	15.6	2.4	267.0	12.5	Heterozygous	117
CL, F, 2	55	Stroke	14.4	4.8	647	5.7	Homozygous	87
VA, M, 2	32	A*	10.7	3.3	390	4	Negative	26
BA, F, 2	55	-	11.0	3.4	198	3.7	Homozygous	11
BN, F, 2	48	-	10.8	6.1	319	4	Heterozygous	56
MS, M, 2	33	Strokes A*	25.6	1.4	170	2.9	Homozygous	78
MM, F,3	46	-	6.5	8.3	819	5.5	Negative	113
RR, M, 3	21	A*	10.3	3.9	884	6.6	Heterozygous	34

*A = Angiocheratoma 344 ; Hcy: homocysteine; FA: folic acid; Lp(a): lipoproteina A.

These results suggest that hyperhomocysteinemia may contribute to the cerebral ischemic events in FD patients. The decreased levels of vitamins, possibly influenced by involvement of the gastrointestinal apparatus, indicate the appropriateness of vitamin administration in these patients.

C022

VITAMIN SUPPLEMENTATION IN PATIENTS WITH MODERATE HYPERHOMOCYSTEINEMIA AND A HISTORY OF THROMBOSIS

Mazzola G, Crippa L, Fattorini A, Fermo I, Viganò D'Angelo S, D'Angelo A

Coagulation Service and Thrombosis Research Unit and Department of Laboratory Medicine, IRCCS H S.Raffaele, Milan, Italy

To evaluate the effects of vitamin supplementation on fasting and post-methionine load (6 h) hyperhomocysteinemia (HHcy), 34 patients with a history of venous and/or arterial thrombosis (17 men and 17 women, mean age 50.8 yrs±14.7) and fasting HHcy (n = 14, group 1), methionine intolerance (n = 11, group 2) or both (n = 9, group 3), were submitted to a one-month course of treatment with either folate (5 mg/day) + vitamin B12 (10 mg/week, groups 1 and 3) or vitamin B6 (300 mg/day) ± folate and vitamin B12 (group 2). Pretreatment PLP levels were not different in the 3 groups of patients, while folate levels were higher in patients with isolated methionine intolerance than in the other groups ($p \leq 0.04$), and vitamin B12 levels lower in patients from group 3 than in the other groups ($p \leq 0.001$, Table 1). After a first course of treatment, normalization of homocysteine levels was observed in 14 of 14 patients with isolated fasting HHcy and in 3 of 9 patients with fasting HHcy + methionine intolerance. Vitamin B6 supplementation normalized methionine intolerance in 4 of 8 patients, and vitamin B6 + folate and vitamin B12 in 2 of 3 patients. Vitamin B6 + folate and vitamin B12 was then administered to 3 patients with methionine intolerance who had not responded to vitamin B6 only and to 6 patients from group 3 who had not responded to treatment with folate and vitamin B12. Normalization of homocysteine levels was observed in 2 of 3 patients and in 3 of 6 patients, respectively (Table 2). Post-methionine load HHcy may respond to treatment with vitamins involved in homocysteine remethylation.

Table 1.

Groups	Fasting tHcy (µmol/L)	PML Δ tHcy (µmol/L)	Folate (ng/mL)	Vit. B12 (pg/mL)	PLP (pmol/L)
Group 1 (n = 14)	27.2±8.1	18.0±3.6	3.4±1.1	324±73	21±12.2
Group 2 (n =11)	8.4±1.7	37.3±13.7	5.4±2.8	369±148	30.1±18.5
Group 3 (n = 9)	43.1±29.0	35.4±12.6	3.5±2.6	192±43	21.0±10.0
p	0.0001	0.0001	0.036	0.001	ns

Table 2.

	B6 (n =3)	Folate, B12, B6 (n = 3)	Folate, B12 (n = 6)	Folate, B12, B6 (n = 6)
Fasting tHcy µmol/L	7.1±0.4	4.5±0.5	28.7±28.9	21.5±22.1
% of pretreatment levels	84±11	54±11	64±26	47±11
p	ns	0.03	0.03	0.001
PML δtHcy µmol/L	29.1±0.5	16.7±4.5	28.1±8.7	21.6±13.1
% of pretreatment levels	70±25	38±3	87±41	70±54
p	ns	0.002	ns	ns

C023

LOW RISK OF THROMBOSIS IN FAMILY MEMBERS OF PATIENTS WITH HYPERHOMOCYSTEINEMIA

Bucciarelli P, Martinelli I, Zighetti ML, Cafro A, Valsecchi C, Mannucci PM

Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Institute of Internal Medicine, IRCCS Maggiore Hospital, University of Milan, Italy

Mild to moderate hyperhomocysteinemia, a metabolic disorder due to genetic and/or acquired factors, is associated with an increased risk of venous and arterial thrombosis. Beside being a thrombotic risk factor *per se*, hyperhomocysteinemia increases the risk of venous thrombosis in patients with factor V Leiden, the most common inherited determinant of thrombophilia. To answer the question on the usefulness of measuring homocysteine in members of families of hyperhomocysteinemic patients, we investigated relatives of patients who developed arterial or venous thrombosis and were found to have hyperhomocysteinemia (n=171) or the factor V Leiden or the G20210A pro-

thrombin mutation (n=325). In the first group, the prevalence of hyperhomocysteinemia was 16.4%, and the relative risk of thrombosis in relatives with hyperhomocysteinemia compared to those without was not increased (odds ratio 1.2; 95% CI 0.24-4.2), with similarly low absolute annual incidences of thrombosis of 0.28% and 0.24%. Among relatives of index patients with factor V or prothrombin mutations, the prevalence of hyperhomocysteinemia was 8.6%. Compared to relatives with no coagulation factor mutation and normal homocysteinemia, the relative risk of thrombosis for relatives with both a coagulation factor mutation and hyperhomocysteinemia was 9.5 (95% CI 0.9-105) and that for relatives with a coagulation factor mutation but normal homocysteinemia was 7.5 (95% CI 1.0-58), with similarly low absolute annual incidences of thrombosis of 0.19% and 0.15%. We conclude that the low prevalence of hyperhomocysteinemia among the relatives of the two groups of index patients chosen for this study, as well as the observation that hyperhomocysteinemia is not associated with an increased risk of thrombosis in these individuals, does not justify family screening either in relatives of index patients with hyperhomocysteinemia or in those with factor V Leiden or prothrombin mutation.

C024

VITAMIN SUPPLEMENTATION REDUCES THE PROGRESSION OF ATHEROSCLEROSIS IN HYPERHOMOCYSTEINEMIC RENAL TRANSPLANT RECIPIENTS

Marcucci R, Zanazzi M,* Bertoni E,* Salvadori M,* Castellani S, Polidori G, Bagnoli M, Fedi S, Abbate R

*Dipartimento Area Critica Medico-Chirurgica, Università di Firenze; *UO Nefrologia, Dialisi e Trapianto, and Centro Trombosi, Azienda Ospedaliera Careggi, Firenze, Italy*

Cardiovascular diseases are the main causes of morbidity and mortality in kidney transplant recipients. Among these patients, we previously demonstrated a high prevalence of hyperhomocysteinemia which might account for their higher cardiovascular risk. The aim of our study was to document, in hyperhomocysteinemic renal transplant recipients, the effect of vitamin supplementation on carotid intima-media thickness (cIMT), an early sign of atherosclerosis which has been associated with risk factors for cardiovascular disease such as blood pressure, diabetes and smoking. Fifty-six stable hyperhomocysteinemic renal transplant recipients were randomly assigned to either vitamin supplementation (folic acid 5 mg/die; vitamin B6 50 mg/die; vitamin B12 400 mg) (group A) or placebo treatment (group B). All subjects underwent cardiovascular risk factor assessment, including fasting homocysteine (Hcy) levels assay, and high resolution B-mode ultrasound to measure IMT of common carotid arteries, before and after 6 months of vitamin supplementation. Three patients of group A discontinued the therapy and were excluded from the study. No side effects were observed. The prevalence of the common cardiovascular risk factor in the two groups was similar; all patients were on a triple immunosuppressive therapy. Fasting Hcy levels markedly decreased in group A after treatment (29.7±16 μmol/L vs 9.2±1.9 μmol/L; $p<.0001$), whereas no significant changes were observed in group B (22.8±5.4 mmol/L vs 22±5.2 mmol/L;p=ns). In group A, cIMT significantly decreased after treatment (0.95±0.20 mm vs 0.64±0.17 mm: $p<.0001$). All but one patient showed a reduction of cIMT and the mean percentage of cIMT decrease was -32.2±12.9. In hyperhomocysteinemic patients without vitamin supplementation (group B) we documented a significant progression in cIMT after 6 months (0.71±0.16 mm vs 0.87±0.19 mm;$p<0.05$). In 19/28 subjects we observed an increase in cIMT, and in 9/28 the cIMT was unmodified. The mean percentage of cIMT increase was +23.3±21.1.In conclusion, our results demonstrate a beneficial effect of the treatment of hyperhomocysteinemia by vitamin supplementation on an early signs of atherosclerosis in a group of renal transplant recipients.

Oral Communications
Genetic Determinants of Atherothrombosis I

C025

THE C807T POLYMORPHISM IN THE PLATELET GLYCOPROTEIN IA GENE AND THE RISK OF ISCHEMIC STROKE IN THE YOUNG

De Stefano V, Chiusolo P, Paciaroni K, Rossi E, Di Lazzaro V,* Rasura M,° Fieschi C,° Leone G

*Departments of Hematology and *Neurology, Catholic University, and °1st Dept. of Neurology, University "La Sapienza", Rome, Italy*

Membrane glycoprotein (GP) Ia/IIa mediates platelet adhesion to collagen; the C807T polymorphism in the GP Ia gene correlates with a variable expression of the platelet surface receptor, the TT genotype being associated with a higher receptor density. We evaluated the possible role of the GP Ia C807T polymorphism as a risk factor for ischemic stroke. We investigated 256 patients (M/F 108/148) with a history of ischemic stroke before 50 years documented by CT or NMR scan; the mean age at the thrombotic event was 36 years (median 37, range 1 to 50). In 137 of them ischemia occurred in the absence of acquired risk factors (smoke, hypertension, diabetes, dislipidemia, oral contraceptive intake, antiphospholipid antibodies). The control group consisted of 312 healthy individuals (M/F 212/100, mean age 47 years, median 49, range 14 to 93). All individuals were of Italian ancestry. The 807 GP Ia genotype was TT in 28 patients (10.9%) and 15 controls (4.8%, p= 0.006), CT in 133 patients (51.9%) and 142 controls (45.5%, p= 0.12), and CC in 95 patients (37.1%) and 155 controls (49.7%, p= 0.001). No significant difference was found between the distribution of the genotypes TT (p= 0.84), CT (p= 0.70), and CC (p= 0.51) among the patients with the presence or the absence of acquired risk factors. The odds ratio for ischemic stroke among the 807 TT homozygotes was 2.4 (95% CI 1.3-4.7) in comparison with C-allele carriers and 3.0 (95% CI 1.5-6.0) in comparison with 807 CC homozygotes; in these past the risk of ischemic stroke was 0.6 (95% CI 0.4-0.8) in comparison with T-allele carriers. In conclusion in the Italian population the GP Ia 807 TT genotype is associated with an increased risk for ischemic stroke before 50 years of age; conversely, the GP Ia CC genotype seems to represent a protective factor.

C026

THE 715PRO ALLELE OF P-SELECTIN THR715PRO POLYMORPHISM INFLUENCES MORTALITY IN PATIENTS WITH ACUTE ISCHEMIC DISEASE REFERRED TO A CARDIOLOGIC INTENSIVE CARE UNIT

Gori AM, Pepe G, Giusti B, Evangelisti L, Falai M, Margheri M, Sofi F, Brogi D, Abbate R, Gensini GF

Dipartimento Area Critica Medico-Chirurgica, Universita' degli Studi di Firenze; Centro Trombosi, Azienda Ospedaliera Careggi, Florence, Italy

The ECTIM study, involving different regions of Europe, showed that Thr715Pro P-Selectin polymorphism was not homogenously distributed in Europe and that this polymorphism was significantly associated with a reduced risk of myocardial infarction in patients from United Kingdom, but not in patients from France. It has been suggested that the Pro715 allele of the P-Selectin polymorphism influenced the cardiovascular mortality, but the effective role of this polymorphism remains to be elucidated. In 274 acute coronary syndromes (ACS) patients, referred to a Cardiologic Intensive Care Unit (178M/96F, median age 62, 34-81 years) and in 290 controls, age and sex-matched, recruited from the same geographical areas, we evaluated the prevalence of the P-selectin polymorphism and the role of this polymorphism on cardiovascular mortality. P-selectin polymorphism was analyzed by PCR and RFLP methods. The Pro715Pro and Thr715Pro genotype distributions in ACS patients were similar (23.0%) to that found in controls (21.7%). No significant association between the genotypes Pro715Pro and Thr715Pro and the clinical manifestations (unstable angina and myocardial infarction) of the atherosclerotic disease was observed. Similarly, the frequencies of the Pro715Pro and Thr715Pro genotypes were not significantly different among the patients with one or more stenosed vessels. During the clinical follow-up, after a mean of 16 months, 11.4% of ACS patients died of cardiovascular causes. The Kaplan-Meier survival curves showed that the Pro715Pro and Pro715Thr genotypes were significantly (p<0.05) associated with survival in patients with ACS, with higher rate of event-free survival in patients carrying the 715 Pro allele. In conclusion, our data did not show a significant different distribution of the P-Selectin polymorphism between patients with ACS and control subjects; the positive association between the Pro allele and the increased rate of event-free survival suggests a protective role for this genetic variant on cardiovascular mortality.

C027

INTERLEUKIN-1 GENE CLUSTER POLYMORPHISMS AND RISK OF CORONARY ARTERY DISEASE

Vohnout B, Di Castelnuovo A, Trotta R,* D'Orazio A, Panniteri G,† Montali A,‡ Donati MB, Arca M,‡ Iacoviello L

*"Angela Valenti" Laboratory of Genetic and Environmental Risk Factors for Thrombotic Disease, Istituto di Ricerche Farmacologiche Mario Negri, Consorzio Mario Negri Sud, Santa Maria Imbaro, *Dipartimento di Scienze Cardiovascolari, Università 'Federico II', Napoli; †II° Cattedra di Cardiologia; ‡Dipartimento di Terapia Medica, Università 'La Sapienza', Rome, Italy*

We studied the association of a variable number tandem repeat (VNTR) (86bp) polymorphism in intron 2 of interleukin-1 receptor antagonist (IL1-RA) and the –511 C/T polymorphism of IL-1ß with the risk of coronary artery disease (CAD). We compared 335 case (CAD+) patients with angiographically documented CAD (stenosis >50% in at least one major coronary artery) with 205 unrelated individuals free of CAD signs at angiogram (CAD- controls): 102 (30.5%) CAD+ patients had single- (SVD) and 233 (69.5%) multiple-vessel disease (MVD). There was no statistically significant difference in either genotype distribution or allele frequency of both IL-1 RA and IL-1β –511 C/T polymorphisms between CAD+ cases and CAD-controls. However, there was a significant difference in IL-1 RA genotype distribution between SVD and either MVD (p=0.024) or controls (p=0.04). As compared to allele 1 homozygotes the risk of SVD was reduced in heterozy-

gotes for 1 and 2 IL-1 RA alleles, but not in homozygotes for allele 2. Our study does not give sufficient evidence to support an association between IL-1 RA Intron 2 VNTR and IL-1β –511 C/T polymorphisms and the risk of CAD. A trend for different genotype effects in simple and multiple vessel coronary disease was observed that deserves further investigation.

C028

ENDOTHELIAL NITRIC OXIDE SYNTHASE POLYMORPHISMS IN CORONARY ARTERY DISEASE PATIENTS

Gensini F,* Fatini C, Brogi D, * Sticchi E, Sofi F, Margheri M, Giglioli C, Comeglio M, Abbate R, Gensini GF

*Dipartimento Area Critica Medico Chirurgica; *Dipartimento di Fisiopatologia Clinica, Unità di Genetica Medica; Università di Firenze; Centro Trombosi A.O. Careggi, Florence , Italy*

Nitric oxide (NO), produced by endothelial nitric oxide synthase (eNOS) encoded by the eNOS gene on chromosome 7, plays important roles in normal vascular homeostasis and its continuous generation serves to mantain basal vascular tone. It has been suggested that endothelial NO may have an important atheroprotective role beyond its effect on vessel tone and blood pressure. An alteration in the activity of the vascular NO system could contribute to the pathogenesis of atherosclerosis. In the eNOS gene two polymorphisms have been identified: in the 5'-flanking region a T-786C polymorphism has been demonstrated to reduce the eNOS gene promoter activity predisposing the patients carrying the C variant to coronary spasm. Moreover in exon 7 of eNOS gene a G894T polymorphism, which encodes a Glu298Asp amino acid substitution, has been hypothesized to modulate eNOS activity. The aim of our study was to investigate the role of these polymorphisms in 304 patients (192 males and 112 females) with coronary artery disease referred to the Coronary Intensive Therapy Unit of the University of Florence, and 320 matched healthy controls. eNOS polymorphisms were analyzed by RFLP analysis. The genotype distribution and allele frequency were significantly different between patients and controls for both polymorphisms (T-786C: p=0.0002 and p=0.0002; G894T: p<0.0001, p<0.0001). At univariate analysis the –786C and 894T variants were associated with the risk of coronary artery disease (-786C: OR=1.99, p<0.0001; 894T: OR=2.15, p=0.0003). Our results suggest a role of eNOS polymorphisms as risk factors for coronary artery disease and permit a better evaluation of the contribution of NO in the pathogenesis of the disease.

C029

A TISSUE-FACTOR POLYMORPHISM REDUCES THE RISK OF FAMILIAL MYOCARDIAL INFARCTION IN SMOKERS

Di Castelnuovo A, Cappuccilli ML, D'Orazio A, Bomba S, Donati MB, Iacoviello L

"Angela Valenti" Laboratory of Genetic and Environmental Risk Factors for Thrombotic Disease, Department of Vascular Medicine and Pharmacology. Istituto di Ricerche Farmacologiche Mario Negri, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy

Tissue factor (TF) is a transmembrane protein which forms a complex with factor VII and thus initiates cell-surface assembly and propagation of the coagulation protease cascade. The genetic variants of tissue factor can be considered of functional importance and candidates as thrombotic risk factor. We investigated the role of the Msp1 polymorphism located in intron 5 of the TF gene in a case-control study performed on 131 cases and 220 controls. Cases were MI patients over 45 years, selected among the GISSI-2 trial population on the basis of an interview regarding their family history of thrombosis. Controls were consecutive patients over the age of 45 years, without a personal or family history of vascular disease. Genotype distributions were in Hardy-Weinberg equilibrium, and they were similar in cases and in controls (p=0.19). The frequencies of the rare allele were 0.19 in cases and 0.25 in controls (p=0.067). We focused on the possible interactions of TF/Msp1 polymorphism with an important risk factor for MI such as smoke. A multivariate logistic regression analysis was used including a term for interaction of genotype with smoking, and age, sex, dyslipidemia, hypertension and diabetes as covariates. We found that TF/Msp1 polymorphism is associated with a reduced risk of MI in smokers. Smokers not carrying the mutation had a risk of MI of 6.19 (95%CI: 2.95-12.99), whereas in smoking carriers the risk was reduced to 2.74 (95%CI: 1.19-6.30), thus suggesting a protective role of TF/Msp1 polymorphism on the risk of MI in smokers by decreasing the effect of smoking. The Synergy Index for Interaction was 0.32 (95%CI: 0.11-0.95). Although our findings indicate a significant interaction between the carriership of the mutant allele and smoking habits, this protective effect should be explored in larger studies.

C030

GENETIC AND METABOLIC RISK FACTORS IN PATIENTS WITH ACUTE CORONARY SYNDROMES REFERRED TO A CARDIOLOGIC INTENSIVE CARE UNIT

Marcucci R, Fedi S, Pepe G, Fatini C, Margheri M, Giglioli C, Falai M, Valente S, Chechi T, Gensini GF

Dipartimento Area Critica Medico-Chirurgica, Università di Firenze; Centro Trombosi, Azienda Ospedaliera Careggi, Florence, Italy

Contrasting data are available on the prevalence of emerging risk factors in patients with acute coronary syndromes (ACS). The aims of our study were: 1) to evaluate the prevalence of two thrombophilic polymorphisms, FV Leiden and G20210A prothrombin polymorphism; and 2) to establish the role of two metabolic risk factors, lipoprotein (a) [Lp(a)] and homocysteine (Hcy) levels. We investigated 602 patients [425 M/177 F; age: 66 (34-87)] with ACS referred to a Cardiologic Intensive Care Unit and 320 controls [226 M/94 F; age: 64 (30-80)], age and sex-matched, recruited from friends or partners of patients. All patients underwent a coronary angiography: 508 underwent subsequent coronary revascularization (402 by coronary angioplasty, 106 by cardiac surgery) and 94 were treated by medical therapy. At the univariate analysis, the OR for the ACS were: FV Leiden 1.06 (0.5-2.2), p=ns; FII G20210A 2.5 (1.1-5.9), p<.05; Lp(a) >300 mg/L 3.1 (2.2-4.4), p<.001; Hcy >95th percentile of controls 10.2 (6-17.4), p<.001. At the multivariate analysis, adjusted for sex, age, and the traditional cardiovascular risk factors, only elevated Lp(a) and Hcy levels remained independent risk factors for ACS (Lp(a): 2.9 (1.3-6.5), p<.005; Hcy: 16.6 (6.4-43.1); p<.001). Furthermore, Hcy levels were significantly associated with the extent of CAD, in terms of number of vessels with stenosis >75%. Patients underwent clinical follow-up: after a mean of 16 months we documented 22/193 cardiovascular

deaths. The rate of event-free survival, in terms of cardiovascular death, was significantly lower in patients with hyperhomocysteinemia with respect to those with Hcy levels within the normal range (p<.05); Kaplan-Meier survival curves for vascular death showed a similar trend for patients with Lp(a) levels >300 mg/L with respect to patients with lower Lp(a) levels (p<.05). In conclusion, our data demonstrated the high prevalence of metabolic emerging risk factors, such as Hcy and Lp(a), in patients with ACS and their influence on cardiovascular death at folllow-up. This study stresses the need of including the determination of these parameters in the evaluation of the cardiovascular risk profile of the patient with ACS.

C031

ROLE OF GENETIC THROMBOPHILIA IN 254 CASES OF JUVENILE ISCHEMIC STROKE

Brancaccio V, Mandarini A,* Iannaccone L, Ames PRJ, Scenna G, Fasanaro AM,* Margaglione M°

*Unità Emostasi-Trombosi e *Divisione Neurologia, Ospedale "A. Cardarelli", Napoli; °Unità Aterosclerosi e Trombosi, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy*

Background. While genetic thrombophilic defects are well known risk factors of venous thrombosis, their role in arterial occlusion is still controversial. Nonetheless, the mutation A20210 of the prothrombin gene has been associated with ischemic stroke (IS) in some series. Moreover hyperhomocysteinemia is recognized as a risk factor of arterial thrombosis. *Methods*. We assessed genetic thrombophilia in 254 patients with juvenile IS (M/F 123/131; median age 39 yrs, range 4-50) and in 431 normal subjects: antithrombin, protein C, protein S deficiency, activated protein C resistance, factor V Leiden (FVL), prothrombin (FII) A20210, TT677 genotype of MTHFR. Levels of homocysteine (Hcy) were measured in 189 of 254 patients by an EIA method (BIO-RAD). One hundred and two patients (40%) were smokers, 51 (20%) hypertensive, 24 (9%) hyperlipidemic, 4 (2%) obese, 5 (2%) diabetic; five patients (2%) had ischemic cardiac disease or diffuse atherosclerosis. Twenty-three (17%) of the females were on oral contraception and 8 (6%) were pregnant or puerperae. Nine cases (3.5%) had had a previous venous thrombosis. Sixty patients (24%) had 2 or more risk factors. Ischemic stroke was confirmed by CT scan or MRI. *Results*. Two cases (0.8%) of deficit of natural anticoagulant (1 antithrombin, 1 protein C) were found. Among thrombophilic factors, genotype FVL was present in 19 patients (7%) and 22 controls (5%, NS); A20210/FII in 18 patients (7%) and 20 controls (4.7%, p=NS); TT677/MTHFR in 58 patients (23%) and 78 controls (18,1%, p=NS). Six patients (2.5%) had combined genotypes: FVL+A20210/FII 1, FVL + TT677/MTHFR 3, A20210/FII +TT677/ MTHFR 2. Forty-nine of 189 patients (26%) had high levels of Hcy: of these 51% had TT677/MTHFR genotype. Mean levels of Hcy were higher in TT677/MTHFR subjects (19.3±15.1 vs 10.7±4.4 μmol/L, p<0.0001). Among patients with previous venous thrombosis 2 had FVL, 2 A20210/FII, 1 FVL+A20210/FII, 1 FVL+ TT677/MTHFR. *Conclusions*. Natural inhibitors of coagulation deficiencies are rare in juvenile IS. FVL and A20210/FII genotypes identify patients at risk of venous thrombosis. Hyperhomocysteinemia is significantly associated with the TT677/MTHFR genotype and seems to be a relevant risk factor requiring an adequate therapeutic approach.

C032

INHERITED PRO-THROMBOTIC CONDITIONS IN YOUNG ADULTS WITH A HISTORY OF CEREBRAL VEIN THROMBOSIS. SIMILARITIES WITH AND DIFFERENCES FROM SUBJECTS WITH ARTERIAL ISCHEMIC STROKE

Madonna P, Tufano A, Coppola A, De Stefano V, Cimino E, Cirillo F, Cerbone AM, di Minno G

*Centro di Coordinamento Regionale per le Emocoagulopatie, Dipartimento di Medicina Clinica e Sperimentale, *Dipartimento di Scienze Neurologiche, Università degli Studi di Napoli "Federico II", Naples, Italy*

Factor V Leiden (FVL), G20210A mutation of prothrombin gene (FII20210A), and homozygosity for 677TT mutation of methylene-tetrahydrofolate reductase gene (MTHFRTT) have been associated with an abnormally high risk of thrombotic diseases. At variance with patients with arterial ischemic stroke (IS), an increased risk of cerebral vein thrombosis (CVT) has been associated with FII20210A and oral contraceptive drugs. We have evaluated the prevalence of FVL, FII20210A and MTHFRTT in 14 patients (5 M, 9 F; first event 31.7±13 years, range 14-48 years) with juvenile CVT, in 132 patients (66 M, 66 F; first event 34.8±10.9 years, range 1-50 years) with juvenile IS and in 262 apparently healthy subjects (117 M, 145 F; mean age 36±13.2 years). The frequency of these polymorphisms was not statistically different between IS patients and controls, being 7/132 (5.3%) vs. 17/262 (6.5%) for FVL, 10/132 (7.6%) vs 16/262 (6.1%) for FII20210A and 30/132 (22.7%) vs 45/262 (17.2%) for MTHFRTT; (p always>.05, χ^2-test). Among CVT patients, the frequency of heterozygous FII20210A was 5/14 (35.7%) that was significantly higher than that found in IS and in controls (p=0.005, OR 6.8, CI 1.6-28.5; p=0.0004, OR 8.5, CI 2.2-32.7 respectively: χ^2-test). The frequency of FVL and of MTHFRTT in patients with CVT was 7.1% (1/14) and 28.6% (4/14), respectively, higher than that found in IS patients and in controls, though not statistically different. In our female patients, 3/6 experienced CVT while using oral contraceptives: FII20210A was not present in any cases. Despite the limitations of the sample size, these data confirm the role of the FII20210A variant as a predisposing factor for CVT. The role of prothrombotic genetic polymorphisms in IS remains controversial. Whether and the extent to which thrombosis at this unusual site reflects a sustained hypercoagulable state needs to be evaluated in larger sample sizes.

Oral Communications
Thrombophilia I

C033

A TRIAGE TEST, BASED ON STANDARDIZED CLINICAL PROBABILITY AND D-DIMER, FOR EXCLUDING ACUTE VENOUS THROMBOEMBOLISM IN THE EMERGENCY WARDS

Siragusa S,* Granzow K, Buonanno C, Anastasio R,* Falaschi F, Porta C, Bressan MA

**Unità Malattie Tromboemboliche ed Emorragiche, Cattedra di Ematologia, Università di Palermo and Servizio Pronto Soccorso Accettazione Policlinico S. Matteo, Pavia, Italy*

The use of standardized clinical probability (SCP), either alone or in combination with other tests, has been claimed to be an important tool in the hands of physicians operating in emergency wards (EW) to safely identify those patients without acute venous thromboembolism (VTE) who do not require further examinations and can, therefore be discharged. In ordet to evaluate the safety of this approach in an EW, we evaluated prospectively 358 outpatients clinically suspected of deep vein thrombosis (DVT) and 89 of pulmonary embolism (PE). SCP and the D-dimer test (semi-quantitative latex assay, Dimertest®, Dade Behring) were performed immediately. Validated objective tests (compression ultrasonography for DVT patients and ventilation/perfusion lung scanning and/or spiral CT and/or pulmonary angiography for PE patients) were applied afterwards, within 48 hours in all patients. According to the test results, acute VTE was confirmed in 114 patients (84 DVT, 30 PE, 25.5% the whole case report, 95% CI 14.9-36.1). The prevalence of VTE was 8.4% (95% CI, 4.3-12.5) in low, 26% (19.3-32.7) in moderate and 48.3% (43.4-53.2) in high SCP assessed patients. In the table below the diagnostic accuracy of SCP is reported; patients with negative SCP and negative D-dimer were considered as not having VTE. We further investigated the accuracy of other combinations and, particularly, that of considering as *negative* also those patients with moderate probability; the sensitivity, specificity, positive and negative predictive values were 50.8%, 87.9%, 68.8% and 78.5%, respectively. At 3rd month follow-up, none of the patients with low SCP and negative D-dimer developed symptomatic recurrent events. The combination of low SCP and negative D-dimer can be safely used as a *triage test* for excluding acute VTE in the EW. All other combinations require mandatory objective tests.

	SCP alone	D-dimer alone	SCP + D-dimer
Sensitivity	87.7% (81-93.8)	84.2% (61.4-107)	99.1% (98.2-100.9)
Specificity	45.6% (40.3-50.9)	71.7% (67.2-76.2)	37.5% (32.4-42.6)
Positive PV*	64.4% (58.8-70)	49.4% (37.1-61.7)	64.8% (58.6-71)
Negative PV	89.9% (85.2-94.6)	92.9% (89.6-96.2)	99.2% (97.5-100.9)

*Predictive value

C034

THE ORAL DIRECT THROMBIN INHIBITOR XIMELAGATRAN AND ITS ACTIVE FORM MELAGATRAN FOR PROPHYLAXIS OF VENOUS THROMBOEMBOLISM AFTER TOTAL HIP OR TOTAL KNEE REPLACEMENT

Eriksson BI, Agnelli G, Cohen A, Dahl OE, Mouret P, Rosencher N, on behalf of the Methro Study Group

Sezione di Medicina Interna e Cardiovascolare, Dipartimento di Medicina Interna, Università di Perugia, Italy

Introduction. The anticoagulant ximelagatran is an oral direct thrombin inhibitor intended for the prophylaxis and treatment of thromboembolic complications. The efficacy and safety of ximelagatran, and its active form melagatran, were evaluated in patients undergoing total hip replacement (THR) or total knee replacement (TKR). *Methods*. Study 1 was a randomized, double-blind, controlled, dose-response study in which patients received 1 of 4 doses of subcutaneous (sc) melagatran (1, 1.5, 2.25, or 3 mg bid), followed by oral ximelagatran (8, 12, 18, or 24 mg bid) or sc dalteparin (5,000 IU od). Melagatran was given immediately before surgery and ximelagatran was started the morning after surgery. Study 2 was a randomized, double-blind, controlled study in which patients received sc melagatran (3 mg bid) started 4-12 h after surgery followed by oral ximelagatran (24 mg bid), or sc enoxaparin (40 mg od). In both studies, LMWH was started the evening before surgery, and all treatments were continued for 8-11 days. Bilateral venography was performed on the final day of treatment. *Results*. In Study 1, 1876 patients underwent THR (n=1270) or TKR (n=606). A highly significant dose-dependent decrease in venous thromboembolism (VTE) was seen with melagatran + ximelagatran for both THR (p<0.0001) and TKR (p<0.0014). The rate of VTE was significantly lower with the highest dose of melagatran + ximelagatran when compared with dalteparin (p<0.0001). In Study 2, of 2788 patients in the ITT population (n=1923 [THR] and n=865 [TKR]), 2268 (81.3%) had an evaluable venogram. The VTE rate was 31% in the melagatran + ximelagatran group and 27% in the enoxaparin group. In Study 1, total bleeding and transfusion rates were similar in the melagatran + ximelagatran groups, and, in both studies, comparable to those of LMWH. *Conclusion*: Sc melagatran followed by oral ximelagatran is efficacious and well tolerated for the prophylaxis of VTE following THR or TKR.

C035

THE INCIDENCE OF VENOUS THROMBOEMBOLISM IN ASYMPTOMATIC FAMILY MEMBERS WHO ARE CARRIERS OF FACTOR V LEIDEN. A PROSPECTIVE COHORT STUDY

Simioni P, Tormene D, Prandoni P, Zerbinati P, Gavasso S, Cefalo P, Girolami A

Department of Medical and Surgical Sciences, 2nd Chair of Internal Medicine, University Of Padua Medical School, Italy

In a prospective cohort study, we assessed the incidence of spontaneous and risk period-related venous thromboembolism (VTE) in asymptomatic family members of consecutive and unselected probands who experienced VTE and had the factor V Leiden mutation. No continuous anticoagulant prophylaxis was given to the study participants, except during risk periods for VTE. All thrombotic events were objectively documented by standard diagnostic tests. A total of 561 family members (older than 15

years of age) of 131 probands were included, 313 of whom were carriers (299 heterozygous and 14 homozygous) and 248 non-carriers of the factor V Leiden mutation. The average follow-up was 4 years (range, 4 months to 6 years). There were 1255 and 984 observation-years of follow-up in carriers and non-carriers, respectively. Eight episodes of VTE occurred in heterozygous carriers, resulting in an annual incidence of 0.67% (95% confidence interval [CI], 0.29 to 1.33). Two events occurred in the absence of associated risk factors determining an annual incidence of spontaneous VTE of 0.17% (95% CI, 0.02 to 0.6). Only one VTE (risk period-related) occurred in non-carriers, with an annual incidence of 0.1% (95% CI, 0.003 to 0.56). The relative risk for VTE in heterozygous carriers as compared to non-carriers of the factor V Leiden mutation was 6.6 (95% CI, 1.1 to 39.8). Risk period-related VTE occurred with an incidence of 18% and 5% per risk period in heterozygous carriers and in non-carriers, respectively. Thus, the low rate of thromboembolic events in asymptomatic family members carrying the mutation does not justify continuous anticoagulant prophylaxis. Screening families of symptomatic probands with the factor V Leiden mutation has the potential to identify those asymptomatic carriers who might benefit from thromboprophylaxis during risk-periods.

C036

INCREASED RISK FOR PULMONARY EMBOLISM COMPLICATING DEEP VENOUS THROMBOSIS OF THE LEGS ASSOCIATED WITH INHERITED ANTITHROMBIN III DEFICIENCY

De Stefano V, Paciaroni K, Rossi E, Leone G

Dept. of Hematology, Catholic University, Rome, Italy

To investigate the risk of embolization among patients with inherited thrombophilia and deep venous thrombosis (DVT) of the legs we studied 522 unrelated patients, 387 with DVT of the legs (M/F 174/213, median age 35 years, range 1 to 84), and 135 with DVT and pulmonary embolism (PE) (M/F 77/58, median age 43 years, range 3 to 80). Diagnoses of DVT and PE were objectively proven; in 207 cases (40%) DVT was unprovoked. No patient had overt neoplasia or autoimmune disease. Inherited thrombophilia was diagnosed in 133 (34.3%) of the patients with isolated DVT: 2 antithrombin (AT) deficiency, 21 protein C (PC) or protein S (PS) deficiency, 71 factor V Leiden (FV-L), 25 prothrombin G20210A (PT-GA), and 14 combined defects, in 3 cases associated with AT deficiency. Among the patients with DVT + PE 47 (34.8%, p=1.00) had inherited thrombophilia: 6 AT deficiency, 7 PC or PS deficiency, 19 FV-L, 11 PT-GA, 4 combined defects. The patients with AT deficiency were overrepresented in the group with PE (4.4%) in comparison with the group without PE (1.3%, p=0.03), with a relative risk for embolization 3.4-fold increased (95% CI 1.1-10.9). After adjustment for other thrombophilic defects, the relative risk for embolization was 8.2-fold increased (95% CI 1.7-39.8) in patients with DVT and isolated AT deficiency in comparison to those with DVT and normal genotype. No difference was found between the two patient groups as regards the distribution of other thrombophilic defects. The increased risk of embolization can be due to the more severe clinical penetrance of AT deficiency in comparison with other thrombophilic traits and to longer periods of inadequate anticoagulation during heparin treatment in comparison with patients with normal AT levels. Thus, patients with acute DVT are recommended to be screened early for the presence of AT deficiency in order to have the special care appropriate for individuals at higher risk of embolization.

C037

CLINICAL CHARACTERISTICS OF THE FIRST VENOUS THROMBOTIC EPISODE AMONG HOMOZYGOTES FOR FACTOR V LEIDEN

De Stefano V, Martinelli I, Legnani C, Rossi E, Grandone E, Castaman G, De Stefano V, Palareti G, Mannucci PM, Leone G

On behalf of the GIRTE (Gruppo Italiano per la Ricerca sulla Trombofilia Ereditaria)

In the frame of an Italian survey on patients with severe thrombophilia we collected the clinical data concerning 68 unrelated homozygotes for factor V Leiden (FV-AA), 11 of them also being heterozygous for prothrombin G20210A (PT-GA). All the individuals were the index patients of the respective kindreds and had been referred to the Thrombosis Centers for a history of objectively proven venous thrombosis. The individuals also carrying the PT-GA had their first clinical event at a younger age than the others; the two groups did not differ in the rate of unprovoked events or in the rate of superficial vein thrombosis (SVT) in respect to deep vein thrombosis (DVT) as heralding event (Table 1).

FV-AA + PT-GG	FV-AA + PT-GA	
Sex (M / F)	33 / 24	6 / 5
Age at first thrombosis (median, range)	33 (16 — 79)	26 (17 — 47)
Site of first thrombosis		
deep veins of the legs (DVT)	41 (72%)	9 (82%)
DVT+pulmonary embolism	5 (9%)	-
superficial veins (SVT)	10 (17%)	2 (18%)
hepatic vein thrombosis	1 (2%)	-
Unprovoked first thrombosis	27 (47%)	5 (45%)

The percentage of proximal vein thrombosis was 96% among FV-AA / PT-GG carriers and 83% among FV-AA / PT-GA carriers; the rate of pulmonary embolism among the 55 patients who had suffered from DVT was 9%. The event was unprovoked in 38% of the cases with DVT and in 83% of the cases with SVT (p= 0.008). The median age at the first event was 39 years (range 20 to 78) among the men and 26 years (range 16 to 79) among the women. The rate of unprovoked first events was higher among the men than among the women (67% vs. 21%, p< 0.001). Oral contraceptives (n= 12) or pregnancy (n= 7) triggered 65% of the first events among the women; in all such cases except one thrombosis involved the deep veins. In conclusion the risk for major clinical events associated with homozygosity for factor V Leiden seems increased by the presence of an acquired risk factor, in particular the use of oral contraceptives or pregnancy, both circumstances enhancing the plasma resistance to activated protein C.

C038

INHERITED THROMBOPHILIA: DIFFERENT CONTRIBUTION TO DEEP VEIN THROMBOSIS OF UPPER EXTREMITIES AND LOWER LIMBS

Coppola A, Madonna P, Tufano A, Cirillo F, De Stefano V, Loffredo F, Cerbone AM, Di Minno G

Centro di Coordinamento Regionale per le Emocoagulopatie, Dipartimento di Medicina Clinica e Sperimentale, Università "Federico II", Naples, Italy

Two hundred and eleven consecutive patients referred to our Center because of deep vein thrombosis of the lower limbs (LLDVT, n=181, 84 men, 97 women, mean age 38.3, mean age at event 32.6, range 6-49) or of the upper extremities (UEDVT, n=30, 14 men, 16 women, mean age 32.5, mean age at event 29.7, range 16-49), were screened for inherited thrombophilia (plasma AT, PC, PS; FV Leiden, FIIG20210A gene mutations). Patients with superficial vein thrombophlebitis or apparently isolated pulmonary embolisms were excluded. Ten patients, belonging to 7 unrelated families, carried natural anticoagulant deficiencies (6 AT, 1 PC, 1 PS): all but one were in the LLDVT group. After excluding these patients plus one (consanguinity with a propositus), the prevalence of FV Leiden was significantly higher in LLDVT than in a population (n=280, 118 males, 162 females, mean age 36.2) of controls (37/171, 21.6%, vs. 15/280, 5.4%, $p<0.00001$), the increase in the risk being of 4.8 (OR, 95%C:I. 2.5-9.7). FIIG20210A mutation was significantly more prevalent (29/171, 17.0% vs. 17/280, 6.1%, $p=0.0004$) in this setting, and associated with increased risk (OR 3.2, 1.6-6.2) as well. The association was stronger (OR 15.5, 1.0-87.5) when the coexistence of two mutations was found (n=9, 5.3%). At variance, in UEDVT patients, the prevalence of these polymorphisms was not significantly different than in controls (FV Leiden 2/29, 6.9%, FIIG20210A 3/29, 10.3%). Moreover, the overall prevalence of thrombophilic gene mutations was significantly higher in LLDVT than in UEDVT (59/171, 34.5% vs. 4/29, 13.8%, $p=0.04$, OR 3.3, 1.0-11.75). In both groups no difference was found (29.2% vs. 20.9%) in *idiopathic* events; in the latter, the prevalence of gene mutations was indistinguishable from that of *non-idiopathic* cases. Thus, in the present setting, inherited thrombophilia is only involved in LLDVT, while its contribution to UEDVT remains to be further investigated.

C039

RISK FACTORS FOR UPPER EXTREMITY DEEP VEIN THROMBOSIS

Verso M, Agnelli G, Nenci GG

Sezione di Medicina Interna e Cardiovascolare, Dipartimento di Medicina Interna, Università di Perugia, Italy

Background. Open questions remain with respect to prevalence, pathogenesis, diagnosis and prognosis of upper limb deep vein thrombosis (UL-DVT). *Study objective*. To assess the risk factors associated with UL-DVT. *Methods*. Consecutive patients referred to our Thrombosis Service between January 1995 and January 2002, with UL-DVT, were included in the study. *Results*. The diagnosis of UL-DVT was confirmed in 59 patients, 29 males and 30 females (mean age 52.1 yr, range: 20-88 yr). The distribution of thrombosis was 27 in right arm and 32 in left arm. All DVT extended to the axillo-subclavian axis. Edema, pain and visible collateral vein were the major clinical manifestations (respectively in 81%, 54% and 23.7% of patients). The diagnosis was confirmed by CUS in 58 patients and by venography in 1 patient. We found a risk factor in 50 patients (84.7%): a thrombophilic state in 10 (16.9%), pacemaker in 4 (6.7%), central venous catheters (CVC) in 10 (16.9%), malignancy in 16 (27.1%), miscellaneous risk factors in 11 (18.6%). The investigations for the Outlet Thoracic Syndrome ware conducted in 18 patients, and in 5 of these cases a positive test was identified (8.4 %). Six patients (10%) had previous DVT (2 patients of upper limbs and 4 of lower limbs). In 12 patients multiple risk factors were identified, in particular 6 patients had cancer and a CVC. In the remaining 9 patients (15.2%), DVT was idiopathic. Two cases (3.3%) of symptomatic, not fatal PE were observed during the hospital period. Two patients (3.3%) died during the hospital stay, but not of VTE. Fifty-two (88%) of the patients had an echographic follow-up (mean follow-up 12 months). *Conclusions*. In most patients with UL-DVT one or more risk factors can be found. Cancer (especially with an *in situ* CVC) and thrombophilic state are major risk factors for UL-DVT.

C040

PREVALENCE OF THROMBOPHILIC GENETIC MUTATIONS IN PATIENTS UNDERGOING THROMBOTIC EVENTS WHILE ON ORAL ANTICOAGULANTS: A CASE-CONTROL STUDY

Taliani MR, Castori L, Agnelli G, Nenci GG, Gresele P

Dipartimento di Medicina Interna, Sezione di Medicina Interna e Cardiovascolare, Università di Perugia, Italy

Background. Pooled data from studies assessing the efficacy of antithrombotic therapy for atrial fibrillation (AF) revealed an annual stroke rate of 1.4% in patients treated with oral anticoagulants (OAs). Myocardial infarction or recurrent venous thromboembolism also occur in a small proportion of patients undergoing chronic OA therapy for AF and/or prosthetic heart valve (PHV). No data evaluating thrombophilia as a risk factor for recurrent thromboembolism in anticoagulated patients are available. *Aim of the study*. To evaluate whether patients chronically treated with OAs for AF and/or PHV and a history of recurrent thromboembolism while on treatment have a higher prevalence of thrombophilic genetic abnormalities as compared to patients without recurrent thromboembolism. *Methods*. In a case-control study, patients with AF and/or PHV attending our anticoagulation clinic were carefully questioned about previous episodes of TIA, stroke and systemic embolism while on OAs. Patients with a history of thromboembolism (cases) were matched for gender, age, reason for anticoagulation, INR target, duration on anticoagulant therapy, family history of thromboembolism and personal history of thromboembolism with an equal number of controls followed in the same period in our clinic. A comparison of the prevalence of FVR506Q and Pt20210GA mutations between cases and controls was performed. *Results*. Sixty-seven out of 1,300 patients (5.15%) experienced a thromboembolic event (44 had a TIA or stroke, 23 had an arterial or venous thromboembolism) while on treatment. The mean age of these patients (28 males and 39 females) was 69.2±10.0 years with respect to 69.2±9.3 years of the control patients (28 males and 39 females). A genetic thrombophilic abnormality was revealed in 5 cases (7.5%; all patients had the FVR506Q mutation) and in 13 controls (19.4%; 11 had the FV R506Q mutation and 2 the Pt20210GA mutation) with an OR of 0.33 (95% CI 0.11-1.00). *Conclusions*. The presence of FVR506Q and Pt20210GA mutations is not a risk factor for thromboembolic complications in patients treated with OA.

Oral Communications
Neoplasia

C041

IN VITRO STUDY OF PROCOAGULANT ACTIVITY MODULATION BY RETINOIDS IN FRESHLY ISOLATED HUMAN ACUTE PROMYELOCYTIC LEUKEMIA BLASTS

Falanga A, Balducci D, Suardi S, Vignoli A, Marchetti M, Barbui T

Hematology Division, Ospedali Riuniti, Bergamo, Italy

Differentiating therapy with all-trans-retinoic acid (ATRA) induces the complete remission of human acute promyelocytic leukemia (APL) and a simultaneous rapid resolution of the associated coagulopathy of this disease. ATRA downregulates the expression of two cellular procoagulants, tissue factor (TF) and cancer procoagulant (CP), in human APL cells. Selective retinoids for CP and TF downregulation have been previously identified in the APL NB4 cell line, however it is not known whether these procoagulants are similarly regulated in APL cells freshly isolated from patients. ATRA and three synthetic retinoid analogs, Am580 (selective for the retinoic acid receptor [RAR] α), CD2019 (selective for RAR β) and CD437 (selective for RAR γ) were tested. APL cells were obtained from bone marrow specimens of four APL patients at the onset of disease. Isolated blasts were incubated with each retinoid (0.01 to 1 μmol/L) for 120h, then TF and CP were characterized and quantified in cell-sample preparations by chromogenic and immunological assays. The results (median of % inhibition) show that ATRA downregulates both TF (58%) and CP (74%) in fresh APL blast cells. Treatment with the synthetic retinoids shows that TF and CP were reduced by the RAR α agonist (Am580), 48 and 61%, respectively, by the RAR β agonist (CD2019), 37 and 85%, respectively, and by the RAR γ agonist (CD437), 0 and 23%, respectively. These data indicate that in freshly isolated APL cells, ATRA regulation of TF involves RAR α and β, as shown for the NB4 cell line. In contrast, CP modulation involves all the three types of RARs, differently from the NB4 cell line where RAR α plays a major role.

C042

CHARACTERIZATION OF PROCOAGULANT ACTIVITY INHIBITION BY ALL-TRANS RETINOIC ACID IN HUMAN BREAST CANCER CELLS

Marchetti M, Balducci D, Suardi S, Barbui T, Falanga A

Hematology Division, Ospedali Riuniti, Bergamo, Italy

All-trans retinoic acid (ATRA) inhibits tumor cell proliferation and downregulates cellular procoagulant activity (PCA), i.e. tissue factor (TF) and cancer procoagulant (CP). ATRA acts by binding to nuclear retinoic acid receptors (RARs) α, β, and γ. Selective RAR agonists have helped identifying receptors involved in CP and TF regulation in human acute promyelocytic leukemia NB4 cells. It is not known whether the same mechanisms are involved in breast cancer cell PCA inhibition. In this study we evaluated the effect of ATRA and three synthetic retinoid analogs, Am580, CD2019 and CD437 (selective for RAR α, β and γ, respectively) on the PCA and proliferation of the human breast cancer cell line MDA-MB-231. The cells were incubated for up to 96h with each retinoid (0.001 to 1 μmol/L) and tested for PCA (by chromogenic and immunological assays), apoptosis (by annexin V staining and Bcl-2 protein expression), and cell proliferation (by growth curves analysis). The results (% mean±SD inhibition vs control cells) show that ATRA significantly inhibited CP (36±1.4%, $p<0.05$) and TF (29±1.1%, $p<0.05$) activities. In the same experiments, ATRA had little or no effects on cell proliferation and apoptosis. The three RAR agonists were able to significantly reduce TF (Am580: 23±1.3%, CD2019: 22±1.6%, CD437: 25±1.4%, $p<0.05$) and CP (32±1.2%, 46±3.1%, and 38±1.6%, respectively; $p<0.05$) expression. Similarly to ATRA, Am580 and CD2019 slightly reduced cell growth, while CD437 significantly inhibited proliferation (1 μmol/L: 28±4.3%; $p<0.05$). None of the retinoids induced apoptosis. This study indicates that, differently from NB4 cells, in these breast cancer cells, ATRA modulates CP and TF expression through all RARs, and that effect occurs independently from retinoid antiproliferative effects. This may suggest that, on the basis of its anticoagulant capacity on tumor cells, ATRA might be considered also in the care of tumors not sensitive to its antiproliferative and/or apoptotic actions.

C043

EFFECT OF THE RETINOID RO41-5253 ON PROLIFERATION AND PROCOAGULANT ACTIVITY OF MDA-MB-231 HUMAN BREAST CANCER CELLS

Balducci D, Marchetti M, Suardi S, Barbui T, Falanga A

Hematology Division, Ospedali Riuniti, Bergamo, Italy

Retinoids are anti-tumor agents that can affect the procoagulant activity procoagulant activity (PCA) of malignant cells. Ro41-5253 is a selective antagonist of the retinoic acid nuclear receptor (RAR) α, that binds RARα but does not induce transcriptional activation and does not influence RAR/RXR heterodimerization and DNA binding. This retinoid does not inhibit proliferation or induce apoptosis in human estrogen receptor negative (ER-) MDA-MB-231 breast cancer cells. The effect of Ro 41-5253 on tumor cell PCA of these cells [i.e. tissue factor (TF) and cancer procoagulant (CP)] is yet unknown. In this study we investigated whether Ro41-5253 may affect the PCA of these cells, and the relation of such effect to cellular apoptosis and proliferation. MDA-MB-231 cells were incubated with increasing concentrations of Ro41-5253 [10^{-10} M to 10^{-6} M] for 96h and then tested for PCA (by functional chromogenic and immunological assays), apoptosis (by Annexin V staining and Bcl-2 protein expression) and cell proliferation (by growth curves analysis). Ro41-5253 was able to inhibit significantly both TF (by 32%±1.2%, $p<0.05$) and CP (by 34±1.4%, $p<0.05$) expression. In the same experiments, virtually no apoptosis and no inhibition of cell proliferation occurred. These results indicate that: 1. Ro 41-5253 modulates PCA in ER- cells and this reduction is independent from apoptosis and inhibition of cell proliferation; and 2. modulation of PCA by retinoids may occur at least in part independently from receptor activation for gene transcription by RAR. This retinoid antagonist is of potential clinical interest, as it may not induce the toxic side-effects linked with the most therapeutically active retinoids, which need retinoic-acid response elements activation.

C044

ENOXAPARIN FOR PROLONGED THROMBOPROPHYLAXIS IN PATIENTS UNDERGOING ABDOMINAL CANCER SURGERY: THE ENOXACAN II STUDY RESULTS

Bergqvist D, Agnelli G, Cohen AT, Eldor A, Nilsson PE, Lemoigne-Amrani A, Dietrich-Neto F, for the Enoxacan 2 Study Group

Academic Hospital, Uppsala, Sweden; Università di Perugia, Perugia, Italy; King's College School of Medicine, London, UK; Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; Malmö University Hospital, Malmö, Sweden; Aventis Pharmaceuticals

Background. The optimal duration of prophylaxis with LMWH after cancer surgery is unknown. *Methods.* A multicenter, double-blind, randomized, placebo-controlled study was conducted to evaluate the efficacy and safety of prolonged thromboprophylaxis with enoxaparin in patients undergoing elective curative surgery for abdominal malignancy. A standard 1-week thromboprophylactic regimen of enoxaparin was compared with a prolonged 4-week regimen. In the open phase of the study, patients received enoxaparin 40 mg SC OD for 8±2 days starting 10–14 hours before surgery. Patients were randomly assigned in a double-blind manner to either enoxaparin 40 mg SC OD or placebo for a further 21 days, for a total treatment duration of 28±3 days. Bilateral venography was performed on day 28±3 on asymptomatic patients; objective documentation of venous thromboembolism (VTE) was required in symptomatic patients. The primary efficacy endpoint of the study was the incidence of postoperative VTE verified on day 28±3 or earlier. *Results.* In the 332 patients evaluable for efficacy (n=165 for enoxaparin; n=167 for placebo), there was a significant 60% relative risk reduction in the rate of VTE among patients receiving prolonged prophylaxis (4.8% vs. 12.0%; [95% CI, 10.0%-81.5%]; p=0.02). This difference was maintained at 3 months (13.8% vs. 5.5%; p=0.01). During the double-blind period, proximal DVT occurred in 1 patient in the prolonged prophylaxis group and 3 patients in the placebo group, and there was 1 PE in the placebo group. The primary safety endpoint, the incidence of bleeding during the double-blind period, was reached in 13 (5.1%; n=253) of the prolonged prophylaxis patients and 9 (3.6%; n=248) of the placebo patients (p=NS). One major hemorrhage occurred in the prolonged prophylaxis group; it was nonfatal. *Conclusions.* Prolonged prophylaxis with enoxaparin should be considered in patients undergoing surgery for malignant disease.

C045

EXTENSIVE SCREENING FOR OCCULT MALIGNANT DISEASE IN IDIOPATHIC VENOUS THROMBOEMBOLISM. A PROSPECTIVE, CONTROLLED, RANDOMIZED STUDY

Piccioli A, Lensing AW, Prins MH, Falanga A, Scannapieco G, Ieran M, Cigolini M, Ambrosio GB, Girolami A, Prandoni P, for the SOMIT Investigators Group

University Hospitals of Padua, Bergamo, Venice, Reggio Emilia, and Verona; Academic Medical Center, University of Amsterdam, The Netherlands

Although there is convincing evidence that the risk of subsequent malignancy is increased among patients with idiopathic venous thromboembolism (VTE), whether an extensive diagnostic screening for occult cancer is indicated in these patients is currently unknown. Two-hundred and one patients with idiopathic VTE and initial negative routine battery tests were allocated to either extensive diagnostic procedures (99) or no further testing (102), and were followed-up for two years. In the extensive screening group, 13 patients (13.1%) had the early detection of a confirmed malignancy (in 10 cases revealed by CT scan), and a further malignancy (1.0%) became apparent during follow-up. Therefore, 13 of the 14 malignancies were identified at the time of screening, for a sensitivity of initial screening of 93% (95 CI, 66 to 100%). In the control group, 10 (9.8%) malignancies became symptomatic during follow-up. Cancer- related mortality occurred in 2 (2.0%) of the 99 patients of the extensive screening group versus 4 (3.9%) of the 102 control patients, for an absolute difference of 1.9% (95% CI, -5.5 to 10.9%). The cluster of cancer-related mortality, presence of residual or recurrent malignancy occurred in 5 (5.1%) of the 99 patients of the extensive screening group as compared to 8 (7.9%) of the 102 control patients, for an absolute difference of 2.8% (95% CI, -6.3 to 13.4%). A selected diagnostic work-up is capable of identifying the majority of occult cancers, while earlier detection is likely to be associated with improved treatment possibilities and thus prognosis.

C046

THE RISK OF RECURRENCE AFTER A FIRST EPISODE OF VENOUS THROMBOEMBOLISM IN CANCER PATIENTS

Cosmi B, Legnani C, Coccheri S, Palareti G

Cardiovascular Department, Division of Angiology, S.Orsola - Malpighi Hospital, University of Bologna, Italy

After a first episode of venous thromboembolism (VTE) in cancer patients, oral anticoagulant therapy (OAT) is recommended while the disease is active or in case of chemotherapy. *Aim of the study*: To evaluate the recurrence rate after a first episode of VTE and OAT withdrawal in cancer patients. *Methods.* Cancer patients with a first objectively documented episode of deep vein thrombosis (DVT) and/or pulmonary embolism (PE) were evaluated prospectively. After an initial course of heparin or low molecular weight heparin, patients received OAT. In the first cohort, OAT was withdrawn at the discretion of the attending physician. In the second cohort, OAT was discontinued when clinical and instrumental evaluation excluded active disease and if no chemotherapy was planned. Follow-up was performed at 3, 9, 15 and 21 months after OAT withdrawal. DVT recurrence was diagnosed by compression ultrasound in case of symptoms and non- or partial compressibility of a previously compressible venous segment and PE recurrence on the basis of V/Q scan. *Results.* In the first cohort, 38 patients (M/F: 15/23, mean age: 71) were evaluated. Cancer site was prostate (4), breast (12), gastrointestinal (7), hematologic (7), other (8). Mean OAT duration was 6 months, range 3-28. Thirteen VTE recurrences occurred during 55.2 patient/years (pt/y) of follow-up (23.5%pt/y). In the second cohort, 36 patients (M/F: 20/16, mean age: 62) were evaluated. Cancer site was prostate (8), breast (9), gastrointestinal (3), lung (4), hematologic (4), other (8). Mean OAT duration was 10 months, range 1-40. OAT was withdrawn in 24 patients and prolonged in 12 patients (metastatic disease in 6, planned chemotherapy in 6). One patient developed VTE during 15.6 pt/y of follow-up (6.4% pt/y). *Conclusions.*

After a first episode of VTE in cancer patients, VTE recurrence rate is low if OAT is withdrawn when clinical and instrumental evaluation exclude active disease.

C047

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

Prandoni P, Lensing AW, Piccioli A, Noventa F, Bagatella P, Bernardi E, Girolami B, Marchiori A, Sabbion P, Simioni P, Prins MH, Girolami A

Department of Medical and Surgical Sciences, 2nd Chair of Internal Medicine, University of Padua, Italy; Academic Medical Center, University of Amsterdam, The Netherlands

Whether cancer patients with deep venous thrombosis (DVT) exhibit a higher risk of recurrent thromboembolism and/or bleeding complications while on anticoagulant treatment is controversial. Consecutive outpatients with confirmed symptomatic DVT with or without cancer participated in the study. Type and stage of cancer were classified as severe, moderately severe or less severe according to accepted guidelines. Heparinization and subsequent oral anticoagulation was given in therapeutic dosages and duration of treatment depended on the presence of risk factors. Patients were prospectively followed for a maximum of one year. Of the 842 included patients, 181 had known cancer at entry. Cancer was severe in 37.6%, moderately severe in 22.1%, and less severe in 40.3% of patients. The 12-month cumulative incidence of recurrent VTE in cancer patients was 20.7% (95% CI, 15.6 to 25.8%) versus 6.8% (95% CI, 3.9 to 9.7%) in patients without cancer, for a HR of 3.25 (95% CI, 1.9 to 5.4; p=0.0001). The 12-month cumulative incidence of major bleeding was 12.4% (95% CI, 6.6 to 18.2%) in patients with cancer and 5.0% (95% CI, 2.5 to 7.5%) in patients without cancer, for a HR of 2.2 (95% CI, 1.2 to 4.1; p=0.015). Recurrence and bleeding were both related to cancer severity but could not be explained by sub- or overanticoagulation. Cancer patients with DVT are more likely to develop recurrent thromboembolic complications and major bleeding than those without malignancy. These risks increase with cancer severity.

C048

CENTRAL VENOUS CATHETER-RELATED COMPLICATIONS IN ONCOHEMATOLOGIC PATIENTS: A RETROSPECTIVE ANALYSIS OF RISK FACTORS

Moia M,° Fracchiolla NS,* Maisonneuve P,^ Cortelezzi A*

*°Hemophilia and Thrombosis Center, *Department of Haematology, IRCCS Ospedale Maggiore and University of Milan, ^European Institute of Oncology of Milan, Italy*

Background: Central venous catheters (CVC) are commonly used in patients receiving chemotherapy. CVC-related thrombosis causes significant morbidity in patients with solid tumors. Few data are available in oncohematologic patients with thrombocytopenia. Thrombocytopenia might decrease the risk of thrombosis on the one side, and may contraindicate the use of anticoagulant drugs on the other side. *Aim of the study*: To assess the incidence and the risk factors for CVC-related thrombosis in oncohematologic patients; to assess the feasibility and the hemorrhagic risk of prophylaxis with anticoagulant drugs. *Methods*: Retrospective analysis in 126 consecutive oncohematologic patients, most of whom with severe thrombocytopenia (median platelet count 76×10^9/L at CVC insertion, 10×10^9/L at nadir). A total of 207 CVC were implanted. Of these, 137 were centrally inserted (CIC) and 70 peripherally inserted (PIC). CVC median duration was 19 days (4,051 catheter days). Antithrombotic prophylaxis was unfractionated heparin (UFH, 2,500 IU/day by continuous intravenous infusion) for 169 CVC, low molecular weight heparin (LMWH, nadroparin, 3,800 IU by single daily intravenous injection) for 21 CVC, low-doses of warfarin for 1 CVC; no prophylaxis for 16 CVC. *Results*: Symptomatic CVC-related thrombotic complications were observed in 15.5% of CVC (7.9 events/1,000 catheter days). CVC-related thrombotic complications were more frequent and earlier in PIC vs CIC (p=0.0001), in patients older than 50 years (p=0.049) and in women taking oral contraceptives (p=0.0001). No major CVC-related hemorrhagic event was recorded. Fewer thrombotic complications were observed with LMWH than with UH prophylaxis (4.7% vs 16.6 %) but this difference did not reach statistical significance. *Conclusions*: The incidence of symptomatic CVC-related thrombotic complications in oncohematologic patients is comparable to that reported in the literature in patients with solid tumors. Thrombocytopenia does not prevent this risk. Prophylaxis with anticoagulant drugs is feasible and does not appear to induce clinically relevant bleeding.

Oral Communications
Physiopathology of Platelet and Leukocyte Activation

C049

THROMBIN INTERACTION WITH PLATELET GLYCOPROTEIN IB: EFFECTS ON FACTOR VIII ACTIVATION

De Cristofaro R,* De Candia E,* Hall Scott W,° Landolfi R*

**Hemostasis Research Center, Catholic University School of Medicine, Rome, Italy; °Division of Hematology, Stanford University, CA, USA*

The interaction of platelet glycoprotein Ib α (GpIb-α) with thrombin contributes to thrombin-induced platelet activation. Recent studies have shown that the thrombin domain referred to as heparin binding site (HBS) is involved in the interaction with the region 268-288 of platelet GpIb-α, containing three peculiar sulphated tyrosines. Alanine scanning mutagenesis of the basic HBS residues R93, R97, R101, R233, K236, and K240 showed in fact that the Kd of thrombin-GpIb interaction was reduced 22-fold for R93A, 8-fold for R97A, 13-fold for R101A, 29-fold for R233A, 21-fold for K236A, and 5-fold for K240A. Thrombin HBS interacts also with other macromolecular ligands, such as prothrombin fragment F1+2, the natural inhibitor hemadin, and factor VIII. The last is transformed into an active form by limited proteolysis by thrombin or factor X. Cleavage at Arg740 removes the size-heterogeneous B-domain from the FVIII heavy chain, while further cleavages at Arg372 and Arg1689 produce the active trimeric form of FVIII. In this study we tested the effect of the synthetic peptide 268-282 of GpIb-α on thrombin-induced factor VIII activation, as evaluated by 1) a chromogenic assay of the generation of FVIIIa cofactor activity in Factor X activation; 2) reversed-phase HPLC measurements of cleavage of FVIII 344-375 synthetic peptide (NH2-E-E-A-E-D-Y-D-D-D-L-T-D-S-E-M-D-V-V-R-F-D-D-D-N-S-P-S-F-I-Q-I-R-S-V-A-K-COOH). The 268-282 GpIb-α peptide exerted a competitive inhibition of full length FVIII activation by thrombin. This effect is likely due to inhibition of the thrombin hydrolysis of Arg372, according to the results of the kinetic experiments using the 344-375 FVIII peptide. These data suggest that thrombin binding to GpIb-α makes the enzyme not available for FVIII activation. The physiological implications of these findings need further studies to be fully unraveled.

C050

MECHANORECEPTOR FUNCTION OF GLYCOPROTEIN Ibα LEADING TO SEQUENTIAL CALCIUM SIGNALS, INITIAL PLATELET ACTIVATION AND THROMBUS FORMATION IN ARTERIAL FLOW

Mazzucato M, Pradella P, Cozzi MR, De Marco L, Ruggeri Zaverio M*

*C.R.O.-I.R.C.C.S. Aviano, PN, Italy; *The Scripps Research Institute, La Jolla, CA, USA*

Platelet activation, which modulates the ligand binding function of αIIbβ3 and is required for stable adhesion and cohesion, may be coupled to the interaction between glycoprotein (GP) Ibα and the von Willebrand factor A1 domain (A1VWF). We analyzed concurrently the instantaneous velocity and $[Ca^{++}]i$ in single platelets interacting with VWF using a new video-imaging method, characterized by high-speed image acquisition and high performance software. Perfusion of blood with a wall shear rate of 1500 s^{-1} or higher resulted in platelet adhesion and formation of aggregates on surface-bound VWF. The interaction of glycoprotein (GP) Ibα with the A1 domain of immobilized VWF leads to Ca^{++} release from intracellular stores (type α/β peaks), which precedes stationary platelet adhesion. Type α/β peaks appear to be directly modulated by tensile stress on GP Ibα bonds during the initial tethering of platelets to VWF. Force above a threshold value of approximately 2 Pa is required to induce an appreciable Ca^{++} response, suggesting that GP Ibα may act as a proper mechanoreceptor. Raised cAMP/cGMP levels, as well as membrane permeable calcium chelators, inhibit these $[Ca^{++}]^i$ oscillations and prevent stable adhesion without affecting the dynamic characteristics of the typical platelet translocation on VWF mediated by GP Ibα. Once adhesion is established through the integrin αIIbβ3, new $[Ca^{++}]^i$ oscillations (type γ) of greater amplitude and duration, and involving a transmembrane ion flux, develop in association with the recruitment of additional platelets into the aggregates. Degradation of released ADP to AMP or inhibition of phosphatidylinositol 3-kinase prevents this response without affecting adhesion, and blocks aggregation. Thus, inside-out signals induced by stressed GP Ibα-VWF bonds activate αIIbβ3 for localized adhesion. Then, outside-in signals from ADP receptors and ligand-occupied αIIbβ3, with the contribution of a pathway involving phosphatidylinositol 3-kinase, amplify platelet activation to the level required for aggregation.

C051

DOES ASIALO-VON WILLEBRAND FACTOR STIMULATE HUMAN PLATELETS THROUGH THE G1 PATHWAY?

Lecchi A,* Mazzucato M,# De Marco L,# Cattaneo M*°

**A. Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, IRCCS Ospedale Maggiore; °Department of Medicine, Surgery and Dentistry, Ospedale San Paolo, University of Milano; #Centro di Riferimento Oncologico IRCCS, Aviano, PN; Italy*

Von Willebrand factor (vWF) induces platelet activation and aggregation under flow conditions characterized by high shear stress. The biochemical pathways responsible for vWF-induced platelet activation are incompletely understood. Asialo-vWF stimulates human platelets independently of mediators and of high shear: therefore, it is a useful model for studying the vWF-platelet interaction. Typically, the Asialo-vWF-induced platelet aggregation is not preceded by shape change. This pattern is shared by agonists that interact with Gi-coupled receptors (epinephrine; ADP interacting with P2Y12 in P2Y1-knock-out platelets). The aim of the study was to evaluate whether the activation of human platelets by Asialo-vWF is mediated by the Gi pathway, which is negatively associated with adenylyl cyclase. The levels of cyclic-AMP were measured in citrated platelet-rich plasma (PRP) from 3 healthy subjects, after its incubation at 37°C for 2 min with: a) Tyrode buffer; b) Tyrode buffer and PGE1 (1 mM); c) Tyrode buffer, Asialo-vWF (3.6 mg/mL), yohimbine (10 mM, antagonist of α2-adrenoceptors) and AR-C69931MX (1

mM, antagonist of P2Y12 receptor); d) ADP (10 mM) and PGE1; e) epinephrine (10 mM) and PGE1. Yohimbine and AR-C69931MX were added to PRP samples in order to prevent the stimulation of the Gi pathway by epinephrine and ADP that are secreted by platelets exposed to Asialo-vWF. The mean (±SD) platelet levels of cyclic-AMP was increased from 3.8±1.3 nmoles/10^8 platelets to 41.2±9.4 by PGE1. This increase was inhibited by ADP (6.9±2.8) or epinephrine (7.9±1.1), but was not affected by Asialo-vWF in the presence of yohimbine and AR-C69931MX (42.3±8.8). This study shows that interaction of Asialo-vWF with human platelets does not inhibit the platelet adenylyl cyclase. Therefore, at variance with ADP and epinephrine, the activation of human platelets by Asialo-vWF is not mediated by the Gi pathway.

C052

CONVULXIN INDUCES PLATELET SHAPE CHANGE THROUGH MYOSIN LIGHT CHAIN KINASE AND RHO KINASE

Riondino S, Gazzaniga PP, Pulcinelli FM

Department of Experimental Medicine and Pathology, University La Sapienza, Rome, Italy

One of the first events that follow platelet functional response to various stimuli is the rearrangement of cytoskeletal proteins that results in a rapid change in shape. It has been demonstrated that a crucial event triggering such a phenomenon is the phosphorylation of myosin light chain (MLC). The agonist-induced signal that results in platelet shape change leads to MLC phosphorylation through two distinct pathways, one Ca^{2+}/calmodulin-dependent, the other subsequent to RhoA activation, mediated by Rho-kinase. The Ca^{2+}-dependent pathway depends upon stimulation of a Gq-coupled receptor and phospholipase C (PLC) activation. Cyclic AMP exerts a potent action on cytoskletal rearrangement by inhibiting the phosphorylation of various proteins among which MLC. The purpose of this study was to investigate how convulxin, a PLCγ2 activator, causes platelet shape change and to asses cAMP involvement in the earliest step of the activatory cascade through the study of MLC phosphorylation. *In vitro* platelet shape change was evaluated in an aggregometer in the presence of high concentrations of iloprost on samples treated with the tetrapeptide RGDS in order to prevent aggregation. The degree of myosin light chain phosphorylation was evaluated with protein electrophoretic studies on urea gel. Our results demonstrated that iloprost treatment did not inhibit platelet shape change induced by convulxin while it strongly affected MLC phosphorylation acting on the Ca^{2+}-dependent pathway. The treatment with the RhoA inhibitor HA1077 was without any effect both on platelet shape change and on MLC phosphorylation induced by convulxin. When HA1077 and iloprost were concomitantly used, both platelet shape change and MLC phosphorylation were completely abolished. Similar results were obtained when the Ca^{2+}/calmodulin pathway was inhibited by the Ca^{2+} chelator BAPTA or by the calmodulin inhibitor W-7. Taken together our findings suggest that convulxin-induced platelet shape change occurs via both a Ca^{2+}/calmodulin-dependent and a Rho A-dependent mechanism.

C053

BOTH P2Y1 AND P2Y12 RECEPTORS ARE NECESSARY FOR NORMAL ADP-INDUCED MOBILIZATION OF THE PLATELET FREE CYTOPLASMIC CALCIUM

Lombardi R, Lecchi A, Cattaneo M

A. Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine IRCCS Ospedale Maggiore and University of Milan, Italy

Concomitant activation of both the P2Y1-driven Gq and the P2Y12-driven Gi pathways is necessary for normal ADP-induced platelet aggregation. It is generally accepted that the ADP-induced increase in free cytoplasmic calcium ($[Ca^{2+}]i$) is mediated by the P2Y1 receptor only. However, we found that the first patient with P2Y12 deficiency described (VR) had borderline-low increases in platelet $[Ca^{2+}]i$ induced by ADP. The aim of the study was to evaluate whether P2Y12 plays any role in the ADP-induced increase in platelet $[Ca^{2+}]i$. We studied 13 normal subjects and 2 patients with P2Y12 deficiency (VR and MG). We studied the ADP-induced $[Ca^{2+}]i$ in washed human platelets in the presence of EDTA (1 mM) and in the presence or absence of the antagonists for P2Y1 (A2P5P 0.5mM) or P2Y12 (AR-C69931MX 1 mM). The mean (±SD) increase in $[Ca^{2+}]i$ induced by ADP (10 mM) in normal platelets was 376±95 nM. It was completely abolished by the P2Y1 antagonist A2P5P, while it was only partially inhibited by the P2Y12 antagonist AR-C69931MX (278±68, $p<0.01$). The ADP-induced increase in $[Ca^{2+}]i$ in VR's and MG's platelets was borderline-low (130 and 282). It was not further decreased by AR-C69931MX (158 and 266), but was completely abolished by A2P5P. The adenylyl-cyclase inhibitor SQ22536 did not inhibit the ADP-induced increase in $[Ca^{2+}]i$ in normal or patients' platelets. In conclusion, concomitant activation of both the P2Y1-driven Gq and the P2Y12-driven Gi pathways is necessary for the normal ADP-induced $[Ca^{2+}]i$ increase. Like for platelet aggregation, P2Y1 triggers the initial platelet $[Ca^{2+}]i$ response, while P2Y12 amplifies it. The mechanism by which P2Y12 amplifies the $[Ca^{2+}]i$ increase is not mediated by inhibition of adenylyl cyclase.

C054

EFFECTS OF PHARMACOLOGIC INHIBITION OF THE P2Y1 AND P2Y12 ADP RECEPTORS ON SHEAR-INDUCED PLATELET AGGREGATION AND PLATELET THROMBUS FORMATION ON A COLLAGEN-COATED SURFACE UNDER FLOW CONDITIONS

Cattaneo M, Savage B, Ruggeri Zaverio M

Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, USA

Concomitant activation of both the P2Y1-driven Gq and the P2Y12-driven Gi pathways is necessary for normal ADP-induced platelet aggregation. In this study we attempted to clarify the relative roles of the two platelet P2 receptors for ADP in platelet aggregation under high shear. We studied the effects of the P2Y1 antagonist MRS-2216 and the P2Y12 antagonist AR-C69931MX alone and in combination on shear-induced platelet aggregation (SIPA) and the development of platelet thrombi on a collagen-coated surface exposed to flowing blood. Normal PRP anticoagulated with PPACK was exposed to a shear stress of 108

dynes/cm^2 in a cone-and-plate viscometer at RT for 6 min: this resulted in irreversible platelet aggregation. Both P2Y antagonists dose-dependently inhibited SIPA (16±9.3% of normal with 10 mM MRS-2216, 10.4±7.0 with 1 mM AR-C69931MX). In the presence of both antagonists, SIPA was 9.7±4.3%. The interaction of platelets with immobilized type 1 collagen was studied in real time by means of confocal epifluorescence videomicroscopy, using a flow chamber maintained at 37°C and mepacrine-labeled platelets in PPACK-anticoagulated whole blood. Two min after the start of perfusion at a flow rate of 6,000 or 1,500 s-1, platelet thrombi formed on the collagen-coated surface, which were partially inhibited by the P2Y1 or the P2Y12 antagonist. When the two antagonists were added together, the inhibition of thrombus formation was almost complete. At a flow rate of 100 s-1, very few platelet thrombi formed, and the two P2 antagonists, even when added together, caused only a marginal inhibition of thrombus formation. Therefore, pharmacological inhibition of P2Y1 or P2Y12 inhibits both shear-induced platelet aggregation and platelet thrombus formation on a collagen surface at high shear. The contemporary inhibition of the two P2 receptors accomplished an almost complete inhibition of these platelet responses, confirming the essential role played by ADP in hemostasis and thrombosis.

C055

PMN ADHESION TO ACTIVATED PLATELETS: ROLE OF cAMP AS A GATING ELEMENT OF P-SELECTIN-INDUCED MAC-1 ADHESIVENESS AND OF TYPE-4 PHOSPHODIESTERASES (PDE) AS A NEW TARGET FOR ANTI-INFLAMMATORY AND ANTI-THROMBOTIC DRUGS

Evangelista V, Manarini S, Totani L, Piccoli A, Bagoly Z, Pecce R, Martelli N, de Gaetano G, Cerletti C, Piccardoni P

"G. Bizzozero" Laboratory of Blood and Vascular Cell Interactions, Consorzio Mario Negri Sud, Santa Maria Imbaro and Università Cattolica del Sacro Cuore, Centro di Ricerca e Formazione ad Alta Tecnologia nelle Scienze Biomediche, Campobasso, Italy

Adhesion of PMN to activated platelets, requires a P-selectin-triggered, SRC kinases- and cytoskeleton-dependent adhesiveness of Mac-1 and is accompanied by tyrosine phosphorylation of a 110 kD protein (P110) in PMN (*Blood 2001; 98:108*). Cyclic AMP may *gate* integrin function through inhibition of Rho-A, a small GTP-binding protein playing an important role in the assembly of *focal adhesions*. We investigated whether by increasing cytoplasmic cAMP in PMN, by PDEs blockade, Mac-1 adhesiveness triggered by P-selectin is inhibited. IBMX, a non specific inhibitor of PDEs, prevented PMN-platelet adhesion and P110 phosphorylation (IC50 500 μM). IBMX-inhibited adhesion and P110 phosphorylation was restored by H89, a specific inhibitor of cAMP-activated PKA (IC50s 5 μM). Interestingly H89 restored PMN adhesion but not tyrosine phosphorylation inhibited by SRC-blockers indicating that after removal of the cAMP-dependent *gating*, the activity of SRC kinases as well as of P110, is no longer required. SRC-inhibitors did not modify cytoplasmic cAMP levels. To better understand the nature of the SRC-dependent signalling, we investigated the possibility that P-110 corresponds to PYK2, a focal adhesion kinase able to promote the function of Rho proteins. Western blot analysis of the immunoprecipitated protein revealed that tyrosine phosphorylation of PYK2 was strongly increased in a SRC and β-2 integrin-dependent manner in PMN challenged by P-selectin. PDE-4 is the main enzyme involved in the metabolism of cAMP in PMN. Rolipram, RO2017-24 (both specific inhibitors of PDE-4) and zardaverine (inhibitor of PDEs-4 and-5), blocked PMN-platelet adhesion (IC50s 0.1, 1 and 0.1 μM, respectively). In contrast adhesion was not modified by MQ and Cilostamide (inhibitors of PDE-5 and-3, respectively). These results provide new molecular clues to the understanding of the intracellular mechanisms regulating PMN-platelet adhesion. Specific inhibitors of PDE-4 may represent new pharmacological tools to prevent leukocyte recruitment and vascular inflammation triggered by platelets at a site of vascular damage.

C056

E-SELECTIN STIMULATES TYROSINE KINASE-DEPENDENT ADHESIVE FUNCTION OF β-2 INTEGRINS IN POLYMORPHONUCLEAR CELLS: ROLE OF AN UNIDENTIFIED RECEPTOR

Totani L, Piccoli A, Manarini S, Martelli N, Vestweber D, de Gaetano G, Cerletti C, Evangelista V

"G. Bizzozzero" Laboratory of Blood and Vascular Cell Interaction, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy; Università Cattolica del Sacro Cuore, Centro di Ricerca e Formazione ad Alta Tecnologia nelle Scienze Biomediche, Campobasso, Italy, Institute of Cell Biology, University of Muenster, Muenster, Germany

Using soluble recombinant E-selectin-IgG chimera and E-selectin-transfected CHO cells (CHO-E) we tested the hypothesis that E-selectin promotes β-2-integrin adhesiveness in polymorphonuclear cells (PMN). PMN homotypic aggregation was evaluated by optical counting, and the formation of mixed conjugates in sheared (1000 rpm) cell suspensions (PMN: CHO ratio=5), by double color flow cytometry. E-selectin-IgG (1-20 μg/mL) dose-dependently stimulated PMN aggregation that was accompanied by tyrosine phosphorylation of a protein of 110 kD (P110). β-2 integrin blockade by specific antibodies prevented both aggregation and P110 phosphorylation. Genistein (1-100μM) and the specific inhibitor of SRC kinases PP1 (1-20 μM) dose-dependently inhibited P110 phosphorylation and aggregation. Inhibitors of Syk/Zap-70 and MAPK were ineffective. Similarly, after 2 min of shear 25±1.5% of PMN adhered to 60±10% of CHO-E by a β2-integrin dependent mechanism. Phosphorylation of P110 accompanied PMN adhesion and PP1 blocked both P110 phosphorylation and PMN recruitment on CHO-E cells. Neuraminidase treatment prevented PMN recruitment indicating a role for sialylated carbohydrates. Among the E-selectin ligands PSGL-1 and L-selectin can trigger intracellular signaling leading to β2 integrin activation. In order to investigate their involvement, we used different approaches including specific antibodies (PL1 and PL2 to PSGL-1 and DREG-56 to L-selectin), a soluble recombinant form of PSGL-1-IgG chimera or chymotrypsin treatment, which removes PL1, PL2 and DREG-56 binding. None of these treatments modified PMN adhesion to CHO-E. Moreover PMN adhesion to CHO-E was not affected by the combination of PL1 and DREG-56 with Affi-6O, a rabbit polyclonal antibody to ESL-1. This study demonstrates that E-selectin promotes a SRC-kinase dependent adhesiveness of the β2 integrins in PMN, and indicates the involvement of an unknown receptor.

Oral Communications
Diagnosis and Treatment of Atherothrombosis

C057

SIMULTANEOUS DECREASE OF PROINFLAMMATORY CYTOKINES AND TISSUE FACTOR BY SIMVASTATIN

Ferro D, Parrotto S, Loffredo L, Caroselli C, Violi F

Institute Of Clinical Medicine I, University "La Sapienza", Rome, Italy

Statins reduce the monocyte expression of tissue factor, but the mechanism has not been investigated. The purpose of this study was to assess whether there is a relationship between proinflammatory cytokines and tissue factor decrease after simvastatin treatment. Twenty patients with polygenic hypercholesterolemia were randomly allocated to diet (n=10) or diet plus 20 mg/day simvastatin (n=10) for eight weeks. Before and at the end of treatment period, lipid profile and lipopolysaccharide (0.4 ng/mL)-induced monocyte expression of tissue factor antigen (American Diagnostica Inc. Greenwich, CT, UA) and activity, tumor necrosis factor α (T cell Diagnostics, Cambridge, MA, USA) and interleukin-1 β (Genzyme Diagnostic, Cambridge, MA, USA) were measured. At baseline no differences in lipid profile and monocyte expression of tissue factor, tumor necrosis factor and interleukin-1 β were observed between the two groups. A significant correlation was found among all subjects between tissue factor and tumor necrosis factor (p<0.0001) and tissue factor and interleukin-1 β (p<0.0001). At the end of treatment patients treated with simvastatin had lower monocyte expression of tissue factor, tumor necrosis factor and interleukin-1 β than did patients assigned to diet alone (ANOVA: p<0.0001). In the simvastatin treated group, the two way interaction between the decrease of tissue factor, as dependent variable, and decrease of tumor necrosis factor as covariate (p<0.0001) and between the decrease of tissue factor, as dependent variable, and decrease of interleukin-1 β as covariate (p<0.002) was statistically significant. An *in vitro* study using an exogenous stimulus of tumor necrosis factor and interleukin-1 β demonstrated that the monocyte expression of tissue factor was dependent upon the concentration of cytokines used.

	Simvastatin, 600 ng/mL		
Stimulus	VCAM-1 expression % of stimulated expression	E-Selectin expression, % of stimulated expression	ICAM-1 expression % of stimulated expression
AGEs, 200 µg/mL	153±23	217±20	128±10
LPS, 1 µg/mL	150±10	130±13	133±14
TNF α,10 ng/mL	135±23	150±23	130±10

This study suggests that in patients with hypercholesterolemia, inflammation is implicated in enhancing activation of clotting system, and that inhibition of monocyte tissue factor expression by simvastatin could be mediated by inhibition of the tumor necrosis factor and interleukin-1 β.

C058

POST-TREATMENT WITH SIMVASTATIN AFFORDS BRAIN PROTECTION AFTER FOCAL ISCHEMIA IN RATS AS ASSESSED BY MAGNETIC RESONANCE IMAGING

Sironi L, Cimino M, Guerrini U, Calvio AM, Lodetti B, Asdente M, Balduini W, Paoletti R, Tremoli E

Department of Pharmacological Sciences, Milan, and Institute Of Pharmacology and Pharmacognosy, Urbino, Italy

The HMG-CoA reductase inhibitors, or statins, represent a promising class of drugs for the prevention and treatment of strokes. Data obtained in a murine model of stroke suggest that pre-treatment with statins exerts neuroprotective effects. In this study we investigated the effects of the administration of simvastatin prior to and after ischemia, induced by middle cerebral artery occlusion (MCAO), might affect the development of brain damage. Simvastatin (20 mg/kg per day) was administered to male Sprague Dawley rats for 3 days before (n=6) or for 3 days after (n=10) permanent MCAO. The brains of both groups of rats, pre- or post-MCAO simvastatin-treated, were imaged 2h, 24 and 48 h after MCAO by using the apparent water diffusion coefficent (ADC) maps, a magnetic resonance imaging (MRI) technique that allows identification of ischemic areas in the brain. Simvastatin-treated rats were compared with sham-operated animals (n=10). Quantification of ADC maps showed that initial ischemic volumes (2h after MCAO) were similar in the untreated (37±5.3 mm^3; mean±SE) and in the simvastatin-treated rats (pre-treated: 40.9±9 mm^3 and post-treated: 32.2±4.4 mm^3) animals. At 24 and 48 h after MCAO, an increase in the damaged areas was observed (47% and 83%, p<0.05 and p<0.01 respectively compared with the damage at 2h), as evaluated by ADC maps. As expected, pre-treatment of rats with simvastatin significantly reduced cerebral infarct size, compared to the initial damage, by 33.8% (p<0.05) and 47.5% (p<0.01) at 24 and 48 h respectively. Neuroprotective effects of simvastatin were observed also in rats treated with the drugs after MCAO. In these animals, after 24 h, only a slight decrease in the infarcted area was observed but 48h after MCAO cerebral infarct areas were decreased by 46.6% (p<0.01). The protective effects of simvastatin also when administered after cerebral ischemia suggests a potential effect for statins not only in the prevention but also in the treatment of acute stroke.

C059

STATIN THERAPY REDUCES CD40L-DEPENDENT PROTHROMBOTIC STATE IN HYPERCHOLESTEROLEMIA

Cipollone F,° Mezzetti A,° Porreca E,* Di Febbo C,* Nutini M,* Fazia M,* Falco A,* Cuccurullo F,* Davì G*

*From the *Center of Excellence on Aging and °Department of Medicine & Aging, "G. D'Annunzio" University, Chieti, Italy*

Background. Hypercholesterolemia is associated with chronic inflammation and a prothrombotic state. CD40-CD40L interactions promote a prothrombotic response in nucleated cells *in vitro*. The aim of this study was to characterize the *in vivo* expression of sCD40L in hypercholesterolemia, to correlate it with the extent of prothrombotic state, and to investigate whether it may be modified by lipid-lowering strategies. *Meth-*

ods and Results. We studied 80 hypercholesterolemic subjects and 80 matched healthy subjects. Hypercholesterolemic subjects had enhanced levels of sCD40L (8.3±5.2 vs 2.4±1.3 ng/mL, $p<0.0001$), factor VIIa (37±13 vs 24±4.8 mU/mL, $p<0.001$) and prothrombin fragment 1+2 (F1+2) (1.52±0.95 vs 0.55±0.22 nmol/L, $p< 0.001$) compared to healthy subjects. sCD40L correlated with total cholesterol (Rho=0.353, $p=0.0018$) and LDL-cholesterol (Rho=0.379, $p=0.0008$). Moreover, sCD40L was positively associated with *in vivo* platelet activation, as reflected by plasma P-selectin (Rho=0.455, $p=0.0204$) and urinary 11-dehydro-thromboxane B2 (Rho=0.443, $p=0.024$), and with a procoagulant state as reflected by factor VIIa (Rho=0.79, $p<0.0001$) and F1+2 (Rho=0.80, $p<0.0001$). Inhibition of cholesterol biosynthesis by pravastatin or cerivastatin was associated with comparable, statistically significant reductions in sCD40L, factor VIIa and F1+2. *Conclusions*. This study suggests that sCD40L may represent the molecular link between hypercholesterolemia and a prothrombotic state, and demonstrate that statin therapy may significantly reduce circulating sCD40L and prothrombotic state.

C060

STATINS INHIBIT CYCLO-OXYGENASE-2 PROTEIN AND ACTIVITY IN HUMAN ENDOTHELIAL CELLS POSSIBLE CONTRIBUTING TO PLAQUE STABILITY

Massaro M,* Zampolli A,° Madonna R, Carluccio MA,#
Storelli C, *° De Caterina R

*CNR Institute of Clinical Physiology, *Pisa And Lecce, #University of Lecce; °"G. D'Annunzio" University, Chieti, Italy*

The 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (statins) are currently being investigated for cholesterol-independent properties, among which anti-inflammatory effects. Cyclo-oxygenase(COX)-2 expression within atherosclerotic plaques has been linked to plaque instabilization through COX-2-dependent expression of matrix metalloproteinases. Accordingly, statins reduce basal and stimulated metalloproteinase expression by macrophages. Since vascular endothelium plays a crucial role in the development of atherosclerotic lesion, we investigated whether statins modulate endothelial COX-2. *Methods*. Simvastatin and atorvastatin, activated by alkaline hydrolysis *in vitro*, were incubated with human umbilical vein endothelial cells (HUVEC) for 6 h, followed by stimulation with tumor necrosis factor (TNF), lipopolysaccharide (LPS) or phorbol myristate acetate (PMA) for a further 12 h. After this time, COX-2 activity and protein were assessed by a radioimmunoassay for 6-keto-PGF1α (the stable hydrolytic metabolite of prostacyclin) and Western analysis, respectively. *Results.* At 0.1-10 µmol/L, and in the absence of any toxicity, both simvastatin and atorvastatin reduced COX-2 expression and prostacyclin production without any effect on COX-I expression. Results for COX-2 protein expression at Western blot densitometry are shown in the Table below.

Stimulus	Simvastatin 1 µmol/L (% of control)	Atorvastatin 1 µmol/L (% of control)
PMA 6.3 ng/mL	15±4	38±10
LPS 1 µg/mL	67±22	79±25
TNFα 10 ng/mL	13±4	34±9

The effect of both statins on COX-2 protein expression was totally reversed by the addition of 200 µmol/L mevalonate, indicating the requirement of a prenylated intermediate in the signal transduction pathway leading to COX-2 expression. 6-keto-PGF1α production (pg/1,000 cells) was 0.22±0.01 in control conditions, 4.88±1.72 after PMA 6.3 ng/mL, 2.60±1.34 after PMA in the presence of simvastatin ($p<0.01$ vs PMA), and 4.42±1.36 after PMA with simvastatin + mevalonate (p=N.S. vs PMA). *Conclusions*. Statins reduce the induced expression and activity of COX-2 in human vascular endothelial cells. This effect may contribute to clinical benefits of statins that are independent of cholesterol-lowering.

C061

PLATELETS RELEASE MATRIX METALLOPROTEINASE-2 *IN VIVO* IN HUMANS AT A LOCALIZED SITE OF PLATELET ACTIVATION

Falcinelli E, Ciferri S, Gresele P

Department of Internal Medicine, Section of Internal and Cardiovascular Medicine, University of Perugia, Italy

Tissue matrix metalloproteinases (MMP) are involved in the breakdown and remodelling of extracellular matrix. Recently, release of MMP-2 from human platelets stimulated *in vitro* with collagen and thrombin was shown. Active, but not latent, MMP-2 enhances platelet activation triggered by several agonists. To investigate whether MMP-2 is released in blood from activated platelets *in vivo*, we measured MMP-2 levels in the blood emerging from a skin wound inflicted for the measurement of the bleeding time and in venous blood in 17 human healthy volunteers. In a subgroup of volunteers, the same measures were carried out before and 1 hr after oral intake of 500 mg aspirin. MMP-2 concentrations were measured by zymography and by immunoblotting; active MMP-2 was measured using an activity assay system. MMP-2 in shed blood was significantly higher than in venous blood (proMMP-2: 329±14 ng/mL vs 173±23 ng/mL, $p< 0.05$; MMP-2: 27.1±5.4 vs 6.3±2.3, $p<0.05$) and Western blotting analysis showed increased expression. Expression of MMP-2 on platelet surface, by flow cytometry, increased time-dependently in bleeding time blood. A significant correlation was evident between platelet number in shed blood and MMP-2 ($r2= 0.93$, $p<0.0001$). Washed white blood cells and HUVEC did not release MMP-2 when stimulated *in vitro*, thus excluding a significant contribution of these cells to the raised levels detected in shed blood. Aspirin intake did not reduce MMP-2 release while causing an inhibition (-33%) of P-selectin expression on platelets. Our data show that significant amounts of enzymatically active MMP-2 are released by platelets at a localized site of vessel wall damage. The amounts of released MMP-2 (~6 ng/10^8 platelets) are in the range of those that we found to potentiate platelet aggregation *in vitro*. Our data suggest that platelet- released MMP-2 contributes to *in vivo* platelet activation; aspirin intake does not reduce this release and this might represent one reason for the partial inefficacy of this drug.

C062

NA^+/H^+ AND $NA^+/CA2^+$ EXCHANGE INVOLVEMENT IN PMA-INDUCED PLATELET AGGREGATION

Pulcinelli FM, di Santo S, Pesce G,* Treppiccione AP, Di Renzo LM, Gazzaniga PP

*Dept. of Experimental Medicine and Pathology, and *Dept. of Cellular Biotechnology and Hematology, University La Sapienza, Rome, Italy*

The biochemical pathways leading to changes of the fibrinogen affinity state of the integrin αIIb-β3 in platelets, from a low affinity into a high affinity state responsible for platelet aggregation, are not completely clear yet. It was previously suggested that PKC activation was an important step in such a process, but we later demonstrated that fibrinogen receptor exposure induced by ADP is independent of PKC activation. PKC activation is able to determine platelet activation if platelets are simultaneously activated with ADP or adrenaline. It is well known that PKC activation induces Na^+/H^+ exchange (NHE), with consequent cytosolic alkalinization, in platelets. To verify whether NHE activation is necessary for PMA to induce integrin αIIb-β3 dynamic changes, we studied whether ethylisopropylamiloride (EIPA; 50 μM), a NHE inhibitor, reduces platelet aggregation induced by PMA (0.2 μM). The reported results show that EIPA slightly inhibited platelet aggregation in response to PMA (62.4%±11.9 versus 77.4±4.3), while preventing the cytosolic alkalinization, investigated using the fluorescent dye BCECF. As in other cellular systems it has been shown that PKC activates $Na^+/Ca2^+$ exchange (NCE), we demonstrated, by using Bepridil (50 μM), one of its inhibitors, an evident inhibition of PMA-induced platelet aggregation (40.4±12.5). The combination of Bepridil and EIPA caused a strong reduction in PMA-induced aggregation (23.2±11.5). These results demonstrate that both exchangers are involved in PK-dependent platelet aggregation and that in this respect NCE is more important than NHE.

C063

TUMOR NECROSIS FACTOR-α INDUCES PLATELET AGGREGATION AND SUPEROXIDE ANION PRODUCTION: A POSSIBLE ROLE IN HEART FAILURE

Pignatelli P,* Lenti L,* Tocci G,** Lambiasi MG,* Pulcinelli FM,* Gazzaniga PP,* De Biase L,° Violi F*

**Dept. of Experimental Medicine and Pathology; °Dept of Cardiology II Faculty University La Sapienza, Rome, Italy*

Introduction. Several studies have demonstrated a state of systemic inflammation in patients with chronic heart failure, and recent observations suggested that proinflammatory cytokines, such as TNFα, are capable of modulating cardiovascular functions by different mechanisms, including generation of reactive oxygen species. As it has recently been demonstrated that platelets possess TNFα-receptors and TNFα-mRNA, aim of the present study was to evaluate the ability of TNFα in inducing platelet aggregation and superoxide anion formation. *Methods*. We analyzed a population of 44 patients with chronic heart failure, due to idiopathic dilated cardiomyopathy (20 men and 2 females, mean age 54±12 years), coronary artery disease (15 men and 1 females, mean age 61±8 years), and valvular dysfunction (5 men and 1 female, mean age 63±9 years). Clinical severity was defined according to the New York Heart Association (NYHA) classification, with 10 patients in class I, 9 in class II, 15 in class III, and 10 in class IV. Blood samples were drawn to evaluate platelet aggregation, basal and collagen-induced platelet O2- production, serum TxB2, serum TNFα. *In vitro* studies were performed using TNFIα as agonist, and the specific TNFα inhibitor, WP9QY. *Results*. An increased platelet O2- production in NYHA class III and IV with a positive (r = 0.8) correlation with TNFα plasma levels was observed. An *in vitro* study, showed that TNFα was able to induce platelet aggregation and superoxide anion production in a dose-dependent manner (1^{-10}), and that the specific TNFα-inhibitor WP9QY (1 mmol) inhibited the TNFα-mediated platelet activation. *Conclusion*. Our results show, for the first time, that TNFα itself is able to induce platelet aggregation and superoxide anion production and suggest that TNFα plays a key role in enhancing oxidative stress in patients with heart failure.

C064

AGGREGOMETRIC EVIDENCE OF A PLATELET ASPIRIN RESISTANCE IN LONG-TERM TREATED PATIENTS

Pulcinelli FM, Pignatelli P, Celestini A, Violi F, Gazzaniga PP

Dept. of Experimental Medicine and Pathology, University La Sapienza, Rome, Italy

Acetylsalicylic acid is the most commonly used antiplatelet drug in the prevention of atherothrombotic diseases. While the clinical efficacy of aspirin in acute coronary syndrome has been well established, chronic use of aspirin has been long debated. Here we report for the first time that aspirin resistance is a phenomenon that can appear after a prolonged treatment with this drug. We performed a retrospective analysis in 150 patients in whom maximal percentage and the lag phase of platelet aggregation induced by collagen (2 μg/mL) before and after 2, 6, 12, and 24 months of aspirin treatment were evaluated. Lag-phase changed from 36.1±18.1 sec to 76.6±46.1 sec after two months' therapy; it progressively decreased at 6 (65.5±3.5 sec), 12 (58.5± 39.8 sec) and 24 (42.5±23.8 sec) months. Maximal percentage decreased from 88.2±21.8% to 37.9±24.4% after 2 months' treatment; a progressive reduction of aspirin effect was observed at 6 (46.1±27.1%), 12 (48.7±27.6%) and 24 months (61.9± 23.9%). Treatment with ticlopidine (250-500 mg/die) did not show any change of platelet aggregation during two years of follow-up. These results show that aspirin resistance can occur after prolonged treatment. These changes may be dependent upon pharmacologic property of aspirin because another antiplatelet drug, ticlopidine, did not elicit resistance. In conclusion we showed that prolonged aspirin treatment is associated with progressive restoration of platelet function. Identification of patients with aspirin resistance may be useful to tailor individual therapy in patients with atherothrombosis.

Oral Communications
Molecular Basis of Inherited Coagulopathies

C065

DYSFIBRINOGENEMIA: RESULTS FROM THE MOLECULAR ANALYSIS OF 7 PATIENTS

Santacroce R, Bossone A, Vecchione G, Brancaccio V,^ Papa ML,* De Lucia D,§ Di Minno G, Margaglione M°

*Unità di Aterosclerosi e Trombosi, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo; ^Divisione di Ematologia, Unità di Coagulazione, Ospedale "A. Cardarelli"; §Istituto di Patologia Generale e Oncologia, Seconda Università di Napoli; *Centro di Emofilia e Trombosi, Ospedale "San Giovanni Bosco", °Genetica Medica, Università di Foggia, Italy*

Congenital dysfibrinogenemia is a rare autosomal disorder characterized by an inherited clotting defect caused by the production of structurally abnormal fibrinogen. Individuals with congenital dysfibrinogenemia are often asymptomatic and are detected during routine screenings or may suffer from a lifelong hemorrhagic diathesis of variable severity or thrombosis. To elucidate the molecular basis of dysfibrinogenemia, we screened for mutations 7 probands, who presented decreased functional, but not immunologic, fibrinogen plasma levels together with prolonged thrombin and reptilase times. Amplifications of all coding regions of fibrinogen chain genes and intron/exon boundaries were achieved using sense and antisense oligonucleotides designed on the basis of known sequences of fibrinogen gene loci (Genebank accession numbers M64982, M64983, and M10014). Amplified DNA fragments were purified and subjected to direct cycle sequence analysis. In 6 out of the 7 patients, a possible causative mutation was identified. Within the Aα-chain gene, 2 previously unreported missense mutations were identified: a C-to-T transition leading to a Arg/Cys substitution at position 104 and a C-to-A transvertion leading to a Pro/Thr substitution at position 270. In addition, a new heterozygous single nucleotide deletion (C) at position Ala499 within the Aα-chain gene was identified, which predicted changes of the corresponding aminoacids encoded by the subsequent portion of the exon V and the appearance of a premature stop codon at position 517. The remaining 3 patients were found to carry a common reported mutation within the γ-chain gene, a C-to-T transition leading to a Arg/Cys substitution at position 275 (Matsumoto III). In conclusion, we have identified a spectrum of unreported mutations that may be of value to unravel the role of specific regions of fibrinogen chains. In addition, we suggest that the common Arg275Cys substitution within the γ-chain gene is a frequent finding in Italians.

C066

NATURALLY OCCURRING MUTATIONS IN THE HUMAN FACTOR VII GENE, ALTERING EITHER THE FORMATION OF THE ACTIVE SITE CLEFT (S363I-W364C) OR THE TISSUE FACTOR LINKED-CONFORMATIONAL EQUILIBRIA (P303T), CAUSE SEVERE FUNCTIONAL ENZYME DEFICIENCY

Peyvandi F,* De Cristofaro R,° Akhavan S,* Garagiola I,* Menegatti M,* Landolfi R,° Mannucci PM*

**Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione Luigi Villa, IRCCS Maggiore Hospital and University of Milan, Italy; °Hemostasis Research Center, Catholic University School of Medicine, Rome, Italy*

Factor VII (FVII) requires the cleavage of an internal peptide bond and binding to tissue factor (TF) to attain its fully active conformation (FVIIa). Free FVIIa remains in a zymogen -like state of relatively low specific activity, but the TF-induced allosteric enhancement of FVIIa activity contributes to the procoagulant activity of the complex. We have identified three naturally occurring mutations (P303T, S363I - W364C) in the FVII gene in three patients with unmeasurable FVII coagulant activity and normal FVII:Ag levels in plasma associated with a moderate to severe bleeding history. To understand the mechanism(s) of the deficiency, *in vitro* expression analysis and biochemical characterization of the expressed recombinant proteins of all three mutants (FVII-303T, FVII-363I - FVII-364C) and wild type (FVII-WT) constructs were carried out. The results recapitulated the patients' plasma data with normal FVII:Ag levels and no measurable coagulant activity. The chromogenic substrate S2238 was used to evaluate the amidolytic activity of WT and mutant recombinant FVII forms in presence and absence of human recombinant tissue factor (rTF). Binding of FVII to rTF was studied by a solid phase binding assay. The result of the amidolytic assays showed that while rTF enhanced 28-fold the value of the specificity constant (kcat/Km) for FVII-WT and only 15 fold for FVII-303T, no activity under any condition was detectable in FVII-363I and FVII-364C constructs. The equilibrium dissociation constant of the rTF-FVII interaction in the activated form showed Kd values equal to 4.4±0.2 nM, 4.9 ±0.5 nM, 6.0±0.9 nM and 17.3± 2.6 nM, for WT, FVII-363I, FVII-364C and FVII-303T, respectively. For the corresponding non-activated forms, Kd values were equal to 24.7±3.3, 24.4±3.1, 24.9±4.1 and 20.6±4 nM, respectively. These data demonstrate that, compared to the WT form, FVII-363I and FVII-364C have no significant affinity change for TF and that the detrimental effect of these two mutations is attributable to the loss of an efficient catalytic machinery in the FVII molecule. Moreover, these experimental data allowed calculation of the difference of the free energy of binding to TF, referred to as δGc, between the activated and non activated FVII forms. The δGc value was 1 kcal/mol higher in the WT-FVII than in the FVII-303T mutant. This result implies indeed that the mutation at Pro303 quenches the TF-induced *active* FVII conformation, opposing the TF-linked allosteric stabilization of the FVIIa *active state*. These data show that Pro303 is involved in regulating the allosteric equilibrium between the *active* and *inactive* FVIIa conformations, and may explain the severe coagulant defect found in the FVII-303T mutant.

C067

RESIDUAL FACTOR VII ACTIVITY AND DIFFERENT HEMORRHAGIC PHENOTYPES IN CRM+ FACTOR VII DEFICIENCIES (GLY331SER AND GLY283SER)

Pinotti M,[#] Etro D,[#] Marchetti G,[#] Papa ML,* Rodorigo G,[&] Rocino A,* Mariani G,° Ciavarella N,^ Bernardi F[#]

*[#]Dipartimento di Biochimica e Biologia Molecolare-CIBF, Università di Ferrara; *Centro Emofilia e Trombosi, Ospedale Nuovo Pellegrini, Naples; [&]Divisione di Angiologia, Azienda Ospedaliera, Policlinico S. Orsola-Malpighi, Bologna; °Divisione di Ematologia, Università di Palermo; ^Centro Emofilia e Trombosi, Ospedale Consorziale, Policlinico, Bari, Italy*

Two CRM+ factor VII (FVII) mutations, associated with similar reduction in coagulant activity (<2.5%) but with mild to asymptomatic (Gly331Ser, c184) or severe (Gly283Ser, c140) hemorrhagic phenotypes, were investigated. The affected glycines belong to structurally conserved regions in the c184-193 and c140s activation domain loops, respectively. Since the low sensitivity of the routine laboratory assays hampers the comparative evaluation of FVIIc values, the residual FVII activity in patient plasma was further evaluated by measuring FXa generation using a FXa fluorogenic substrate. We also expressed the natural mutants 331Ser-FVII and 283Ser-FVII, and in addition the 331Ala-FVII and 283Ala-FVII variants because three functional serine-proteases bear alanine at these positions. Only the 331Ser-FVII showed detectable FXa generation activity in patient plasma (0.7±0.2%) and in the reconstituted system with the recombinant molecules (2.7±1.1%). The 331Ser-FVII residual activity would trigger coagulation, thus preventing severe bleeding symptoms in the several Gly331Ser-homozygotes. On the other hand the undetectable activity of the 283Ser-FVII is in accordance with the severe phenotype observed in the homozygous patient. Although clearly lower than that of Wt-FVII, FXa generation by 331Ala-FVII and 283Ala-FVII was remarkably higher than that of the corresponding natural variants. This suggests that the full activity of FVII is not compatible with the presence of side-chains at positions 331 and 283. The appreciable activity of 283Ala-FVII, compared to the impaired function of the 283Ser-FVII, suggests that the oxydrile group of Ser283, potentially affecting proper salt bridge formation and c140s loop conformational changes, participates in producing a clinically severe form of CRM+ FVII deficiency. Furthermore, in a plasma system with limiting thromboplastin concentration, the 283Ser-FVII inhibited the Wt-FVIIa activity in a dose-dependent manner, a finding which might have implications for substitution therapy of severe CRM+ deficiency.

C068

A FREQUENT HUMAN COAGULATION FACTOR VII MUTATION (A294V) AFFECTS THE INTERACTION WITH ACTIVATORS, TISSUE FACTOR AND SUBSTRATES

Toso R,*[#] Pinotti M,* High K,[#] Marchetti G,* Pollak E,[†] Bernardi F*

**Dipartimento di Biochimica e Biologia Molecolare, Università di Ferrara, Italy; [#]Joseph Stokes Research Institute, Children's Hospital of Philadelphia, PA, USA*

Activated factor VII (FVIIa) is a vitamin-K dependent serine protease that initiates blood clotting after interacting with its cofactor tissue factor (TF). The complex FVIIa–TF is responsible for the activation of factor IX (FIX) and factor X (FX) ultimately leading to the formation of a stable fibrin clot. Activated FX (FXa), a product of FVIIa enzymatic activity, is also the most efficient activator of zymogen FVII. Interactions of FVII/FVIIa with its activators, cofactor and substrates have been extensively investigated to define contact regions and residues involved in the formation of the complexes. Site-directed mutagenesis and inhibition assays led to the identification of sites removed from the FVIIa active site that influence binding specificity and affinity of the enzyme. In this study we define the biochemical properties of the FVII mutant A294V, a frequent naturally occurring variant in Caucasians, responsible for asymptomatic FVII deficiency. FVII levels associated with the A294V mutation in patients were FVII:C 6-18% and FVII:Ag 25-61%. The affected residue (chymotrypsin numbering 152) lies in the Loop 140s, a region that undergoes major rearrangements after FVII activation and that is relevant to the development of substrate specificity. FVII A294V shows delayed activation by FXa as well as reduced activity towards peptidyl and macromolecular substrates without impairing the catalytic efficiency of the triad. Also, the interaction of this FVII(a) variant with TF was altered, suggesting that this residue and more likely the Loop 140s plays a pivotal role not only in the recognition of FX by the FVIIa–TF complex, but also in the interaction of FVII with both its activators and cofactor TF.

C069

FACTOR VII SAN GIOVANNI ROTONDO: IDENTIFICATION OF 3 NEW POSSIBLE CAUSAL MISSENSE MUTATIONS IN TWO UNRELATED SUBJECTS

Bossone A, Colaizzo D, Cappucci G, Vecchione G, Lupone M,^ De Lucia D,* Perricone C,^ Di Minno G, Margaglione M°

*Unità di Aterosclerosi e Trombosi, I.R.C.C.S. "Casa Sollievo della Sofferenza", S. Giovanni Rotondo; ^Centro Regionale per le Emocoagulopatie, Azienda Ospedaliera "Santobono-Pausillipon", *Istituto di Patologia Generale e Oncologia, Seconda Università di Napoli; °Istituto di Medicina Interna e Geriatria, Università di Palermo; and Genetica Medica, Università di Foggia, Italy*

Severe factor VII (FVII) deficiency has an estimated incidence of 1 in 500,000. Severe reduction in plasma FVII activity usually leads to bleeding problems in human patients although the phenotype is heterogeneous. We have investigated 2 young probands, an asymptomatic woman, offspring of a non-consanguineous marriage, and a symptomatic young man both with undetectable levels of functional FVII. In the first proband, a C-to-A transversion leads to a Arg/Ser substitution at position 110, whereas a G-to-T transversion leads to a Asp/Tyr substitution at position 123. Her first-degree relatives, who had approximately half the normal FVII values and showed concordance between functional and immunologic levels, were heterozygtes, father and mother showing the Asp123Tyr and the Arg110Ser substitution, respectively. The only brother of the patient, who also carried approximately half the normal FVII values, exhibited the Asp123Tyr substitution. Both the mutations occur within the epidermal growth factor (EGF-2) domain. This domain is not involved in direct contact between tissue factor and FVII but is necessary for optimal binding, merely imparting structure to the rest of the molecule. Indeed, EGF-1 and

EGF-2 domains are described as forming a single rigid structural unit with no interdomain flexibility. The second proband showed normal FVII antigenic levels. A homozygous G-to-A substitution within exon 8 was identified that leads to a Gly/Ser substitution at position 331. The mutation identified involved the catalytic domain. In summary we have described two patients with a severe FVII deficiency: a compound heterozygous for two substutions within the EGF-2 domain and a homozygous mutation in the catalytic domain. 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 FVII molecule.

C070

MUTATION ANALYSIS IN THE ITALIAN COHORT OF PATIENTS WITH SEVERE HEMOPHILIA A AND INHIBITORS

Salviato R,* Belvini D,* Mancuso G,** Scaraggi A,*** Musso R,° Muleo G°, Berrettini M,°°° Trapani Lombardo V,^ Tamponi G,^^ Tagariello G*

Centri Emofilia di Castelfranco Veneto, Palermo,** Bari,*** Catania,° Catanzaro°,° Perugia°°,° Reggio Calabria^, Torino^^, Italy*

The development of inhibitors against transfused factor VIII still remains the main complication of replacement therapy of hemophilia A. Large gene deletions, factor FVIII gene inversion and nonsense mutations display an incidence of antibodies of approximately 35%, compared with only about 5% in patients with small gene deletions or missense mutations. The objective of this study was to identify the molecular defects in a cohort of 44 severe A hemophiliacs with a history of inhibitor. FVIII gene inversion detection by multiplex long range PCR according to Liu (*Blood, 1998*) revealed the presence of this common mutation in 16 (36%) patients. The others were analyzed by conformation sensitive gel electrophoresis (CSGE), a heteroduplex based method for nucleotide mismatch detection requiring amplification of the gene coding and regulative sequences (26 exons and 5'and 3' flanking regions) as separate fragments. In 3 patients we were unable to obtain any PCR product for a portion of the FVIII gene (exons 2 to 25, 5 to 10 and 14 to 26, respectively), suggesting a large deletion. By long range PCR a specific product was obtained for two of them, using primers for 5' and 3' sequence flanking the breakpoint intronic regions. In the remaining 20 out of 25 patients so far analyzed, 11 nonsense, 2 missense, 2 small deletions (4-pb e 7-pb), 3 small insertions (1-pb) and 2 single nucleotide substitutions in a splice junction were the mutations identified. FVIII gene inversion represents a risk factor for inhibitor development. Nevertheless other severe genetic defects probably interfering with the synthesis of FVIII are well represented in these group especially large deletions, nonsense mutations and FVIII gene inversions (28 out of 39, 72%). These results support the hypothesis that gene defects producing a severe phenotype can frequently be found in association with higher risk for inhibitor development.

C071

IN VITRO EXPRESSION STUDIES OF A NATURALLY OCCURRING MUTATION LOCATED IN THE CATALYTIC DOMAIN ON FACTOR X GENE (GLY222ASP)

Peyvandi F, Menegatti M, Bajetta MT, Mannucci PM

A. Bianchi Bonomi, Hemophilia Centre, IRCCS Maggiore Hospital, Milan, Italy

Phenotype and genotype analysis was carried out in two patients with severe factor (FX) deficiency. Three coagulant assays (PT, PTT, DRVVT) detected no coagulant activity in both patients' plasma, while chromogenic and FX antigen levels (FX:Ag) were respectively 3-6% and 10-15% in patient A and B. Direct sequencing of the factor X gene showed a homozygous mutation in each patient causing substitution of residue Gly222Asp (numbering the + 1 corresponding to Ala at the N-terminus of the mature protein). This mutation is localized in the catalytic domain close to the catalytic triad His236-Asp282-Ser379 of the FX gene. In order to clarify the effect of this substitution on the protein, both wild type (WT) and mutant FX cDNAs were expressed transiently in HEK 293 cells. The FX-WT or FX-222 coagulant activity was measured in conditioned media of cells transfected by mammalian expression vector (pCMV4). The procoagulant activity of FX-WT was normal whereas the mutant protein had no coagulant activity. FX:Ag levels were measured in both cell lysates and conditioned media of transfected cells. FX:Ag in cell lysate transfected by mutant construct was above 40% higher than FX-WT indicating an intracellular accumulation. The conditioned media of cells transfected by FX-222 had only 33% of FX-WT Ag level, confirming a secretion defect. *In vitro* expression analysis of the Gly222Asp substitution of the FX gene demonstrated an intracellular accumulation of the mutant protein associated with an alteration in the secretion pathway. Further biochemical characterization is required to explain why a partially secreted mutant FX protein is not functionally active.

C072

MOLECULAR CHARACTERIZATION OF SEVEN FAMILIES WITH SEVERE FACTOR XIII DEFICIENCY

Peyvandi F, Tagliabue L, Mehran K,* Mannucci PM

*A. Bianchi Bonomi,Hemophilia Centre, IRCCS-Maggiore Hospital, Milan, Italy,*Nemazee Hospital, School of Medicine, Shiraz University of Medical Science, Iran*

Coagulation factor XIII (FXIII) is a plasma transaglutaminase consisting of two catalytic A subunits and two non-catalytic B subunits. Factor XIII deficiency is a rare bleeding disorder (1:2,000,000) transmitted with an autosomal recessive pattern. Seven Iranian patients (born from consanguinous marriages) from unrelated families have been investigated. All had a severe bleeding history. We found two novel and one previously reported homozygous missense mutations in the subunit A of FXIII gene. The first novel mutation was Arg77His located in the ß sandwich of the FXIII gene that probably leads to an improperly folded or unstable structure of the protein. This mutation was present in five of our patients (5/7). Only one family showed an Arg382Ser mutation located in the catalytic core of the protein. This mutation probably leads to an alteration of the catalytic activity of this enzyme. We confirmed the heterozygous state of the parents of each patient by endonuclease restriction analysis. The absence of these two novel mutations in 120 normal tested alleles from the same geographic area suggest that these mutations are responsible for FXIII deficiency in the families.

Oral Communications
Anticoagulation I

C073

INCIDENCE OF NEWLY DIAGNOSED CANCER AFTER THREE MONTHS OR ONE YEAR OF ORAL ANTICOAGULATION FOR A FIRST EPISODE OF IDIOPATHIC VENOUS THROMBOEMBOLISM

Taliani MR,* Agnelli G,* Prandoni P, Becattini C, Moia M, Bazzan M, Tomasi C, Guazzaloca G, Bertoldi A, Ageno W, Ambrosio GB, Salvi R, Poggio R, Silingardi M, Porro F, Zonzin P, Imberti D, Casazza F, Pogliani E, Ria L, Piovella F, De Lucia D, for The Warfarin Optimal Duration Italian Trial Investigators

**Dipartimento di Medicina Interna, Sezione di Medicina Interna e Cardiovascolare, Università di Perugia, italy*

Background. A number of studies have reported the association between idiopathic venous thromboembolism (VTE) and subsequent occurrence of cancer. The risk of newly diagnosed cancer has been reported to be higher in patients with VTE treated with oral anticoagulants (OA) for six weeks than in patients treated for six months. *Aim of the study*. To evaluate in a multicenter, prospective, randomized study if one-year anticoagulation reduces the risk of newly diagnosed cancer in patients with idiopathic VTE with respect to three-month anticoagulation. *Methods.* Patients with a first episode of VTE not associated with temporary risk factors (surgery, trauma, oral contraceptives, etc.) or persistent risk factors (known cancer and thrombophilia) were included in the study. After three months of oral anticoagulation (INR: 2 to 3), patients were randomized to stop anticoagulation or to continue it for 9 additional months. Newly diagnosed cancer was assessed by follow-up visits scheduled every three months for the first year and every 6 months for the following years after randomization. *Results*. Four hundred and nineteen patients (267 presenting with DVT and 152 with PE, 53% males; mean age 65 years) were enrolled in the study at 20 Italian hospitals. During a mean follow-up of 45.7 months, newly diagnosed cancer occurred in 25 patients (6.0%):11 out of 206 patients (5.3%) treated with OA for 3 months and 14 out of 213 patients (6.6%) treated for 12 months (RR=0.81, 95%CI 0.37-1.74). Cancer sites were urogenital tract (7 patients), respiratory tract (5), gastrointestinal system (5), breast (5), hemopoietic system (3). No difference in the incidence of death related to cancer was found between the two groups. *Conclusions*. In patients with idiopathic VTE one-year anticoagulation does not have a protective effect on the development of newly diagnosed cancer with respect to three-month anticoagulation.

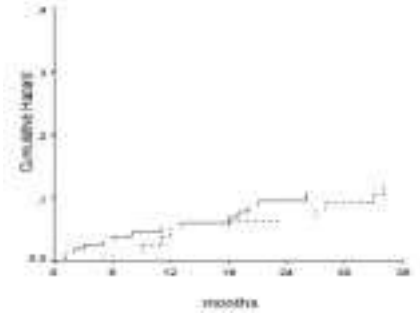
C074

THREE MONTHS VERSUS EXTENDED ORAL ANTICOAGULANT TREATMENT AFTER A FIRST EPISODE OF PULMONARY EMBOLISM

Becattini C, Agnelli G, Prandoni P, Silingardi M, Taliani MR, Miccio M, Poggio R, Imberti D, Pogliani E, Ageno W, Porro F, Zonzin P, Bertoldi A, Tomasi C, Casazza F, Moia M, Ria L, Ambrosio GB, Piovella F, De Lucia D, Pieralli F, for The Warfarin Optimal Duration Italian Trial Investigators

Dipartimento di Medicina Interna, Sezione di Medicina Interna e Cardiovascolare, Università degli Studi di Perugia, Italy

Background. The optimal duration of oral anticoagulant treatment after a first episode of pulmonary embolism has never been evaluated. *Methods.* Patients with a first episode of pulmonary embolism who had completed three months of oral anticoagulant therapy were randomly assigned to discontinuation of anticoagulation or to its continuation for three (pulmonary embolism associated with transient risk factors) or nine (idiopathic pulmonary embolism) additional months. The primary study outcome was recurrence of symptomatic, objectively confirmed venous thromboembolism. *Results.* All the recurrences but one occurred after discontinuation of anticoagulant treatment. Among 164 patients assigned to extended anticoagulation, 14 had a recurrence (3.2% per patient-year; average follow-up 32.0 months), as compared with 17 of 162 patients assigned to treatment discontinuation (4.3% per patient-year; average follow-up 29.6 months), resulting in a relative risk of 0.81 (95% confidence interval 0.41-1.59). In patients assigned to extended anticoagulation the incidence of recurrence after treatment discontinuation was 4.0% per patient-year (average off-treatment period 25.9 months). No major bleeding was observed during extended anticoagulation. Ten of 89 patients with idiopathic pulmonary embolism assigned to extended anticoagulation experienced a recurrence (4.2% per patient-year; average follow-up 32.4 months), as compared with 11 of 92 patients assigned to treatment discontinuation (5.0 percent per patient-year; average follow-up, 28.6 months), resulting in a relative risk of 0.94 (95% confidence interval 0.42–2.10). An incidence of 9.0% of adverse clinical outcome events (recurrence and death) was observed in patients with pulmonary embolism associated with transient risk factors with respect to 17.1 % in patients with idiopathic pulmonary embolism, resulting in a relative risk of 1.91 (95% confidence interval 1.04-3.51; p= 0.04).



Conclusions. In patients with pulmonary embolism, the clinical benefit associated with extending the duration of anticoagulant therapy is not maintained after therapy is discontinued.

C075

INTERACTION BETWEEN THROMBOPHILIC GENETIC MUTATIONS AND CLINICAL BLEEDING IN PATIENTS UNDERGOING CHRONIC ORAL ANTICOAGULANT TREATMENT

Castori L, Talani R, Agnelli G, Nenci GG, Gresele P

Sezione di Medicina Cardiovascolare Dipartimento di Medicina Interna, Università di Perugia, Italy

Recent studies have suggested a protective role of the FV Leiden and PT20210A mutation on primary intracranial hemorrhage, on blood loss during delivery and on hemophilia. We investigated whether these polymorphisms can influence the development of hemorrhagic complications in patients undergoing chronic oral anticoagulant treatment. *Material and Methods*. From a population of patients attending our Anticoagulation Clinic, we selected 78 patients with a history of moderate-severe bleeding complications, 31 of which led to hospitalization, while on oral anticoagulant treatment (6 intracranial hemorrhages, 32 epistaxes requiring nasal packing, 27 macroscopic hematurias, 3 spontaneous muscular hematomas, 9 gastrointestinal bleedings, 1 hemarthrosis). Patients with known hemorrhagic risk factors at the time of the event, e.g. urinary infections, gastric-duodenal ulcer, bowel polyposis or cancer, concomitant use of drugs, potentiating oral anticoagulants were excluded. We compared these patients with a control group matched for gender, age, kind of anticoagulant drugs (warfarin or acenocoumarol), INR range, duration of treatment. Average age of the group of patients was 70.8 years (range 35-89) (69.2 for controls, range 35-87), average duration of anticoagulant therapy was 110.2 months (106.8 for control). Indications for anticoagulant treatment were prosthetic mechanical valve (44), atrial fibrillation (27), thromboembolism (2), arterial by-pass (2), heart valve disease (1), aneurysm of interatrial septum (1). At the time of the bleeding 72 patients were treated with warfarin, 6 with acenocoumarol. *Results*. Among 78 patients with a history of hemorrhagic complications, 12 (15.38%) were carriers of the FV Leiden and 2 (2.56%) carriers of the PT20210A mutation as compared with 5 (6.41%) and 2 (2.56%), respectively, in the control group. The odds ratio for bleeding in carriers of FV Leiden was 2.6 (95% CI 0.889-7.94). *Conclusions.* No significant interaction was observed between these two thrombophilic genetic mutations and bleeding events in patients on chronic oral anticoagulant treatment. Contrary to was what expected, our results suggest a trend towards an increase of the hemorrhagic risk in anticoagulated patients carrying the FV Leiden mutation. Larger studies are required to confirm these results.

C076

EVIDENCE FOR A DIFFERENT LABORATORY QUALITY OF ORAL ANTICOAGULANT TREATMENT IN PATIENTS RECEIVING ACENOCOUMALONE OR WARFARIN

Crippa L, Fattorini A, Pattarini E, Viganò D'Angelo S, Pricolo F, D'Angelo A

Coagulation Service & Thrombosis Research Unit, IRCCS H S.Raffaele, Milan, Italy

We evaluated the laboratory quality of oral anticoagulant treatment over a period of 10 years in 2463 patients (1112 F, 1531 M, mean age at enrollment 67.5±24 yrs) with target INR of 2.5 (n = 1681) and 3.0 (n = 962), and receiving acenocoumalone (4 mg, n = 797) or warfarin (5 mg, n = 1666) pills. Data were included in the analysis after achievement of a first therapeutic INR value, for a total of 80,503 INR determinations classified as below (n = 15,795; 19.6%), within (n = 55,261; 68.6%) and above the therapeutic interval (n = 9,447; 11.7%). Irrespective of the target INR, patients on acenocoumalone treatment received a lower average weekly number of pills (n = 3.8±2.3) than patients on warfarin treatment (n = 5.6±2.8, p<0.0001). INR values within the therapeutic range were more often observed with warfarin (71%) than with acenocoumalone treatment (64.6%, p <0.0001) and the Mantel-Haenszel OR (adjusted for the target INR) of INR values outside the desired range with acenocoumalone was 1.35 (95% C.I.: 1.31-1.39). With both anticoagulant drugs the Mantel-Haenszel RR (adjusted for the target INR) of unsatisfactory INR values was greater with daily dosages of less than 0.25 pills (acenocoumalone: 1.94; warfarin 1.70) and it was significantly lower than 1 with daily dosages ranging from 0.5 to 1.25 pills of acenocoumalone and from 0.75 to 1.50 pills of warfarin. However, the Mantel-Haenszel OR of INR values outside the desired range was consistently significantly greater with acenocoumalone than with warfarin (from 1.17 to 1.49) across daily dosages ranging from less than 0.25 to more than 2 pills of anticoagulant drugs. These data are evidence that a better quality of INR monitoring is easier to achieve with warfarin than with acenocoumalone and strongly suggest that especially patients requiring low doses of acenocoumalone should be shifted to warfarin treatment.

C077

PROLONGED THROMBOPROPHYLAXIS WITH ORAL ANTICOAGULANTS AFTER TOTAL HIP ARTHROPLASTY. A PROSPECTIVE, CONTROLLED, RANDOMIZED STUDY

Prandoni P, Bruchi O, Sabbion P, Tanduo C, Scudeller A, Sardella C, Errigo G, Pietrobelli F, Maso G, Girolami A

Department of Medical and Surgical Sciences, 2nd Chair of Internal Medicine, and Saint Anthony Unit Care of Anesthesiology, University Hospital of Padua, Italy

The optimal duration of thromboprophylaxis following major orthopedic surgery is controversial. Although oral anticoagulants are still widely used for prevention of venous thromboembolism after hip replacement, no study has assessed the benefit of prolonging anticoagulation beyond the hospital stay. Consecutive patients who had received warfarin prophylaxis after total hip arthroplasty were randomized to stop the drug at the time of hospital discharge or to continue it for four additional weeks. The rate of symptomatic and asymptomatic venous thromboembolic events (as shown by compression ultrasonography) arising in the study period was compared between the two groups. The study was prematurely terminated after the inclusion of the first 360 patients because of a statistically significant and clinically relevant superiority of extended over short thromboprophylaxis. Objectively confirmed venous thromboembolic complications were recorded in 10 patients, 9 in the 176 control patients (5.1%), and 1 in the 184 patients who continued the warfarin treatment (0.5%). The absolute difference in the incidence of events was 4.6% (95% CI, 1.15 to 8.0). The RR of developing venous thromboembolism in control patients as com-

pared to patients assigned to extended thromboprophylaxis was 9.4 (95% CI, 1.2 to 73.5). The NNT was 22. Major bleeding developed in 1 patient randomized to extended prophylaxis (0.5%; 95% CI, 0.02 to 3.0) as compared to none in the control group. Extending prophylaxis with warfarin for a few additional weeks beyond the hospital stay has the potential to improve the outcome considerably of patients who have undergone hip arthroplasty.

C078

LOW-MOLECULAR-WEIGHT HEPARIN FOR THE LONG-TERM TREATMENT OF SYMPTOMATIC VENOUS THROMBOEMBOLISM: META-ANALYSIS OF THE RANDOMIZED COMPARISONS WITH ORAL ANTICOAGULANTS

Iorio A, Guercini F, Pini M*

*Sezione di Medicina Interna e Cardiovascolare, Università di Perugia; *Dipartimento di Medicina Interna, Ospedale di Fidenza, Italy*

Background. The standard oral anticoagulant (OA) treatment of venous thromboembolism (VTE) is not ideal because it requires laboratory monitoring, carries a definite bleeding risk, and is contraindicated in some patients. A number of small clinical trials evaluated the efficacy and safety of low molecular weight (LMW) heparins in the long-term treatment of VTE, but they lacked the power to establish equivalency or superiority versus OA. We performed a meta-analysis of the randomized comparisons between the two treatments as a contribution to answer this important clinical question. *Aim of the study*. The objective of this review was to evaluate the efficacy and safety of long-term treatment of VTE with LMW heparins compared with OA. *Materials and Methods*. Computerized searches of MEDLINE and EMBASE were performed. In addition, trials were located through colleagues and the hand-scanning of meeting proceedings and reference lists. Two reviewers reviewed and extracted data independently using a standard form. The analysis was performed on an intention to treat basis for the period of randomized treatment and separately for the subsequent follow up. A meta-regression analysis was employed to investigate the relationship between daily dose and clinical outcome. *Results*. Seven studies were identified that fulfilled our predefined criteria for a total of 1379 patients. A statistically non-significant reduction in the risk of VTE (OR 0.66; 95% CI [0.41, 1.07]) and in the risk of major bleeding (OR 0.45; 95% CI [0.18, 1.11]) in favour of LMW heparin treatment was found. No difference in mortality (OR 1.19; 95% CI [0.78, 1.83]) was observed between the two treatments. *Conclusions*. LMW heparins are possibly more effective and safer than OA in the prevention of recurrent symptomatic VTE. The trade-off of the parenteral administration and higher acquisition cost of LMW heparins has to be balanced with specific requirements from particular type of patients.

C079

THE G20210A MUTATION IN THE FACTOR II GENE IS ASSOCIATED WITH SYSTEMIC EMBOLISM IN PATIENTS WITH NON-VALVULAR ATRIAL FIBRILLATION

Pengo V, Filippi B, Biasiolo A, Pegoraro C, Noventa F,* Iliceto S

*Clinical Cardiology Thrombosis Centre, *5th Medical Clinic, Department of Clinical and Experimental Medicine, University of Padua, Italy*

Atrial thrombus formation and subsequent dislodgment into the systemic circulation are responsible for thromboembolic complications observed in patients with non-valvular atrial fibrillation (AF). Stasis of blood in the left atrial appendage generates fibrin-rich thrombi that resemble those found in the venous circulation. The fact that inherited thrombophilia is a risk factor for venous thromboembolism suggests that it might also contribute to systemic thromboembolism in this clinical setting. We studied 71 consecutive patients with non-valvular AF and a previous documented systemic embolism along with 142 age-and sex-matched control nonvalvular AF patients who had not experienced this complication. Compared to controls, the cases showed an increased frequency of factor V Leiden mutation (OR=1.7; 95% CI, 0.5-5.8) and G20210A prothrombin gene mutations. This latter polymorphism was present in 9 (12.7%) of 71 cases and in 6 (4.2%) of 142 controls (OR= 3.3; 95% CI, 1.1-9.6, $p<0.05$). Two out of these 9 cases and none of the six controls had both the factor V Leiden and factor II mutation (double heterozygosis). When logistic regression analysis was performed taking into account major and minor clinical risk factors, the presence of the G20210A factor II gene mutation was revealed to be independently associated with the occurrence of a previous systemic embolism. The G20210A factor II gene mutation is associated with systemic thromboembolism in patients with non-valvular AF; its detection might aid in identifying patients at risk and in determining the appropriate antithrombotic treatment.

C080

PATIENTS HIGHLY UNSTABLE IN THEIR RESPONSE TO ORAL ANTICOAGULANTS: A COLLABORATIVE CONTROLLED STUDY

Palareti G, Legnani C, Guazzaloca G, Fariselli S, Poli D,* Prisco D* (on Behalf of the Ad Hoc Study Group of The Italian Federation of Anticoagulation Clinics, FCSA)

Dept. Angiology and Blood Coagulation, University Hospital S. Orsola-Malpighi, Bologna; Thrombosis Center, University of Florence, Italy*

Bleeding and thrombotic complications of oral anticoagulant (OA) treatment are often associated with poor anticoagulation control. The FCSA centers were invited to enroll their most unstable cases and stable controls (matched for gender, age and indication for OA). Demographic and educational data, medical history, detailed diet and life habits, and number of visits, INR values, OA doses during the 4 months preceding and following enrollment were collected; blood cell count and tests for liver and renal function were performed; *Abbreviated Mental Test* (AMT) and a questionnaire to assess the degree of attention and comprehension of OA mechanisms were administered. One hun-

dred unstable cases (median age 68 y; 24-87y; 45 M) and 96 controls (median age 68 y; 26-80y; 48 M) were enrolled in 32 Centers. The criteria for case selection were different among the centers. The median number of visits during a 4 month period was 9 (from 4 to 18) in cases and 6 (3-14) in controls (p<0.0001, Mann-Whitney). Cases and controls spent 41.5% and 86.5% of time in range, respectively (p<0.0001); overanticoagulation was more frequent in cases (p<0.0001). Problems with compliance were admitted by 40 cases and 19 controls (p<0.01). An insufficient score for the AMT was recorded in 12.2% and 6.5% of cases and controls, respectively; an excessive number of wrong answers to the questionnaire was obtained in 14.0% of cases and 4.3% of controls (p<0.05). No differences were observed regarding: educational level, type of employment, family composition, diet and life habits, alcohol consumption, blood tests, liver and renal function, use of warfarin or acenocoumarol and mean daily dose. An inadequate comprehension of OA treatment mechanisms, an insufficient degree of attention and problems regarding compliance to the treatment are conditions more frequently found in subjects highly unstable during OA therapy than in matched, stable controls. Overanticoagulation is significantly more frequent in unstable patients.

Oral Communications
Nutrition and Vascular Risk

C081

MEDITERRANEAN DIET AS A PROTECTIVE FACTOR FOR PERIPHERAL ARTERIAL DISEASE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Ciccarone E,*° Di Castelnuovo A,* Salcuni M,# Siani A,@ Giacco A,^ Donati MB,* Capani F,° Iacoviello L* on behalf of the GENDIABE investigators

**"Angela Valenti" Laboratory of Genetic and Environmental Risk Factors for Thrombotic Disease, Department of Vascular Medicine and Pharmacology, Consorzio Mario Negri Sud, Santa Maria Imbaro; °Department of Medicine and Ageing; #Institute of Radiology, University of Chieti, Chieti; @Institute of Food Sciences, National Research Council, Avellino; ^Department of Clinical and Experimental Medicine, "Federico II" University of Naples, Italy*

The Mediterranean diet has protective effects on cardiovascular disease. We investigated the impact of dietary habits on peripheral arterial disease (PAD) in a high risk population, with a standard Mediterranean diet. From a cohort of 944 patients with type 2 diabetes, we selected 144 patients with PAD, confirmed with color-duplex ultrasonography, who were matched for age and sex with 288 type 2 diabetic control patients without micro- or macrovascular complications. In multivariate analysis, diabetes duration(OR >15 years = 2.49; CI95%, 1.45-4.25), hypertension (OR = 2.12; CI95%, 1.31–3.45), butter consumption (OR = 2.6; CI95%, 1.15–3.68) and a healthy dietary score (OR = 0.44; CI95%, 0.24-0.83), were significantly and independently associated with the risk of PAD. Dietary score significantly improved the predictive value of models based on duration of diabetes and hypertension (LSR =2.19, FD=7, p<0.001). The effect of dietary score on the risk of PAD was independent from the duration of diabetes, indeed it influenced the risk of PAD, in the presence of both, a short (<10 years) and a long (>10 years) history of diabetes. Similar results were obtained for presence or absence of hypertension. In patients with type 2 diabetes, a Mediterranean dietary pattern has a protective role against PAD, despite a long history of this disease and the presence of hypertension. The use of butter still increases the risk of PAD in patients with a daily consumption of olive oil. Mediterranean diet should be, therefore, strongly advised for PAD prevention in type 2 diabetes.

C082

IN VIVO EFFECT OF CHIANTI WINE FROM FLORENCE UNIVERSITY'S VINEYARD ON THE TISSUE FACTOR-TISSUE FACTOR PATHWAY INHIBITOR SYSTEM IN *HEALTHY* VOLUNTEERS

Marcucci R, Gori AM, Fedi S, Giusti B, Brunelli T, Bandinelli B, Capalbo A, Bertuccioli M,* Gensini GF, Abbate R

*Dipartimento Area Critica Medico Chirurgica, Università di Firenze; *Facoltà di Agraria, Università di Firenze; Centro Trombosi, Azienda Ospedaliera Careggi, Florence, Italy*

An inverse relation between moderate alcohol consumption and the risk of coronary heart disease has been demonstrated in

several epidemiological studies. A number of studies suggested that moderate consumption of red wine may be more effective than other alcoholic beverages in decreasing the risk of coronary artery disease mortality. Recently, moderate alcohol intake has been found to affect homocysteinemia determining a significant increase in its plasma levels. In addition, it has been demonstrated in *in vitro* studies that polyphenolic compounds of red wine are able to inhibit tissue factor (TF) expression in vascular cells. Chianti red wine (vintage: 1998; vines: Sangiovese toscano, trebbiano toscano, canaiolo nero, malvasia, colorino) was furnished by the University of Florence. Fifteen healthy subjects (7F/8M) (age: 38, 23-58 yrs), who were moderate alcohol drinkers, volunteered for the study. After 2 weeks' alcohol abstinence, they were requested to drink 300 mL of wine per die for two weeks. Peripheral blood samples were withdrawn after two weeks' alcohol abstinence (PRE-wine) and at the end of the alcohol intake period (POST-wine). TF, free-tissue factor pathway inhibitor (free-TFPI) and thrombin-antithrombin complexes (TAT) were determined by ELISA methods and homocysteine levels were assayed by HPLC. After 2 weeks of red wine intake, TF plasma levels were significantly decreased with respect to PRE-wine samples (POST-wine 101.0, 29.7-205.0 pg/mL vs PRE-wine121.0, 72.9-204.7 pg/mL; $p<0.01$). The red wine intake was associated with a reduction of TF levels, in all but two subjects, by about 17.3%. In addition, at the end of wine intake, we observed an increase (+ 10.1 %) of free- TFPI levels (POST-wine:12.4, 7.0-19.0 ng/mL vs PRE-wine:10.0, 6.0-23.2 ng/mL; $p<0.05$) in all subjects. No significant relationship between TF decrease and free-TFPI increase was observed (r=0.12; $p=0.8$). On the other hand, homocysteine plasma levels were significantly affected by wine intake. Infact, we observed significantly raised homocysteine levels after 2 weeks of red wine intake (POST-wine:11.0, 8.2-18.0 μmol/L vs PRE-wine: 9.9, 7.0-13.8 μmol/L; $p<0.01$) with an increase, in all subjects but one, of 11.9%. No significant changes in TAT plasma levels were found in relation to red wine assumption (PRE-wine:1.9, 0.2-4.0 ng/mL vs POST-wine:1.5, 0.2-4.1 ng/mL; $p=0.33$). In conclusion, our results documented an *in vivo* inhibitory effect of red wine on TF circulating levels, suggesting a novel mechanism explaining the protective effect of red wine intake against cardiovascular disease. The free-TFPI increase was likely responsible for TF reduction, but the lack of a strict relationship between TF and free-TFPI levels suggested that other mechanisms might be responsible for TF decrease after wine intake. The contemporary observed increase in homocysteine levels, referred to a tissue damage induced by ethanol, was not associated with blood clotting activation and might only mirror a beneficial tissue repair process.

C083

A META-ANALYSIS OF WINE AND BEER CONSUMPTION IN RELATION TO VASCULAR RISK

Di Castelnuovo A, Rotondo S, Iacoviello L, Donati MB de Gaetano G*

*Department of Vascular Medicine and Pharmacology, Istituto di Ricerche Farmacologiche Mario Negri, Consorzio Mario Negri Sud, Santa Maria Imbaro; *Center for High Technology Research and Education in Biomedical Sciences, Catholic University, Campobasso, Italy*

Many epidemiologic studies have evaluated whether different alcoholic beverages protect against vascular disease. We performed a systematic review and a meta-analysis on the relationship between wine or beer consumption and vascular risk. General variance-based methods and fitting models were applied to data derived from 26 studies that gave quantitative estimations of the vascular risk associated with either beverage consumption. From 13 studies involving 209,418 subjects, the relative risk of vascular disease associated with intake of wine was 0.68 (95%CI: 0.59-0.77) relative to non-drinkers. There was strong evidence from 10 studies involving 176,042 persons to support a J-shaped dose-response relation. A statistically significant protective effect was found up to a daily dose of 150 mL wine. The overall effect of moderate beer consumption, measured in 15 studies involving 208,036 persons, was 0.78 (95%CI: 0.70-0.86). However, the best-fitting model failed to show any dose-response relationship between beer intake and vascular risk. The inverse association of wine or beer intake with vascular risk remained statistically significant when pooling studies in which either coronary heart disease or total non-fatal vascular events were the only events considered. Vascular mortality risk, in contrast, was only significantly reduced by wine intake. Significant risk reduction was also obtained in studies which formally excluded ex or light drinkers from the reference group or in studies that had adjusted for different types of alcoholic beverages or indicators of social class level or to those that compared both wine and beer drinking groups with the same reference group. These findings are strongly supportive of a significant inverse association between light to moderate wine consumption and vascular risk. A similar, though smaller association was also apparent concerning beer consumption. The latter finding however is difficult to interprete, as no dose-response relationship could be found between beer intake and vascular risk.

C084

PAI-1 ANTIGEN AND ACTIVITY DECREASES AFTER VITAMIN E SUPPLEMENTATION IN TYPE 2 DIABETIC SUBJECTS ARE DEPENDENT ON PAI-1 GENOTYPE

Mari D, Testa I, Testa R, Bonfigli AR, Manfrini S, Sirolla C, Coppola R, Sacchi E, Franceschi C

IRCCS Maggiore Hospital, Department of Internal Medicine, University of Milan, Institute of Internal Medicine, University of Ancona; INRCA, Department of Gerontology Research, Ancona; L. Sacco Hospital, Immunology and Transfusion Department, Milan, Italy

Background. PAI-1 4G/5G polymorphism is a predisposing factor to arterial thrombosis. Epidemiological studies have shown that environmental and genetic factors act in a synergistic way to determine PAI-1 plasma levels. In particular, the 4G polymorphism of PAI-1 gene promoter seems to enhance the expression of PAI-1 causing a condition of pathological fibrinolysis. *Objectives*. As type 2 diabetes mellitus is a known cause of increase in PAI-1 plasma levels and vitamin E supplementation is able to lower these levels, we wanted to verify whether the 4G/5G gene polymorphism may be important in these changes. Twenty-eight type 2 diabetic patients were enrolled (19 males and 9 females, mean age±SD, 61,3±5,8 years). The guanine insertion/deletion polymorphism 4G/5G in the promoter of the PAI-1 gene was evaluated. These patients were treated with vitamin E (500 IU/die) for 10 weeks. PAI-1 antigen, PAI-1 activity, and the main fibrinolytic parameters were evaluated at baseline

and after 5, 10 and 30 weeks. *Results.* As expected, decrements were detected for PAI-1 antigen and PAI-1 activity between baseline and the 10th week ($p < 0.01$) followed by an increase at the 30th week. Patients with 4G/4G genotype showed the same profiles as the patients with 4G/5G genotype while those with had significant differences with respect to 4G/4G and 4G/5G genotype ($p < 0.01$). These data evidenced that type 2 diabetic patients with at least one 4G allele showed different decrements in PAI-1 plasma levels compared to patients who were homozygous for the 5G allele. In conclusion, decreased PAI-1 plasma levels occur during vitamin E supplementation and this effect is modulated by a common insertion/deletion polymorphism in the PAI-1 promoter.

C085

EFFECT OF RED WINE AND EXTRA VIRGIN OLIVE OIL ON EXPERIMENTAL ARTERIAL THROMBOSIS IN RATS

De Curtis A, Murzilli S, Brosko S, Donati MB, Iacoviello L

"Angela Valenti" Laboratory of Genetic and Environmental Risk Factors for Thrombotic Disease. Department of Vascular Medicine and Pharmacology, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy

The Mediterranean diet has been shown to extent a protective effect against ischemic vascular disease. We aimed at investigating in rats, the possible antithrombotic role of red wine in a condition predisposing to thrombosis (hypercholesterolemia) and the effect of a diet enriched with extra virgin olive oil in normal animals. A colony of rats (FNL) were fed with a 2% cholesterol-rich-diet (CRD) for six months (FNL+D). After 5 months diet, a group of rats was supplemented for 1 month with alcohol-free red wine (FNL+D+W). On the other hand, a group of normal rats was supplemented with extra virgin olive oil (3%; w/w) for six weeks. Cholesterol, triglycerides, FVII activity and fibrinogen levels were measured. The thrombotic tendency was estimated measuring the occlusion time (OT) of a prosthesis inserted into the abdominal aorta. Five months of CRD in FNL rats induced a dramatic increase in cholesterol and triglyceride levels, with a concomitant shortening of the OT, compared to animals fed with standard diet. Alcohol-free red wine supplementation for 1 month reverted the prothrombotic effect of the diet. Indeed, in FNL+D+W a significant prolongation of OT (116±14 vs 57.6±7.3 hrs, $p<0.01$) was observed compared to FNL+D. The olive oil enriched diet induced a significant delay in the thrombotic occlusion of the *aortic loop* (99±5 h vs 82±5 h, $p<0.04$) compared with animals fed a normal diet. Alcohol-free red wine did not affect the increase in cholesterol and triglyceride levels induced by the CRD; no changes in cholesterol or triglyceride levels were observed also with extra virgin olive oil. Neither fibrinogen nor FVII was modified after treatments with alcohol-free red wine or extra virgin olive oil. In conclusion, a supplementation of alcohol-free red wine reverted the prothrombotic status induced by a cholesterol-rich diet, even in the presence of high levels of cholesterol and triglycerides. Similar prevention properties were observed even in normal animals after olive oil supplementation.

C086

PHARMACOLOGIC AND CELLULAR BASES OF SOME ANTITHROMBOTIC PROPERTIES OF EXTRAVIRGIN OLIVE OIL: STUDIES ON OLEUROPEIN AND HYDROXYTYROSOL

De Curtis A,* Murzilli S,* Rotondo S,° Krauze-Brzósko K,° Di Deo A,# Del Boccio P,# Rotilio D,# Donati MB,* Iacoviello L*

**"A. Valenti" Laboratory of Genetic and Environmental Risk factors for Arterial Thrombosis; °"Giulio Bizzozzero" Laboratory of Blood and Vascular Cell Interactions Department of Vascular Medicine and Pharmacology; #"Gennaro Paone" Center of Environmental Health. Consorzio Mario Negri Sud, S. Maria Imbaro, Italy*

Extra-virgin olive oil is a source of polyphenolic compounds with a strong antioxidant activity. These compounds can have a beneficial effect on processes involved in the pathogenesis of thrombosis. However, few data are available on their pharmacokinetics and pharmacodynamics. We investigated the absorption and metabolism of oleuropein and its major metabolite – hydroxytyrosol - in rats and their biological effect. Rats were treated *per os* with 100mg/kg oleuropein. Plasma samples were collected from the femoral vein 10, 30, 60, 120, and 300 minutes after treatment. Urine was collected at 0 and 24 hrs. The levels of oleuropein were measured by LC-mass-spectometry. Oleuropein reached its maximum plasma concentration within the first two hours after administration (100±11 ng/mL). Analysis of urine showed the presence of oleuropein at the concentration of 26±7 ng/mL. Enzymatic hydrolysis of urine with β-glucuronidase yielded 238±28 ng/mL of oleuropein indicating that the 90% of oleuropein is excreted as glucuronide. This conjugated compound was not found in plasma. The major oleuropein metabolite detected in plasma and in urine was hydroxytyrosol, conjugated in the urine with glucuronic acid. To evaluate the biological activity of these compounds, human washed platelets and PMN were incubated for five minutes with different doses of oleuropein or hydroxytyrosol (5-500 μM). Hydroxytyrosol, but not oleuropein incubation, decreased in a dose-dependent manner, platelets aggregation and TxB2 release by arachidonic acid. This reached the maximum effect at 100 μM (92% of inhibition). At higher concentration hydroxytyrosol (250 μM) also inhibited PMN aggregation and reduced by 53% leukotrienes production. However, to obtain the same decrease in elastase release, a double concentration (500 μM) was required. These data indicate that oleuropein is absorbed after oral ingestion, metabolized in hydroxytyrosol and excreted mainly as glucuronide conjugate. The metabolic product of oleuropein, hydroxytyrosol, has an inhibitory effect on platelet and PMN which may contribute to the antithrombotic effect of olive oil.

C087

INHIBITION OF TISSUE FACTOR EXPRESSION IN ACTIVATED ENDOTHELIAL AND MONONUCLEAR CELLS BY RESVERATROL AND QUERCETIN, TWO POLYPHENOLIC COMPOUNDS: A POSSIBLE MECHANISM CONTRIBUTING TO THE CARDIOPROTECTIVE EFFECT OF RED WINE

Di Santo A, Mezzetti A, Napoleone E, Donati MB, de Gaetano G,* Lorenzet R

*Istituto di Ricerche Farmacologiche Mario Negri, "Antonio Taticchi" Laboratory for Atherosclerosis and Thrombosis, Consorzio Mario Negri Sud, Santa Maria Imbaro, and * Centro di Ricerche e Alta Formazione, Università Cattolica, Campobasso, Italy*

Epidemiological studies suggest that moderate drinking of red wine helps decrease the morbidity and mortality rate from coronary heart disease. Polyphenols, such as resveratrol and quercetin, in red wine, have been suggested to contribute to this protection for their effect in preventing low-density lipoprotein oxidation and inhibition of platelet aggregation. Tissue factor (TF), the cellular receptor that initiates blood coagulation, plays a primary role in hemostasis following tissue injury and in the pathogenesis of atherosclerosis. We decided to investigate the role of resveratrol and quercetin on TF expression by activated endothelial and mononuclear cells (MN). Confluent human umbelical vein endothelial cells (HUVEC) and MN from healthy donors were stimulated with bacterial lipopolysaccharide (LPS), IL-1 β or TNF-α at the presence of increasing concentrations of resveratrol or quercetin (1-50 μM). The agonist-induced TF activity in both cell types, measured by one stage clotting assay, was significantly reduced in a dose-dependent fashion. Inhibition of TF activity was paralleled by a decrease in TF antigen, as assessed by ELISA. The inhibition requires mRNA synthesis, as shown by Northern blot in HUVEC and RT-PCR in MN. To understand the mechanism by which the two compounds downregulate TF activity we studied the translocation of the transacting factor c-Rel/p65 into the nucleus by EMSA (electromobility shift assay). Translocation of c-Rel/p65 induced by LPS, IL-1β or TNF-α was greatly reduced in the presence of resveratrol and quercetin in both cell types. Western blot analysis revealed that the diminished c-Rel/p65 activity was dependent upon inhibited degradation of the inhibitory protein I-κBα. The finding that resveratrol and quercetin, two polyphenolic compounds, downregulate TF expression in vascular cells provides an additional mechanism by which moderate red wine consumption could exert a protective role against cardiovascular disease.

C088

THE Ω-3 FATTY ACID DOCOSAHEXAENOATE INHIBITS TISSUE FACTOR EXPRESSION BY A POST-TRANSLATIONAL MECHANISM IN ACTIVATED HUMAN ENDOTHELIAL CELLS

Del Turco S, Basta G, Lazzerini G, Camera M,* Tremoli E,* De Caterina R°

*CNR Institute of Clinical Physiology, Pisa; *Institute of Pharmacological Sciences, The University of Milan; °Chair Of Cardiology, G. D'Annunzio University, Chieti, Italy*

Background and Objective. Tissue factor (TF) is expressed on endothelium in response to lipopolysaccharide (LPS) or inflammatory cytokines, conferring endothelial cells a pro-thrombotic phenotype. Because Mediterranean-diet Ω-3 fatty acids have been associated with reduced incidence of myocardial infarction, we investigated the endothelial effects of the most abudant Ω-3 FA, docosahexaenoic acid (DHA, 22:6 n-3) on TF expression. *Methods and Results.* We stimulated human umbilical vein endothelial cells (HUVEC) with interleukin-1 (IL-1), tumor necrosis factor (TNF-α) and lipopolysaccaride (LPS) for 4-6 h in the presence or absence of DHA (10-25-50 μM) for 72 h (or stearate as control). TF expression was measured by a TF-dependent clotting assay and a surface EIA ± a blocking antibody. All stimuli induced TF activity and expression in a concentration- and time-dependent manner. DHA pre-incubation concentration dependently reduced TF surface expression (–20±10%, –36±10%, at DHA 25 μM and 50 μM respectively, $p<0.005$), but not total procoagulant activity. The reduced TF surface expression was not associated with decrease of TF m-RNA at Northern analysis and the expression of total cellular protein at Western analysis. *Conclusions.* These results indicate that DHA inhibits TF surface expression in conditions of endothelial activation probably through a post-translational mechanism, potentially accounting for the reduced tendency to thrombosis associated with diets with a high content of Ω-3 fatty acids.

Oral Communications
Venous Thromboembolism and Pregnancy

C089

THE ROLE OF FAMILY HISTORY IN IDENTIFYING WOMEN WITH THROMBOPHILIA AND HIGHER RISK OF VENOUS THROMBOEMBOLISM DURING ORAL CONTRACEPTION

Cosmi B, Legnani C, Bernardi F,* Coccheri S, Palareti G

*Cardiovascular Department, Division of Angiology, Unità di Ricerca Clinica sulla Trombofilia "M. Golinelli", University Hospital S.Orsola-Malpighi, Bologna, and *Centro di Studi Biochimici del Genoma Umano, Department of Biochemistry and Molecular Biology, University of Ferrara, Italy*

Women who are carriers of unrecognized thrombophilic defects are at higher risk of venous thromboembolism (VTE) during oral contraception (OC). *Objectives.* To evaluate the sensitivity and specificity of family history of VTE for the identification of thrombophilia in women before OC and after VTE during OC. *Design.* Thrombophilia screening was performed after obtaining first and second degree family history of VTE by means of a standardized questionnaire. *Setting.* The referral center. *Participants.* Two cohorts: 1-thrombosis-free women before OC and 2 - women after an episode of objectively confirmed VTE during OC. *Main outcome measures.* Sensitivity and positive predictive value of family history of VTE for thrombophilia. *Results. a) thrombosis-free cohort*: we evaluated 479 women (age range: 15-49 y). A positive family history was present in 49 (10.2%). Thrombophilic defects were identified in 36 subjects (7.5%; 95% confidence intervals-CI: 5-10%) of whom 3 had a positive family history (8.3%). The sensitivity and positive predictive value of family history of VTE for thrombophilic defects were 8.3% (95% CI: 2-22%) and 6.1% (95% CI: 1-17%), respectively. b) *Women with history of VTE during OC*. We evaluated 189 women (age range:15-49 y). A positive family history for VTE was present in 48 (25.4%: 95% CI:19-32%) of whom 22 had a thrombophilic defect (46%; 95% CI: 31-61%). Thrombophilic defects were identified in 81 women (43%; 95% CI: 36-50%). The sensitivity and positive predictive value of family history of VTE for thrombophilic defects were 27.2% (95% CI: 18-38%) and 45.8% (95% CI: 31-61%), respectively. *Conclusions.* Family history for VTE has low sensitivity and positive predictive value for identifying women with thrombophilia who are at increased risk of VTE during OC.

C090

FV LEIDEN AND FII G20210A MUTATIONS, PREGNANCY AND VENOUS THROMBOEMBOLISM

Tufano A, Madonna P, Coppola A, De Stefano V, Garofano T, Cirillo F, Cerbone AM, Di Minno G

Centro di Coordinamento Regionale per le Emocoagulopatie, Dipartimento Di Medicina Clinica e Sperimentale, Università degli Studi Di Napoli "Federico II", Naples, Italy

Pregnancy is characterized by a five-fold higher risk of venous thromboembolism (VTE). About 0.013-0.07% of pregnant women suffer from VTE; the incidence in puerperium is 0.23-0.61%. Heterozygous factor V (FV) Leiden and G20210A mutation of the prothrombin gene (FII G20210A) are associated with a 7-fold increased risk of VTE, while the association with homozygosity for 677TT mutation of 5,10-methylene-tetrahydrofolate reductase gene (MTHFR 677TT) is disputed. We evaluated the prevalence of these three gene polymorphisms in 48 consecutive women (mean age at first event 29.1±4.8 years) with a history of VTE during pregnancy/puerperium, in 102 women (mean age at first event 28.7±7.5 years) with non-pregnancy related VTE and in 114 apparently healthy women (mean age 29±6 years). Among women with pregnancy-related VTE, FV Leiden was detected in 13 (27.1%), FII G20210A in 10 (20.8%) and 677TT MTHFR in 6 (12.5%) patients. Among women with non-pregnancy related VTE, FV Leiden was present in 14 (13.7%) FII G20210A in 9 (8.8%) and 677TT MTHFR in 26 (25.5%) patients; (p=0.08, p=0.07, p=0.11 vs pregnancy subgroup respectively; χ^2-test). Among healthy women, FV Leiden was present in 6 (5.3%), FII G20210A in 3 (2.6%) and 677TT MTHFR in 20 (17.5%) subjects; (p=0.0002, p=0.0003, p>0.05 vs pregnancy subgroup respectively; χ^2-test). Our data confirm a high prevalence of FV Leiden (27.1%) and of FII 20210A (20.8%) among women with pregnancy-related VTE and extend it to show a difference (p=0.08, p=0.07 respectively) with a group of non-pregnancy related VTE. Actually the screening for FV Leiden and FII G20210A before pregnancy is thought to be not cost-effective. The high prevalence of these two polymorphisms and recent data on the safety of newly available anticoagulant strategies show that screening should be reconsidered at least in women with a family history of thrombosis during pregnancy.

C091

INHERITED THROMBOPHILIA AND FIRST VENOUS THROMBOEMBOLISM DURING PREGNANCY AND PUERPERIUM

Martinelli I,* De Stefano V,° Taioli E,^ Paciaroni K,° Rossi E,° Mannucci PM*

**Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, and ^Epidemiology Unit, IRCCS Maggiore Hospital, University of Milan, Italy; °Department of Hematology, Catholic University of Rome, Italy*

Venous thromboembolism is a rare but threatening complication of pregnancy. Little conclusive information is available on the actual risk of venous thromboembolism during pregnancy or puerperium in women with inherited thrombophilia, particularly in carriers of factor V Leiden and of the G20210A prothrombin gene mutation. To determine the pregnancy-related and puerperium-related risk of venous thromboembolism in women with inherited thrombophilia, we performed a case-control study on 119 women who had a first episode of deep vein thrombosis and/or pulmonary embolism during pregnancy or puerperium and 232 healthy women who had at least one pregnancy without thrombosis. Inherited thrombophilia was diagnosed in 47 patients (39.5%) and 15 controls (6.5%). The relative risk of venous thromboembolism was 10.6 (95% CI, 5.6 to 20.4) for heterozygous carriers of factor V Leiden, 2.9 (95% CI, 1.0 to 8.6) for heterozygous carriers of the prothrombin mutation and 13.1 (95% CI, 5.0 to 34.2) for those with antithrombin, protein C or

protein S deficiency taken together. Sixty-eight of the 119 women (57%) had thrombosis after delivery, confirming the puerperium as a particularly high-risk period. When women were divided into two groups of those with antenatal or postnatal thrombosis, the relative risks associated with each type of inherited thrombophilia were of similar magnitude. In conclusion, women with inherited thrombophilia have an increased risk of venous thromboembolism during pregnancy. Among thrombophilic abnormalities, the prothrombin mutation was the weakest risk factor. Thrombosis occurred more frequently in puerperium than in pregnancy, whether or not thrombophilia was diagnosed.

C092

FACTOR V LEIDEN MUTATION AND THE RISK OF VENOUS THROMBOEMBOLISM IN PREGNANT WOMEN

Tormene D, Simioni P, Prandoni P, Luni S, Zerbinati P, Sartor D, Franz F, Girolami A

Department of Medical and Surgical Sciences, 2nd Chair of Internal Medicine, University of Padua Medical School, Italy

In this retrospective, single center, cohort study we assessed the risk of pregnancy-related venous thromboembolism (VTE) in women belonging to a large number of families identified because of a symptomatic proband with factor V Leiden mutation. Female family members who had experienced at least one full-term pregnancy were enrolled in the study. Two-hundred and seventy pregnancies occurred in 105 carriers and 215 pregnancies in 81 non-carriers of factor V Leiden mutation. The incidence of VTE per % pregnancies was 2.5 for heterozygous, 7.1 for homozygous, 7.1 for double heterozygous carriers of thrombophilic defects, and 0.5 for non–carriers. *Post-partum* represented the highest risk period for thrombosis in carriers of thrombophilic defects. The relative risks of developing pregnancy-related VTE in women who were carriers of heterozygous and homozygous (or double heterozygous) factor V Leiden mutation as compared to non-carriers were 5.3 (95% CI, 0.6 to 43.9) and 15.4 (95% CI, 1.4 to 164), respectively. Factor V Leiden mutation is a risk factor for pregnancy-related VTE, especially in its homozygous form and in combination with other thrombophilic abnormalities. Screening of families with this mutation may be useful for women in fertile age, as they may take advantage from thromboprophylaxis during pregnancy and *post-partum*.

C094

RETROSPECTIVE ANALYSIS OF THROMBOEMBOLIC RISK IN THROMBOPHILIC PREGNANCIES

Russo U, Arrigoni L, Candolfi R, Carraro MC, Ieri M, Masseroni C, Sacchi E, Rossi E

Hematology And Blood Transfusion Service L. Sacco Hospital, Milan, Italy

Introduction. Inherited or acquired thrombophilia is a well known risk factor for fetal loss, intrauterine growth retardation and eclampsia in pregnancy; these patients have an increased risk of venous thromboembolism in puerperium. We report a retrospective analysis of 244 pregnancies of thrombophilic women, undergoing antithrombotic therapy; as a control group we evaluated 471 previous untreated pregnancies, with obstetric complications. *Patients.* From January 1995 to July 2000 we observed 227 women: 153 with inherited thrombophilia (67 carriers of V Leiden, 55 prothrombin mutation, 27 hyperhomocysteine, 2 antithrombin defect, 8 protein C defect, 11 protein S defect, 3 dysfibrinogenemia) and 74 acquired thrombophilia (19 essential thrombocythemia, 55 antiphospholipid syndrome (APS)); 20 women showed 2 combined defects. 31 women had an history of previous venous thrombosis. Patients were treated with ASA 100 mg/day until 28th week, and heparin (UFH 5000 U × 3 or LMWH 4000 U sc/day) from the 12th until 2nd week after delivery. During this period fetal growth and clotting activation parameters were monitored. Patients were observed 1 month after delivery, reporting anamnestic and ultrasound or angiographic data of clinical evidence of thrombotic events, and testing D-dimer assay. *Results.* The treatment significantly decreased ($p<0.00001$) the percentage of fetal loss, intrauterine growth retardation and pre-eclampsia; during pregnancy no clinical evidence of thromboembolic events were observed. One patient with inherited thrombophilia (dysfibrinogenemia and prothrombin mutation) developed massive pulmonary embolism on the 4th day after delivery. Three patients with APS had thromboembolic events (2 DVT and 1 PE) after interruption of therapy; these women had no previous history of venous thromboembolism. *Conclusions.* These retrospective data confirm the usefulness of prophylaxis with heparin in thrombophilic pregnant women; in patients with higher risk ultrasound monitoring during the puerperium is indicated to identify not clinically evident venous thrombosis. A prospective study is necessary to identify the duration and intensity of treatment.

C094

ROLE OF THROMBOPHILIA IN IMPLANTATION FAILURE AFTER *IN VITRO* FERTILIZATION

Colaizzo D, Vecchione G, Lo Bue A, Cittadini E, Checola G, Pinto A, Di Minno G, Margaglione M, Grandone E

Unità di Aterosclerosi e Trombosi, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, (FG), "Centro di Biologia della Riproduzione", Istituto di Clinica Medica, Palermo, Italy

We hypothesized that inherited, in addition to acquired, thrombophilias could be associated with the occurrence of *in vitro* fertilization-implantation failure (IVF-IF). From February 1999 to July 2000, 18 women with at least 3 IVF cycles with subsequent fetal loss (n=8) or IF (n=10) among 435 women who had undergone 844 treatment cycles at the Centro di Biologia della Riproduzione in Palermo, were consecutively enrolled (group A). Another group of IVF patients (group B, n=24) randomly selected among women concomitantly attending the same Center, and formed by women at their first or second IVF attempt and women with at least one successful pregnancy after an IVF cycle, was also selected. All the women included in groups A and B underwent a complete screening for congenital (FV Leiden and FII A20210 mutations, antithrombin, protein C and protein S deficiencies) and acquired (Lupus anticoagulant, anticardiolipin antibodies) causes of thrombophilia, in addition to karyotype (also of partners), a large panel of autoantibodies and FT3, FT4 and TSH evaluation. Another group of women conceiving naturally with uneventful pregnancies was also considered (Group C,

n=216). Two women in group A showed the factor V Leiden and 3 the FII A20210 mutation. In group B, no mutation was found. Overall, the carriers of one prothrombotic mutation were 5 (27.7%) in the group A and 0 in the group B (p=0.010). Two women in group A had antiphospholipid antibodies. Factor V Leiden mutation was found in 4 (1.9%) women of the group C, while the FII A20210 allele in 9 (4.2%). The difference between group A and group C was significant (p vs group C: 0.007). Prothrombotic mutations can have a role in recurrent IVF-fetal losses or IVF-IF.

C095

RISK OF PREGNANCY LOSS AND VENOUS THROMBOEMBOLISM IN WOMEN CARRYING THROMBOPHILIC GENE POLYMORPHISMS

Coppola A, Tufano A, Madonna P, Cirillo F, Coppola D, Varricchione N, Loffredo F, Cerbone AM, Di Minno G

Centro di Coordinamento Regionale per le Emocoagulopatie, Dipartimento di Medicina Clinica e Sperimentale, Università "Federico II", Naples, Italy

Several reports over the last years have strengthened the association of inherited thrombophilia with risk of pregnancy loss, probably due to placental vascular disease. We evaluated the prevalence and the risk associated with factor V (FV) Leiden and prothrombin (FII) G20210A mutations in women referred to our Center because of at least one unexplained pregnancy loss (PL, n=38, range 1-8 episodes, median age 36 years) and compared with women with early-onset (age at the event <45 years) venous thromboembolism with (PL+VT, n=46) or without (VT, n=106) a history of unexplained unsuccessful gestational outcome (range 1-4) and control women who carried normal pregnancies and were negative for thrombosis (C, n=114). Women with other known causes of thrombophilia were excluded. FV Leiden was found in 15.8% (6/38) of PL women, 17% (18/106) of VT and 13.0% (7/46) of PL+VT groups, always being significantly more prevalent than in control women (5/114, 4.4%; p always <0.05). Similar about 4-fold increases of risk were calculated in these groups (OR and 95%C.I: PL 4.1, 1.0-16.8; VT 4.5, 1.5-14.4; PL+VT 3.9, 1.0-15.3). Prevalence of FIIG20210A mutation was significantly higher in all groups (PL 5/38, 13.2%; VT 11/106, 10.4%; PL+VT 6/46, 13.0%) than in control women (3/114, 2.6%; p always <0.05) as well, with a 4-5-fold associated increase of risk (OR and 95%C.I: PL 5.6, 1.1-28.1; VT 4.3, 1.1-16.9; PL+VT 5.6, 1.2-25.4). Moreover, in the PL group, when women with repeated events were considered (17/38), FV Leiden and/or FIIG20210A were found in 41% of patients, with an overall further double increase of risk (OR and 95%C.I: 9.2, 2.4-36.7). Despite the limitation due to the sample size, our data show a comparable increase of risk of pregnancy loss and venous thromboembolism in women carrying thrombophilic gene polymorphisms, and support the concept of including placental vascular abnormalities among clinical expressions of thrombophilia.

C096

HIGH PLASMA LEVELS OF FACTOR VIII IN WOMEN WITH EARLY RECURRENT PREGNANCY LOSS

Marietta M, Bertesi M, Sgarbi L,* Neri I,* Facchinetti F,* Simoni L,§ Torelli G

*Dept. Medical Sciences, Section of Haematology; *Dept. Obstetrics and Gynecology, University of Modena and Reggio Emilia, §Dept. of Clinical Pathology, Ospedale Policlinico, Modena, Italy*

Inherited and acquired thrombophilia has been associated with recurrent pregnancy loss (RPL). Recently, it has been demonstrated that elevated plasma levels of factor VIII, IX and XI are significant risk factors for venous thromboembolism. The aim of our study was to assess whether RPL in the first trimester is associated with elevated plasma levels of coagulation factors. *Methods.* We studied 52 women with a history of RPL, defined as three or more (2 for women >35 years) first trimester pregnancy losses. Exclusion criteria were documented preclinical and blighted ovum abortions and pregnancy losses resulting from fetal malformation, infectious complication, metabolic or anatomic causes. Controls were 52 women matched for age, without a history of RPL and at least one successful pregnancy. In patients and controls we determined ATIII, protein C and S activity, fasting plasma homocysteine levels, APC resistance (APCr), lupus anticoagulant assays, anticardiolipin antibodies, prothrombin G20210A mutation, factor VIII:c, IX and XI levels (one-stage clotting assay). Tests were performed out of pregnancy and at least one month after pregnancy loss. *Results*. No significant differences were found between cases and controls comparing either the prevalence of thrombophilic defects or factor VIII:c, IX and XI mean levels. However, 44% of patients presented a factor VIII:c level above the 90th percentile of the control population (130 IU/dL). Moreover, a significant difference in factor VIII:c level distribution was found if patients were split up according to parity. Indeed, less women with at least one live birth presented factor VIII:C levels above 130 UI/dL compared to those who had not had a successful pregnancy (26.3% vs 54.5%, p=0.04). *Discussion.* Our data suggest that elevated plasma levels of factor VIII can be associated with early RPL.

Oral Communications
Thrombophilia II

C097

MUTATIONS IN THE PROTEIN C GENE IN THROMBOPHILIA: STRUCTURAL IMPACT OF AMINO ACID CHANGES

Merati G,* Zanardelli S,* Castaman G,§ Rovida E,° Asti D,* Artoni A,* Mannucci PM,* Faioni EM*

**Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Maggiore Hospital and Department of Internal Medicine 2, University of Milan, Italy, §Hemophilia and Thrombosis Center, S. Bortolo Hospital, Vicenza, °Institute for Advanced Biomedical Technologies, National Research Council, Milan, Italy*

Twenty-one heterozygous mutations (10 novel) in the promoter and coding regions of the protein C (PC) gene were identified by PCR and sequencing in 32 patients with thrombosis and PC deficiency type I or II. Table 1 shows the recurrent mutations and the mutations affecting the highly conserved amino acid residues. The sequence of PC heavy chain, vitamin K-dependent serine protease (factors II, VII, IX, X) and factors XI and XII in different species were aligned to evaluate the degree of amino acid conservation.

Position	Amino acid change	No. of patients	Highly conserved	Comments
Promoter (1511)T		4		Disruption of an HNF-1 binding site C(- with impaired expression of the protein
Exon 3	R-11C	4		Localized in the propeptide region
Exon 7	R169Q	1		Loss of the thrombin cleavage site
Exon 8	G197E	2	++	Modification of the electrostatic potential in the active site (introduction of a negative charge)
Exon 8	C212R	2	++++	Impairment of the correct folding (loss of C212-196C disulphide bond)
Exon 9	V297M	2	++++	
Exon 9	G381D	2	++++	Located at the loop involved in the substrate binding
Exon 9	P279L	1	+++	
Exon 9	A259T	1	++++	
Exon 9	D359N	1	++++	Modification of the electrostatic potential in the active site (loss of a negative charge)
Exon 9	S379N	1	++++	Located at the loop involved in the substrate binding

Molecular modelling was employed in order to derive plausible explanations for the impairment in PC stability structure and function for further biochemical studies. Expressions studies are needed to confirm the possible role of the selected mutations in the pathogenesis of thrombophilia. In particular, we are interested in the identification of highly specialized regions in the PC molecule involved in Na^{++}-binding and in the interaction with endothelial cell protein C receptor (EPCR).

C098

PREVALENCE OF THROMBOMODULIN GENE MUTATIONS IN PATIENTS WITH SEVERE THROMBOPHILIA

Franchi F,* Castaman G,§ Biguzzi E,* Rodeghiero F,§ Faioni EM*

**Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Department of Internal Medicine 2, I.R.C.C.S. Maggiore Hospital and University of Milan; §Department of Hematology, S. Bortolo Hospital, Vicenza, Italy*

Several mutations and polymorphisms in the thrombomodulin (TM) gene have been described in patients with venous thromboembolism. Unfortunately, no conclusions can be drawn as to the impact of these mutations, because of the different clinical characteristics of the patients enrolled in the studies. Due to the inconclusive results, we decided to tackle the issue of the relevance of TM in venous thromboembolism by investigating the presence of TM gene mutations in a very selected group of 38 patients with *severe* thrombophilia defined as the simultaneous presence of recurring thrombotic events, the first one at a young age, and a positive family history. We also sequenced the TM gene of 12 individuals with low sTM levels chosen from a larger study group, because low sTM levels have been described to be associated with a TM gene mutation. Finally, we evaluated the allelic frequency of the Ala455Val polymorphism in 192 patients with at least one thrombotic event and in 369 age and sex-matched asymptomatic controls. Two mutations were identified, G/A -201 and G/T 1456, in a severely thrombophilic patient and in a patient with low soluble thrombomodulin levels. The first mutation was reported by some, but not others, to be associated with moderately reduced levels of thrombomodulin. The second was identified previously in a patient with low soluble thrombomodulin, but expression studies failed to show functional changes in the mutant. Thrombomodulin gene mutations thus appear to be rare even in highly selected Italian thrombophilic patients, and possibly functionally irrelevant. The allelic frequency of the Ala455Val polymorphism was identical in patients and controls. Considering the lack of a phenotype and the costly screening procedure, we recommend that TM defects be sought only for research purposes.

C099

GENETIC DETERMINANTS OF PLASMINOGEN ACTIVATOR INHIBITOR-1 LEVELS ARE RISK FACTORS FOR RETINAL VEIN OCCLUSION

Gori AM, Marcucci R, Giusti B, Fatini C, Gensini F, Fedi S, Brunelli T, Betti I, Sodi A,* Abbate R, Prisco D

*Dipartimento Area Critica Medico-Chirurgica, *Clinica Oculistica II, Università degli Studi di Firenze; Centro Trombosi, Azienda Ospedaliera Careggi, Florence, Italy*

Several studies demonstrated that plasminogen activator inhibitor-1 (PAI-1) is a risk factor for both venous and arterial thrombotic disease. PAI-1 levels are modulated by the 4G/5G polymorphism of the PAI-1 gene and the insertion/deletion (I/D) polymorphism of the ACE gene. Scanty data are available on the presence of a thrombophilic state in patients with retinal vein occlusion (RVO). In 123 RVO patients (58M/65F; 60, 18-83 years) and 104 comparable controls (52M/52F; 57, 20-84 years) we evaluated the prevalence of PAI-1 4G/5G and ACE I/D polymor-

phisms and whether these polymorphisms account for elevated PAI-1 levels in RVO. A significant difference in PAI-1 levels between patients and controls (11.0, 1-29 IU/mL vs 9.0, 1.2-16 IU/mL; $p<0.05$) was observed. The 4G allele frequency was similar in RVO patients and in controls (57.3% and 51.4%), whereas the D allele frequency was higher in RVO patients than in controls (61.8% vs 48.6%, $p<0.05$). Homozygosity for ACE DD (OR= 1.7, 95% CI 1.1-2.3; $p<0.05$] was significantly associated with RVO. We observed a significant association between PAI-1 levels and both PAI-1 and ACE genotypes in RVO patients. In the subgroup of patients (71/123) without thrombophilic alterations, the 4G allele frequency was significantly higher than in controls (65.4% vs 51.4%, p<0.05) and the 4G4G PAI-1 genotype was significantly associated with RVO (OR=2.3, 95%CI 1.3-4.4; p<0.005). The contemporary presence of ACE D/D and PAI-1 4G/4G genotype was associated with a further increas of the risk for RVO (OR=2.7; 95% CI 1.2-6.1; $p<0.05$). Our study confirms the role of impaired fibrinolysis and PAI-1 genetic modulation by PAI-1 4G/5G and ACE I/D polymorphisms in RVO. Furthermore, it demonstrates that the ACE DD genotype is a risk factor for RVO and that PAI-1 4G/4G genotype represents a risk factor for RVO in patients without the classic thrombophilic alterations.

C100

INTRA-INDIVIDUAL CONSISTENCY OF THE ACTIVATED PROTEIN C RESISTANCE PHENOTYPE

Tosetto A, Simioni M, Dall'Oste C, Ruggeri M, Rodeghiero F

Department of Hematology, S. Bortolo Hospital, Vicenza, Italy

Background. Resistance to activated protein C (APC) is a risk factor for venous thromboembolism independent from the FV Leiden mutation. The consistency over time of the APC resistance phenotype within the same subject is however not known. *Aim of the Study*. To evaluate the intra-individual consistency of the APC resistance phenotype on two different occasions, several months apart. *Subjects and Methods*. We reinvestigated a sample of 2309 subjects previously enrolled in the VITA Project. Blood sampling, plasma preparation and storage were performed using the same protocol as that of the previous investigation. APC resistance was measured as the APC sensitivity ratio using the same aPTT method (based on IL ellagic acid cephaloplastin). *Results*. Overall, 1043 males and 1266 females were studied, with a mean age of 54 years. Sixty-two subjects were carriers of the FV Leiden allele. The median time of reinvestigation was 61 months from the first visit. Concordance analysis using the Bland & Altman method showed no bias between the two measurements but a significant dispersion (mean difference between first and second measurement±2SD =0.01±0.26). The dispersion was significantly lower in FV Leiden carriers (0.04±0.12; $p<0.001$). There were 318 subjects with an APC resistance phenotype (APC-SR <0.84) independent from FV Leiden at the first examination. Among these subjects, an APC resistance phenotype was confirmed in 84 subjects (26%). *Conclusions*. Although the reproducibility of the APC-SR phenotype may be poor in subjects not carrying the FV Leiden mutation, a significant proportion of subjects with an APC-resistance phenotype still show the same abnormality after a median follow-up of five years. The clinical significance of a persistent APC-resistance phenotype deserves further investigation.

C101

ASSESSMENT OF PERFORMANCE OF CLINICAL LABORATORIES FOR DNA ANALYSES TO DETECT TWO THROMBOPHILIC MUTATIONS

Tripodi A, Peyvandi F, Chantarangkul V, Menegatti M, Mannucci PM

Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, University and IRCCS Maggiore Hospital, Milan, Italy

DNA analyses to detect mutations are being increasingly used. Though the confidence of clinicians on the reliability of these analyses is high, information on performance is scanty. We aimed to assess the performance of laboratories in detecting 2 thrombophilic mutations (FV Leiden and prothrombin 20210). The exercise was carried out as part of the activity of the Interlaboratory Coagulation Survey (ICS) of the CISMEL (Italian Committee for Standardization), which enrolls 250 participants and aims to assess the laboratory performance of basic coagulation and thrombophilia testing. All ICS participants were asked to join this special exercise and 32 accepted. They were provided with aliquots of the same DNA and asked to detect the mutations with their methods. DNA samples were prepared at the organizing center from 6 patients whose genotype was previously identified on the occasion of thrombophilia screening. Genotypes were as follows: 1 wild-type (no mutations), 1 carrier with the FV Leiden and 1 with the prothrombin heterozygous mutations, 1 double-heterozygote and 2 homozygous carriers with the FV Leiden or prothrombin mutations. Upon informed consent, 10 μL of DNA (400ng/μL) were aliquoted. These were coded and stored at 4 °C until shipment. Twenty-eight of 32 participants returned results: 13 used in-house and 15 used commercial methods. Five participants did not complete their assessment on all samples, while 5.4% of the respondents failed to identify the heterozygous FV Leiden and 5.6% the heterozygous prothrombin mutations. 3.7% of the respondents failed to identify the homozygous FV Leiden and 18.5% the homozygous prothrombin mutations. Failures to identify the mutations were associated more frequently with in-house than with commercial methods. There were no false-positive identifications. In conclusion, this exercise shows that DNA analyses meant to detect 2 thrombophilic mutations are not devoid of problems. Standardization and quality control programs aimed at identifying causes of failure are warranted.

C102

CRITERIA OF SCREENING FOR INHERITED THROMBOPHILIA AMONG PATIENTS WITH VENOUS THROMBOEMBOLISM

De Stefano V, Rossi E, Paciaroni K, Leone G

Dept. of Hematology, Catholic University, Rome, Italy

Recently an algorithm for diagnosis of inherited thrombophilia based on family history, frequency of the defects, and the circumstances of thrombosis has been proposed (*N Engl J Med 2001; 344:1222*). Antithrombin (AT), protein C (PC) and S (PS) are screened only in selected patients; no investigation is proposed for patients with provoked distal vein thrombosis. We applied the proposed criteria on 676 patients with a first venous thrombosis and no evidence of overt cancer: the expected likelihood of thrombophilia was high in 269 patients (172 with first unprovoked DVT

before 45 years or recurrent event and 97 with first cerebral- or visceral vein thrombosis), intermediate in 327 (102 with unprovoked DVT after 45 years, 146 with DVT due to oral contraceptives or pregnancy, 79 with proximal DVT due to surgery, trauma, plastering, bed rest), and low in 59 (distal DVT due to surgery, trauma, plastering, bed rest). The overall rate of diagnosis of inherited thrombophilia was 33%, with no significant difference among the groups (p= 0.445) (see Table below). AT, PC, and PS deficiency would have been missed in 4.9% of the patients with intermediate likelihood of thrombophilia and partially screened; 27.1% of the unscreened patients with distal DVT would have been undiagnosed. We suggest that all patients with venous thrombosis should be investigated for inherited thrombophilia.

Likelihood of thrombophilia	AT,PC,PS defect	FV Leiden	PT G20210A	Combined defects	Total
High	25 (9.3%)	51 (18.9%)	10 (3.7%)	9 (3.3%)	95 (35.3%)
Intermediate	16 (4.9%)	57 (17.4%)	23 (7.0%)	10 (3.0%)	106 (32.4%)
Low	2 (3.4%)	7 (11.8%)	6 (10.2%)	1 (1.7%)	16 (27.1%)

Oral Communications
Inflammation and Vascular Risk

C103

ASSOCIATION BETWEEN INTERLEUKIN-6 PROMOTER POLYMORPHISM AND CLINICAL OUTCOMES AFTER CORONARY ARTERY BYPASS SURGERY

Di Castelnuovo A,^ Burzotta F, Andreotti F, Glieca F,° Luciani N, Zamparelli R,* Alessandrini F, ° Schiavello R,* D'Orazio A, Amore C,^ Donati MB, Maseri A,° Possati GF,° Gaudino M, Iacoviello L^

*Department of Cardiology, °Cardiac Surgery and *Cardiac Anaesthesiology, Catholic University, Rome and ^"Angela Valenti" Laboratory of Genetic and Enviromental Risk Factors for Thrombotic Disease, Department of Vascular Medicine and Pharmacology, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy*

Interleukin (IL)-6 plasma levels are predictive of cardiovascular events in healthy subjects and in patients with coronary artery disease (CAD). A variant (-174G/C) within the promoter of the IL-6 gene affects basal levels *in vivo* and transcription rates *in vitro*. The association between this variant, the acute-phase response of IL-6 and clinical outcome after coronary artery bypass grafting (CABG) has not been investigated. Genotyping at position -174, IL-6 plasma levels and clinical outcomes were prospectively assessed in 111 patients with CAD undergoing CABG. Baseline clinical and surgical characteristics did not differ according to -174G/C polymorphism. Distribution of genotype was in Hardy-Weinberg equilibrium; frequency of the G allele was 0.73. IL-6 levels showed an average 17-fold increase from baseline, peaking at 24h (p<0.0001). Major postoperative complications did not differ significantly by genotype (8% in GG genotype and 2% in C-carriers, p=0.16); however, GG (compared to GC+CC) was associated with a longer stay in hospital (6.2±4.0 vs 4.5±1.4 days, p=0.018). Moreover, GG had a worse pulmonary and renal function postoperatively (see the Table). Need for extra diuretic stimulus occurred in 63% of the GG carriers versus 10% of the (CG+CC) group (adjusted odds ratio: 15.1; 95%CI: 4.5-49.9; p<0.0001) and dopaminergic renal stimulation had to be used in 31% of the GG vs 4% of the non-GG patients (adjusted odds ratio: 21.9; 95%CI: 3.2-150; p=0.0017). Atrial fibrillation occurred in 33.9% of the GG homozygotes vs 10.4% of the C allele carriers (adjusted odds ratio: 4.5; 95%CI: 1.4-14.5; p=0.012). In conclusion, among patients undergoing CABG, homozygosity for the G allele of the IL-6 –174 G/C genotype is associated with higher acute-phase levels of IL-6, with longer stays in hospital and in the intensive care unit and with a higher degree of postoperative renal and pulmonary dysfunction, as well as development of atrial fibrillation.

Postoperative indexes of renal and pulmonary function according to IL-6 promoter polymorphism.

	CG + CC (N=49)	GG (N=62)	Multivariate* (p-value)
Respiratory Index at 6 hrs	2.1±0.5	2.9±0.8	0.0001
Respiratory Index at 12 hrs	1.3±0.1	2.8±0.3	0.0001
Mechanical ventilation (hrs)	12.7±6.7	22.5±20.6	0.01
Perioperativef-Creat	0.18±0.14	0.82±0.34	0.0001
Perioperativef-K	0.15±0.48	0.99±0.44	0.0001
Perioperativef-BUN	2.6±4.1	10.1±7.8	0.0001

*Multivariate Analysis of Variance adjusted also for pk_IL-6.

C104

INCREASED THROMBIN GENERATION AND TUMOR NECROSIS FACTOR α CIRCULATING LEVELS IN PATIENT WITH *HELICOBACTER PYLORI*-POSITIVE CHRONIC GASTRITIS

Borgia MC,* Consolazio A,* Paoluzi P,* Violi F, Ferro D

*Istituto I Clinica Medica, Università "La Sapienza", Rome; *Istituto II Clinica Medica, Università "La Sapienza", Rome, Italy*

Previous reports suggested that chronic infection associated with *Helicobacter Pylori* (*H. Pylori*) predispose to cardiovascular disease through the activation of clotting system. In a first study we measured the plasma levels of prothrombin fragment 1+2 (F1+2) (Enzygnost F 1+2, Behringwerke, Marburg, Germany) and tumor necrosis factor α (T Cell Diagnostics, Cambridge, MA) in patients with chronic gastritis negative (5 males, 4 females, aged from 45 to 78 years), or positive (7 males, 12 females, aged from 31 to 72 years) for *H. pylori*. Diagnosis of chronic gastritis was dose by esophagogastroduodenoscopy; the presence of *H. pylori* in gastric biopsy specimens was determined directly by histologic examination. In a second study, the 19 patients positive for *H. pylori* were treated with omeprazole (20 mg u.i.d.), clarithromicin (250 mg b.i.d.) and amoxicillin (500 mg b.i.d.) for two weeks. Patients with gastritis positive for *H. pylori* had significantly higher F1+2 (p<0.02) and tumor necrosis factor (p<0.004) than patients negative for *H. Pylori*. After antibiotic treatment, 16 patients became negative for *H. Pylori*. In these patients we found a significant decrease of F1+2 (p=0.03) and tumor necrosis factor (p<0.01), while no significant change was found in patients with persistent *H. Pylori* positivity, as concerns F1+2 and tumor necrosis factor. The study shows that *H. Pylori* infection in patients with chronic gastritis may represent a trigger for clotting system activation. This mechanism may be mediated by further inflammation, as suggested by the behavior of tumor necrosis factor.

C105

THE INDUCTION OF VASCULAR CELL ADHESION MOLECULE-1 EXPRESSION BY ADVANCED GLYCATION END PRODUCTS IS MEDIATED BY DIFFERENT PATHWAYS GENERATING REACTIVE OXYGEN SPECIES

Basta G, Lazzerini G, Del Turco S, Schmidt AM,^ Ratto GM,* De Caterina R°

*CNR Institute of Clinical Physiology and *Neurophysiology, Pisa, °Chair of Cardiology, G. D'Annunzio University, Chieti, Italy, ^Columbia University, New York, NY, USA*

Interaction of advanced glycation end products (AGEs) with their main receptor RAGE on cultured human umbilical vein endothelial cells (HUVEC) induces intracellular generation of reactive oxygen species (ROS) and the expression of vascular cell adhesion molecule-1 (VCAM-1). AGE-induced ROS generation and VCAM-1 expression was completely and specifically blocked by anti-RAGE IgG. We now explored the potential contribution to VCAM-1 expression induced by AGEs of different ROS-generating pathways including NAD(P)H oxidase, mitochondrial respiratory chain, and xanthine oxidase, through the use of specific inhibitors. HUVEC were stimulated with AGEs (500 μg/mL) and pretreated for 30 minutes with: rotenone and antimycin A (0.01-1 μM), two inhibitors of the mitochondrial respiratory chain, apocynin (0.5-2 mM) and allopurinol (0.1-10 μM), inhibitors of NAD(P)H oxidase and xanthine oxidase respectively, and the superoxide dismutase inhibitor diethyldithiocarbamic acid (DETC) (10-100 μM). Intracellular ROS formation in HUVEC exposed to AGEs was measured by the fluorescent probe 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate, which mainly detects peroxide oxygen. VCAM-1 expression was assessed by cell surface enzyme immunoassay. AGE-induced intracellular ROS production was decreased by apocynin, which also inhibited VCAM-1 expression in a concentration dependent manner. Allopurinol pretreatment affected neither ROS nor VCAM-1 induction. Rotenone, which only partially inhibits electron entry to ubiquinone, and antimycin A, a blocker of ubisemiquinone, increased ROS both basally and after induction by AGEs. In parallel, VCAM-1 was significantly increased by both rotenone and antimycin A. DETC pretreatment completely inhibited ROS production and VCAM-1 expression, indicating that peroxide oxygen but not superoxide anion is involved as a mediator of VCAM-1 expression. The ability of apocynin to inhibit both ROS and VCAM-1 suggests that ROS generated by a NAD(P)H-oxidase may play the role of a second messenger in AGEs-induced VCAM-1 expression. However, the increase of ROS and VCAM-1 by rotenone and antimycin A suggests the involvement of mitochondrial ROS production in VCAM-1 induction.

C106

15-DEOXY-Δ12,14-PROSTAGLANDINJ 2 INHIBITS TISSUE FACTOR EXPRESSION IN HUMAN MACROPHAGES AND ENDOTHELIAL CELLS: EVIDENCE FOR ERK1/2 SIGNALING PATHWAY BLOCKADE

Eligini S, Banfi C, Brambilla M, Camera M, Barbieri SS, Tremoli E, Colli S

Dept. of Pharmacological Sciences, E. Grossi Paoletti Center, University of Milan, Italy

Basic and clinical advances have recently provided insight into the molecular events that link inflammation and blood coagulation. In particular, a variety of clinical conditions associated with either chronic inflammation or sepsis have indicated an increased expression of tissue factor (TF) by circulating and vascular cells as being responsible for the thrombotic complications associated with the inflammatory response. The cyclopentenone prostaglandins are a family of naturally occurring prostaglandin D2 derivatives that comprises prostaglandin J2 (PGJ2) and its metabolites Δ12-PGJ2 and 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2). These compounds have been suggested to possess antiinflammatory properties *in vivo* (*Gilroy 1999, Thieringer 2000*). In this study we investigated the effect of 15d-PGJ2 on TF expression in human monocyte-derived macrophages and in endothelial cells (HUVEC). Our results indicate that 15d-PGJ2 (10-30 μM) down-regulates TF activity, protein levels and mRNA via inhibition of LPS- and TNFα-induced transcriptional activation mediated by the mitogen activated protein kinase ERK1/2 and by the NF-κB/I-κB pathway. 15d-PGJ2 represents a natural low affinity ligand of PPARγ and the activation of this transcription factor has been shown to be responsible for the repression of several inflammatory genes (*Straus 2000*). Therefore the role of PPARγ in TF inhibition by 15d-PGJ2 was explored, taking advantage of the use of the high affinity PPARγ ligand BRL-49653. BRL-49653 (10 μM), did not affect TF activity in macrophages and in HUVEC, thus indicating that 15d-PGJ2 reduces TF through a PPARγ-independent mechanism. We conclude that 15d-PGJ2 negatively affects TF expres-

sion in macrophages and endothelial cells. This down-regulation may be crucial to limit excessive blood clotting activation in immunoinflammatory diseases.

C107

TISSUE FACTOR SYNTHESIS BY ACTIVATED MONOCYTES IS UPREGULATED BY PENTRAXIN PTX3

Napoleone E, Di Santo A, Peri G,* Mantovani A,* Donati MB, Lorenzet R

*"Antonio Taticchi" Laboratory for Atherosclerosis and Thrombosis, Consorzio Mario Negri Sud, S.Maria Imbaro, and *Istituto Mario Negri, Department of Immunology and Cell Biology, Milan, Italy*

The novel inflammatory acute phase reactant pentraxin PTX3 belongs, together with C-reactive protein and serum amyloid P component, to the family of the pentraxins. PTX3, synthesized by monocytes and endothelial cells following exposure to agents such as IL-1β, TNF-α and LPS, was recently found to be elevated in septic shock, and increased in patients with acute myocardial infarction. Strong evidence assigning a central role to tissue factor (TF) in thrombosis and inflammation associated with atherosclerosis has been reported. We have recently reported that PTX3 increases TF activity, protein, and mRNA in IL-1 β, TNF-α and LPS-stimulated HUVEC (*Napoleone et al., Arterioscler Thromb Vasc Biol, in press*). We decided to test whether PTX3 could modulate TF expression in monocytes. Monocytes (MN), obtained from peripheral blood of healthy donors, were incubated with highly purified PTX3 with or without LPS. Cells were then disrupted by freezing and thawing and procoagulant activity was assessed by a one-stage clotting time. PTX3 enhanced TF activity and antigen from MN stimulated by LPS in a dose-dependent way. The effect is specific since other pentraxins, such as CRP and SAP, could not modulate TF activity. Moreover, in contrast with the results obtained with endothelial cells, the increase in activity is specific for LPS, since in the presence of other TF-inducing agents, such as IL-1β, and TNF-α, PTX3 was not effective. The increase in TF activity requires mRNA synthesis, as assessed by PCR. The mechanism by which PTX3 modulates TF synthesis resides in IκBα phosphorylation and degradation, and increased migration of the transacting factor cRel/p65 into the nucleus, as determined by Western blot and EMSA (electromobility shift assay). In the area of vascular injury, during the inflammatory response, cell-mediated fibrin deposition takes place. Our results suggest that PTX3, increasing TF, potentially plays a role in thrombogenesis and ischemic vascular disease.

C108

GENETIC MODULATION OF INFLAMMATORY CYTOKINES AND TISSUE FACTOR RELEASE FROM HUMAN MONOCYTES

Amore C,# Napoleone E,° D'Orazio A,* Lorenzet R,° Donati MB,* Iacoviello L*

**"A. Valenti" Laboratory of Genetic and Environmental Risk factors for Arterial Thrombosis; °"Antonio Taticchi" Unit of Atherosclerosis and Thrombosis Department of Vascular Medicine and Pharmacology, Consorzio Mario Negri Sud, S. Maria Imbaro, Italy*

Monocytes release inflammatory cytokines, such as interleukin-1 (IL-1)β and IL 6, which play an important role in atherogenesis, and express tissue factor (TF), a major contributor to the thrombogenicity of atherosclerotic plaques. We investigated whether the production of these factors from human monocytes upon stimulation with LPS might be genetically regulated. One hundred and twenty-eight healthy volunteers (64 males and 64 females, 28±5 years) were studied. None of the subjects received medication or had suffered from allergic disease or infections within a period of 15 days prior to blood sampling. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood and incubated at 37 °C with or without LPS for 6 or 24 hours to measure procoagulant activity or IL-1 β and IL-6 levels. Polymorphisms -511C/T of IL-1 β, -174 G/C of IL-6 and T10025C and A9925G of TF genes were evaluated. The release of IL-1 β was regulated by -511C/T polymorphism, in particular carriers of TT genotype showed a significantly lower release of IL-1 β (0.8±0.2 ng/mL) as compared with both, CT heterozygotes (2.1±0.3 ng/mL $p<0.006$) and TT homozygotes (4.5±0.8 ng/mL, $p<0.003$). Il-1 β polymorphism also regulated the expression of TF from stimulated monocytes: TT=6.1±1.06 U/3×10^6 cells; TC-4.96±0.74 U/3×10^6 cells; CC=1.51± 0.54 U/3×10^6 cells ($p=0.02$vs TT; $p=0.01$vs CT). Basal levels of either IL-1 β or TF were unaffected by -511C/T IL-1 β polymorphisms. A trend, although not significant, towards a regulation of TF activity after stimulation was also observed for the A9925G polymorphism of the TF gene (AA=6.48±1.11; AG=4.83±0.87; GG=3.48±0.89). In contrast, basal (GG=4.2±0.48 pg/mL; GC=8.9±2.1 pg/mL ($p=0.009$ vs GG); CC= 64.8±28.7 pg/mL ($p=0.001$ vs GG), but not stimulated levels of IL-6 were modulated by a polymorphism in the promoter of IL-6 gene. The observed genetic regulation of the inflammatory/hemostatic response of isolated monocytes contributes to explaining the different susceptibility to cardiovascular disease after exposure to inflammatory stimuli.

Oral Communications
Anticoagulation II

C109

SUBCUTANEOUS ADJUSTED-DOSE UNFRACTIONATED HEPARIN VERSUS FIXED-DOSE LOW-MOLECULAR-WEIGHT HEPARIN IN THE TREATMENT OF VENOUS THROMBOEMBOLISM. A PROSPECTIVE CONTROLLED RANDOMIZED STUDY

Prandoni P, Carnovali M, Marchiori A, Ghirarduzzi A, Girolami B, Tropeano PF, Alatri A, Accorsi F, Moia M, Todini AR, Scannapieco G, Villalta S, Quintavalla R, Cogo A, Imberti D, Parente F, Agnelli G, Sartori D, Cuppini S, Migliacci R, Corà F, Bagatella P, Girolami A

University Hospitals of Padua, Rho, Reggio Emilia, Pordenone, Bologna, Milano, Roma, Treviso, Parma, Vicenza, Piacenza, Lecce, Perugia, Noale, Adria, Cortona, Asiago

While the initial treatment of the first episode of deep-vein thrombosis with unfractionated (UFH) or low-molecular-weight heparin (LMWH) has been extensively investigated, few reports have addressed the value of these agents in treating the full spectrum of venous thromboembolism (VTE), including recurrent VTE and pulmonary embolism. In an open, multicenter clinical trial 620 consecutive patients with acute symptomatic VTE, including 97 patients (15.6%) with pulmonary embolism and 87 (14.0%) with recurrent VTE, were randomly assigned to adjusted-dose subcutaneous UFH, using a weight-based algorithm, or fixed-dose subcutaneous nadroparin. Oral anticoagulant therapy was started concomitantly and continued for three months. We recorded the incidence of major bleeding during the initial heparin treatment, and that of recurrent VTE and death during three months of follow-up. Fifteen of the 310 patients assigned to UFH (4.8%) had recurrent thromboembolic events, as compared with 13 of the 310 patients assigned to nadroparin (4.2%; $p>0.2$). Four patients assigned to UFH (1.3%) and 3 patients assigned to nadroparin (1.0%) had episodes of major bleeding ($p>0.2$). Overall mortality was 4.5 and 4.2%, respectively. Subcutaneous adjusted-dose UFH using a weight-based algorithm is as effective and safe as fixed-dose nadroparin for the initial management of VTE patients, including those with pulmonary embolism and recurrent VTE.

C110

HIGH VERSUS LOW DOSES OF HEPARIN FOR THE TREATMENT OF SUPERFICIAL THROMBOPHLEBITIS OF THE LEG. A PROSPECTIVE CONTROLLED RANDOMIZED STUDY

Marchiori A, Verlato F, Sabbion P, Camporese G, Rosso F, Mosena L, Andreozzi GM, Prandoni P

Dept of Medical and Surgical Sciences, 2nd Chair of Internal Medicine, and Unit Care of Angiology, University Hospital of Padua, Italy

The optimal treatment of superficial thrombophlebitis of the leg is undefined. The main study objective was to assess the efficacy and safety of unmonitored high doses as compared to low doses of unfractionated heparin (UFH) for prevention of venous thromboembolic complications in patients with superficial thrombophlebitis of the thigh. Sixty consecutive patients with acute thrombophlebitis of the great saphenous vein, as assessed by ultrasonography, were randomized to subcutaneous injection twice daily of UFH in high unmonitored doses (12,500 IU for one week followed by 10,000 IU) or prophylactic doses (5,000 IU) for four weeks. The rate of asymptomatic involvement of the deep venous system and/or symptomatic thromboembolic events during a six-month follow-up period was assessed and compared between the two study groups. Six of the 30 patients (20.0%; 95% CI, 7.7 to 38.6) randomized to low-dose UFH developed symptomatic or asymptomatic events as compared to 1 of the 30 patients (3.3%; 95% CI, 0.07 to 17.2) who received high-dose UFH ($p=0.05$ by one-sided Fisher's exact test). No patient experienced major bleeding complications in either group. The results of this study suggest that in patients with acute thrombophlebitis of the thigh unmonitored high doses are more effective than prophylactic doses of UFH for prevention of venous thromboembolic complications without enhancing the risk of bleeding complications.

C111

RELATIONSHIP BETWEEN INR VALUES, FACTOR II CLOTTING LEVELS AND *IN VIVO* PROTHROMBIN ACTIVATION DURING THE EARLY AND STEADY PHASE OF ORAL ANTICOAGULANT TREATMENT

D'Angelo A, Della Valle P, Crippa L, Fattorini A, Pattarini E, Pricolo F, Viganò D'Angelo S

Coagulation Service & Thrombosis Research Unit, IRCCS H S.Raffaele, Milan, Italy

In vitro studies have shown that the rate of prothrombin activation is linearly related to the concentration of factor II (FII) in the assay system, suggesting a key role of prothrombin levels in the expression of the antithrombotic activity of oral anticoagulant treatment (OAT). We investigated the *in vivo* relationship between prothrombin activation and FII levels during the early and steady phase of oral anticoagulation in patients and in healthy volunteers. The changes in INR and in the plasma levels of factor VII (FVII), FX, FII and prothrombin fragment 1.2 (F1+2) induced by OAT were monitored over several days in 10 patients – not on heparin – starting warfarin on the first postoperative day after heart valve replacement (HVR) and in 9 healthy volunteers submitted to a 8-day course of warfarin treatment. FII and F1+2 plasma levels were also measured in 100 patients on stable oral anticoagulant treatment with INRs ranging from 1.2 to 6.84 Because HVR patients had subnormal FVII, FX and FII levels after surgery, INR values > 2.0 were attained already 24 hours after the first warfarin dose. In healthy volunteers, INR values greater than 2.0 were first observed after 96 hours. Nadir levels of FVII, X and II were reached between 39 and 111 hours in HVR patients and between 88 and 183 hours in healthy volunteers. The apparent half-disappearance time (t/2) for FII levels was 74 hours in HVR patients and 70 hours in healthy volunteers (ns). In HVR patients there was no normalization of initially elevated F1+2 levels until day 7 with an apparent t/2 of 153 hours. In healthy volunteers, a decrease to subnormal F1+2 levels was observed by day 8 of treatment (apparent t/2 = 138 hours). In both HVR patients and healthy volunteers, the changes in F1+2 levels were associated with the changes in FII levels ($r\cdot = 0.26$, $p = 0.01$), but not with the changes in FVII or FX levels. In patients on stable OAT, the relationship of F1+2 with FII levels was closer than that observed with INR values. During the early phase of treatment, oral anticoagulants do

not prevent *in vivo* prothrombin activation until some time after the decrease in factor II levels, providing an explanation for the requirement of overlapping heparin and oral anticoagulant treatment for at least 48-72 hours after obtaining *therapeutic* INR values in patients with thromboembolic diseases. In addition, *in vivo* prothrombin activation correlates better with factor II levels than with INR values also in patients on stable oral anticoagulant treatment.

C112

EUROPEAN CONCERTED ACTION ON ANTICOAGULATION (ECAA). COMPARISON OF FRESH PLASMA AND WHOLE BLOOD MULTICENTER ISI CALIBRATION OF WHOLE BLOOD PROTHROMBIN TIME POINT-OF-CARE MONITORS

Tripodi A, Poller L, Keown M, Chauhan N,
Van Den Besselaar AM, Shiach C, Jespersen J

University and IRCCS Maggiore Hospital, Milan I; University of Manchester UK; University of Leiden NL, Manchester Royal Infirmary UK, University of Southern Denmark, Esbjerg, DK

Whole blood point-of-care test (POCT) prothrombin time (PT) monitors are being used on an increasing scale. To ensure their safety in controlling oral anticoagulation these POCT need to be calibrated in terms of their International Sensitivity Index (ISI) to accord with the World Health Organization (WHO) PT standardization scheme. A method for their ISI calibration was described by Tripodi *et al.* (*Thromb Haemost 1993; 70:921*) and depends on the comparison of parallel tests on whole blood samples from patients on oral anticoagulants and healthy subjects on the POCT with the manual PT test using the appropriate International Reference Preparation (IRP) for thromboplastin on plasmas obtained from the same blood samples. If plasmas could be substituted for whole blood, ISI determination of the POCT would be much simplified. A procedure for use of citrated plasmas for ISI calibration of two types of POCT monitor system has been evaluated in a multicenter calibration. Calibration with the CoaguChek Mini (Roche) and the Thrombolytic Assessment System, TAS (Bayer) gave higher ISI with whole blood samples than with plasmas. However, there was good agreement between whole blood and plasma monitor system International Normalized Ratio (INR) and the reference INR of target plasmas obtained with the specific species of IRP using the manual PT technique. It is concluded that reliable INR can be obtained with ISI from both whole blood and plasma samples of these two POCT systems. In the case of the CoaguChek Mini the plasma calibration ISI can also be used to derive INR with whole blood PT and mean normal PT. This was not possible with the TAS.

C113

LOW DOSE ORAL VITAMIN K EXCESSIVELY REVERTS ACENOCOUMAROL-INDUCED COAGULOPATHY

Ageno W, Crowther M, Steidl L, Ultori C, Mera V, Dentali F,
Squizzato A, Marchesi C, Venco A

Department of Medicine, University of Insubria, Varese, Italy; Department Of Medicine, Mcmaster University, Hamilton, Ontario, Canada

Background. Low dose vitamin K has been shown to be effective in the rapid reversal of warfarin-associated coagulopathy without causing warfarin resistance. Yet, the beneficial effect of this strategy has not been proven for patients treated with different oral anticoagulants. *Methods*. We carried out a randomized, controlled trial to compare the efficacy of 1 mg oral vitamin K with treatment withholding in patients receiving acenocoumarol who had an INR value between 4.5 and 10.0 and no concomitant signs of bleeding. The primary end-point of the study was the INR value on the day after study drug administration. Secondary outcomes were the INR value after 5±1 days and the rate of hemorrhagic or thromboembolic events after 1 month. *Results*. Patients receiving oral vitamin K had an excessive rate of sub-therapeutic INR values as compared to controls (36.6% and 13.3%, respectively; RR 1.83, 95% confidence interval 1.16, 2.89; p= non significant) and a lower rate of INR values within the therapeutic range (50% and 66.6%, respectively) after 1 day. After 5±1 days, the proportion of patients with an INR value within the range was higher in the group treated with vitamin K than in controls (74% and 44.8%, respectively). There were neither bleeding nor thrombotic events during follow-up. *Conclusions.* The omission of a single dose of acenocoumarol is sufficiently effective for a rapid reduction of the INR in asymptomatic patients presenting with coagulopathy. The addition of low dose vitamin K may produce an excessive correction. A potential stabilizing effect of vitamin K in a longer-term deserves further investigation.

C114

VITAMIN K ADMINISTRATION IN ASYMPTOMATIC AMBULATORY PATIENTS WITH EXCESSIVE COUMARIN ANTICOAGULATION

Poli D, Antonucci E, Lombardi A, Falciani M, Ilari I, Rizzuti G,
Sofi F, Chioccioli M, Gensini GF, Prisco D

Dipartimento di Area Critica Medico-Chirurgica, Università degli Studi di Firenze, Centro di Riferimento Regionale per la Trombosi, AO Careggi, Florence, Italy

Optimal management of outpatients with asymptomatic elevation of INR remains uncertain. In our center, in case of INR >7, therapy is stopped for one day and 2 mg of vitamin K1 (VK1) is orally administered, according to the recommendations from the *Federazione Italiana Centri Sorveglianza Anticoagulati* (FCSA). VK1 is also administered in patients with intrinsically high risk of bleeding or older than 75 years, if INR>6. The aim of this study was to evaluate the rate of vitamin K administrations and the safety and effectiveness of this practice. From June 1995 to December 2001 we observed 1112 patients, followed for 2329 patients/years. During this period 128 patients (61 males and 67 females) received 2 mg of oral VK1 on 172 occasions. The mean age of patients was 64±13.7 years. VK1 administration was significantly more frequent in women (rate 9.7 ×100 pt/yrs) than in men (rate 5.8 ×100 pt/yrs) and the Incidence Rate Ratio (RR) was 1.6 (1.2-2.2 95% C.I.), p=0.0009. Patients older than 75 years received VK1 administration more frequently than younger patients [RR 2.7 (1.9-3.9 95% C.I.), p=0.0000]. Patients treated with acenocoumarol had a significantly higher rate of VK1 administrations in comparison to patients treated with warfarin (rate 16 vs 5.1 ×100 pt/yrs respectively); [RR 3.1 (1.5-5.6 95% C.I.), p=0.001]. No differences were found in relation to mean weekly dose for both drugs. Patients with a target INR ≥ 3 received VK1 administrations more frequently than patients with a target INR < 3 [RR 1.8. (1.3-2.5 95% C.I.), p=0.0001]. The mean

interval between VK1 administration (T0) and the first INR measurement was 1.5 days (T1). The mean value registered at T1 was 2.8±0.9. Eighteen percent of patients had an INR ≤ 1.8 at T1; this figure is similar to that of the whole population followed by our Center. The second INR measurement (T2) was done 5.5 days after T0; the mean INR value registered was 2.9±1.1. No adverse events were registered during the 2 weeks after VK1 administration. In conclusion, our data confirm that low dose VK1 administration is safe and effective in the management of asymptomatic elevation of INR and is not followed by an excessive reduction of anticoagulation.

Oral Communications
Homocysteine II

C115

HOMOCYSTEINE PLASMA LEVELS IN CHILDREN: DIFFERENT MEASUREMENT METHODS AND ROLE OF POLYMORPHISMS OF ENZYMES INVOLVED IN THE METABOLISM

Vecchione G, D'Angelo F, Colaizzo D, Pellegrino M, Pinto A, Margaglione M, Grandone E

"Unità di Aterosclerosi e Trombosi", and Divisione di Pediatria, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, (FG), Cattedra di Genetica Medica, Università di Foggia, Italy

Homocysteine (tHcy) levels can be influenced by the presence of some genetic variants in addition to nutritional factors. A recent study compared three different methods for dosing tHcy: high pressure liquid chromatography (HPLC), Enzyme Immunoassay, fluorescence polarization immunoassay (FPIA) and capillary electrophoresis. FPIA is able to discriminate between two close concentrations within the normal range. It was also demonstrated that liquid chromatography electrospray tandem mass spectrometry (LC-MS/MS) is a sensitive and specific method for measuring tHcy. Moreover, immunological methods provided results with little bias compared with HPLC and MS. We evaluated tHcy, folic acid and B12 plasma levels in a group of 43 children (age 2 months-12 years). In the same group we studied three different polymorphisms: the C677T for 5,10 MTHFR, the A1298C substitution in the MTHFR and the haplotypes 844ins68 /833T→C. Moreover, we compared two different methods: the FPIA and MS. The median tHcy was 4.54 (range 1.91-9.59) when measured by means of EIA and 4.36 (range 2.12-9.71) using LC-MS/MS. As far as the MTHFR genotype is concerned, the TT was recorded in 8 (18.6%) children and the CT in 24 (55.8%). The 1298 C gene variant was present in 3 (7.1%), while the haplotype heterozygosis 844ins68 /CBS in 4 (9.3%). Mean tHcy using LC-MS/MS was 4.84 + 1.74 μmol/L in TT MTHFR individuals, 4.6 + 1.72 in CT children. Median folic acid and B12 plasma levels were 698. 33 pg/mL (range 86-2000) and 4.8 ng/mL (range 0.7-20.0), respectively. In conclusion, LC-MS/MS appears to be reliable and cheaper than the EIA test for measuring tHcy. Mean values of tHcy in a group of apparently health children appear to be lower than those of adults. Moreover, a factorial ANOVA analysis, including genetic and nutritional determinants, showed that only age (months) significantly accounts for differences (p= 0.031).

C116

DOES PLASMA HOMOCYSTEINE INFLUENCE THE *IN VITRO* ANTICOAGULANT RESPONSE TO ACTIVATED PROTEIN C?

Podda G, Zighetti ML, Lecchi A, Lombardi R, Faioni E, Cattaneo M

A. Bianchi Bonomi Hemophilia and Thrombosis Center, Dept. of Internal Medicine, IRCCS Ospedale Maggiore, University of Milan, Italy

Resistance to activated protein C (APC) is the most frequent risk factor for venous thrombosis among Caucasians. It may be congenital (in most instances, associated with factor V Leiden) or acquired. Recently, it has been shown that homocysteine may impair the inactivation of factor Va by APC. The aim of the study was to investigate whether the *in vitro* anticoagulant response to APC is impaired in subjects with moderately high plasma levels of homocysteine (tHcy). We studied 1254 subjects: 434 of them had had previous episodes of venous or arterial thrombosis and 820 had a negative personal history for thrombosis. The plasma levels of tHcy were measured before and after an oral methionine load (3.8 g/m2 b.s.a.) (PML). Subjects with factor V Leiden were not included in the study. The anticoagulant reponse to APC was measured only in the fasting state using a standard method, and was expressed as the APC ratio. There was no statistically significant difference between the mean APC ratio of subjects with and without hyperhomocysteinemia. There was no statistically significant correlation between APC ratio and fasting tHcy levels. However, there was a statistically significant correlation between APC ratio and PML tHcy (r= -0.093, p=0.001) or its PML increases above fasting levels (r= -0.127, p<0.0001). The above correlations remained unmodified after adjustment for factor VIII levels and APTT ratio. Our study does not support the hypothesis that moderate hyperhomocysteinemia negatively affects the *in vitro* anticoagulant response to APC. However, the finding of a negative correlation between APC ratio and PML tHcy suggests the existence of an association between abnormal anticoagulant response to APC and abnormal Hcy metabolism. The nature of this association is presently unknown.

C117

HOMOCYSTEINEMIA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Loffredo L, Fimognari FL, Marcoccia A, Perrone A, Maranghi M, Piccheri C, Simeoni I, Mugnaini L, Portalone L, Violi F

Institute of Clinical Medicine I, University "La Sapienza", Rome, Italy

Background. Experimental and epidemiologic studies showed a positive association between chronic obstructive pulmonary disease (COPD) and risk of coronary disease; we speculated that hyperhomocysteinemia, an independent risk factor for cardiovascular disease, could have a pivotal role in this process. *Methods.* We measured plasma total homocysteine (t-Hcy) levels in 16 patients with acute exacerbation of COPD (11 males, 5 females, age 70.94±7.78), in 17 patients with stable COPD (15 males, 2 females, age 70.39±6.83) and in 16 healthy subjects (11 males, 5 females, age 70.27±6.62). Exclusion criteria were: neoplasm, smoking, diabetes mellitus, cardiovascular disease and renal failure. Venous blood was collected from the fasting subjects into vacutainers containing EDTA. Samples were collected on ice, and plasma was separated within 15 minutes and frozen at –80°C for subsequent analysis. Homocysteine levels were measured by HPLC with fluorescent detection. *Results.* The plasma levels of t-Hcy were significantly higher in patients with acute exacerbation of COPD than in controls (p=0.005) and in patients with stable COPD than controls (p=0.015). No significant difference was observed between patients with acute exacerbation of COPD and patients with stable COPD. Thirteen patients with acute exacerbation of COPD were treated with theophylline, a drug potentially affecting homocysteine metabolism; we compared their plasma levels of t-Hcy (15.66±5.18) with the levels of 17 patients with stable COPD without theophylline treatment (17.49±9.5) but we did not observe significative differences (p=0.537).

	Patients with acute exacerbation of COPD	Patients with stable COPD	Controls
N	16	17	16
HCY (mmol/L)±SD	15.49±5.08	17.49±9.50	11.19±2.53
Pa O2 (mmHg)±SD	59.7±9.0	74.3±10.5	88±7.5
PaCO2(mmHg)±SD	48.8±8.2	43±5.3	40±4.9
pH±SD	7.35±3.3	7.39±3.5	7.4±1.5
FEV1 (liters)±SD	1.18±0.44	1.55±0.93	2.71±0.53
FVC (liters)±SD	2.15±0.7	2.76±1.3	3.01±0.42
Theophilline treatment	13	-	-

Conclusions. The study shows, for the first time, that patients with COPD have high homocysteine plasma levels and suggests a potential role for homocysteine as a risk factor for the premature atherosclerosis observed in these subjects. The mechanism that relates hyperhomocysteinemia to COPD deserves further investigation.

C118

METABOLIC DETERMINANTS OF FASTING PLASMA HOMOCYSTEINE IN A NORTHERN ITALIAN REGION: THE CREMONA STUDY

Mazzola G,* Garancini P,§ Sampietro F,* Fermo I,**
Testa S,° D'Angelo A*

**Coagulation Service and Thrombosis Research Unit, §Epidemiology Unit; **Department of Laboratory Medicine, IRCCS H S. Raffaele, Milan; °Istituto di Patologia Cinica, Istituti Ospitalieri, Cremona, Italy*

A large population study was conducted in 1990-1991 in 2096 subjects aged > 40 years from the Cremona area (Lombardia, Italy) to evaluate the prevalence of recognized and unrecognized cardiovascular risk factors and their impact on 7-year morbidity and mortality. Aliquots of citrated plasma and serum for the determination of glucose and lipids were stored at –70°C until assay. We report plasma total homocysteine (fasting tHcy, IMX homocysteine, Abbott), folate (IMX folate, Abbott), vitamin B12 (IMX B12, Abbott), pyridoxal-5'-phosphate (PLP) and cystatin C levels in a preliminary analysis of 999 subjects (455 men, 544 women, aged 58.7±11.0 years). At blood sampling, women were older than men (59.9±11.3 vs 57.4±10.5 years, p = 0.002) and had lower tHcy (12.1± 6.3 vs 18.1±13.5 μmol/L, p = 0.0001), higher folate (9.5±3.8 vs 8.3±4.8 nmol/L, p = 0.0001) and vitamin B12 levels (405±396 vs 320±380 pmol/L, p = 0.0001). A substantial proportion of subjects (15.6%) had tHcy levels >20 μmol/L and 3.7% of them had tHcy levels >40 μmol/L). The study population was divided in 2 groups according to tHcy levels < or >15 μmol/L. The two groups differed for proportion of male subjects, age, vitamins and cystatin C levels (all p values <0.0001, see table). The percentage of subjects with PLP, folate and vitamin B12 levels <15 nmol/L, <3.7 nmol/L and <150 pmol/L in the two groups were respectively 13% vs 23% (p

=0.0001), 2.5% vs 5.1% (p = 0.034) and 16.% vs 23% (p=0.012). Independent predictors of tHcy levels were analyzed in a generalized linear model for the two groups of subjects. In subjects with tHcy <15 μmol/L, 18.5% of the variation in tHcy levels was explained by age (p = 0.0001), gender (p = 0.0001) folate (p = 0.0001) and vitamin B12 (p = 0.002). In subjects with tHcy >15 μmol/L, 12.2% of the variation in tHcy levels was explained by gender (p = 0.008) and folate (p = 0.001). PLP was not an independent determinant of tHcy levels in either group of subjects. These results highlight the relevance of the vitamin status, and particularly of folate levels, in the modulation of fasting tHcy levels.

Table 1.

	n	Men (%)	Age (yrs)	PLP (nmol/L)	Folate (nmol/L)	Vit. B12 (pmol/L)	Cystatin C (μmol/L)
THcy < 15 μmol/L	683	37.5%	57.4±10.5	34.9±31.6	10.7±22.1	403±459	56.6±15
tHcy ≥ 15 μmol/L	316	65.3%	62.1±11.5	27.1±17.9	7.6±5.5	311±278	68.2±23

C119

THE PLASMA LEVELS OF VITAMIN B6 ARE LOW IN WOMEN ON ORAL CONTRACEPTION

Lussana F, Zighetti ML, Bucciarelli P, Cugno M, Cattaneo M

A. Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine IRCCS Ospedale Maggiore and University of Milan, Italy

Women using oral contraceptives (OC) are at increased risk of arterial and venous thromboembolic events (TE). High plasma levels of total homocysteine (tHcy) and low levels of vitamin B6 are associated with heightened risk for TE. In a case-control study, we investigated the effects of OC use on the plasma levels of tHcy and vitamin B6. Two hundred and nineteen healthy women were enrolled in the study. The study population was divided into two groups: group 1) 159 women who had not used OC for at least 12 months prior to their enrollment in the study (median age, 34y, range 18-45); group 2) 60 women on regular OC treatment (31y, 21-45). The plasma levels of tHcy (before and 4 h after a standardized oral methionine load [PML]) and vitamin B6 were measured. The serum levels of folate and vitamin B12 (which, like vitamin B6, are involved in Hcy metabolism) were also measured. None of the subjects had a positive family or personal history of TE, or overt neoplastic or autoimmune diseases. The median levels of vitamin B6 and B12 were significantly lower in OC users than in non users (24.2 pmol/L and 278 ng/mL, vs 32.9 pmol/L and 429 ng/mL; p=0.027 and p<0.001). The observed differences remained statistically significant after adjustment for age and cigarette smoking. There were no statistically significant differences in the levels of tHcy (fasting and PML) and folate in the two groups of subjects. In our case-control study, we found no effects of OC use on the plasma levels of tHcy. However, OC use was associated with low vitamin B6 levels. Since low vitamin B6 levels are independently associated with heightened risks for arterial and venous TE, they could partly account for the high TE risk of OC users.

C120

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

Giusti B, Porciani MC, Brunelli T, Evangelisti L, Fedi S, Attanasio M, Gensini GF, Abbate R, Sani G, Yacoub M,* Pepe G°

*Dipartimento Area Critica Medico-Chirurgica, Università degli Studi di Firenze; *Department of Cardiothoracic Surgery, National Heart and Lung Institute, Imperial College of Science, Technology and Medicine, Heart Science Centre, Harefield Hospital, Uxbridge, United Kingdom; °Dipartimento di Medicina Interna, Università degli Studi di Roma "Tor Vergata", Rome, Italy*

Elevated homocysteine (Hcy) levels have been reported to be associated with abdominal aortic aneurysm and with the mutation C677T in 5,10-methylenetetrahydrofolate reductase (MTHFR) gene coding for an enzyme of the homocysteine metabolism. Thoracic aortic aneurysm is the major cardiovascular manifestation in Marfan's syndrome, an inherited connective tissue disease due to mutations in fibrillin-1 gene. The aim of this study was to evaluate homocysteinemia and the prevalence of the C677T MTHFR gene mutation in patients with Marfan's syndrome and to determine whether this mutation influences Hcy levels and the severity of cardiovascular manifestations. We studied 189 control subjects, and 107 patients with Marfan's syndrome subdivided into 3 subgroups based on the severity of cardiovascular manifestations: A) any (n=4); B) involvement or major criterion (mild aortic dilatation <2.2 cm/m^2 body surface area) (n=45); C) major criteria (moderate to severe aortic dilatation >2.2 cm/m^2 body surface area, or aortic dissection) (n=58). Hcy levels were significantly higher in patients with Marfan's syndrome than in controls (10.6±4.1 versus 8.9±3.7 μmol/L, p<0.0003). In subgroup A Hcy levels were 7.7±0.9 μmol/L. In subgroup C Hcy levels (11.5 ±4.5 μmol/L) were significantly higher (p<0.04) than in subgroup B (9.8±3.5 μmol/L). The prevalence of homozygotes for the C677T mutation in patients with Marfan's syndrome was higher (22.4%) than in controls (14.3%), but the difference did not reach the statistical significance (p=0.08). In subgroup C the prevalence of homozygotes (25.9%) for the C677T mutation was significantly higher than in controls (p<0.05), and was higher but not significantly (p=0.35) than in the subgroup B (17.8%). In the whole Marfan population, in the subgroups B and C, and in the controls a significant genotype-phenotype correlation between Hcy levels and C677T mutation was observed (p<0.000001, p<0.03, p<0.000001 and p<0.05, respectively). Our data indicate an association between the severity of cardiovascular manifestations in patients with Marfan's syndrome and elevated Hcy plasma levels and provide further information on the pathophysiologic mechanisms of extracellular matrix involvement in vascular damage due to hyperhomocysteinemia.

Oral Communications
Pathologic Hemostasis in Humans II

C121

NQO1 POLYMORPHISM AS A MODIFIER OF THE ORAL ANTICOAGULANT THERAPY DOSE REQUIREMENT

Manotti C,* Tagliaferri A,* Pattacini C,* Guarnieri D,* De Palma G,# Mozzoni P,# Scotti E,# Buzio L,# Scarpa S#

**Dip. Medicina 3, Centro per le Malattie dell'Emostasi e Cura dell'Emofilia, Azienda Ospedaliera di Parma; #Dip. di Clinica Medica, Nefrologia e Scienze della Prevenzione, Lab. di Tossicologia Industriale, Università di Parma, Italy*

Vitamin K is bioactivated by reduction of the quinone form (K) to the corresponding hydroquinone (KH2) [1], which is then oxidised into the 2,3 epoxide (KO) [2], and further reduced to the quinone (K) [3]. The reaction [2] is coupled with the ƒ-carboxilation of glutamic acid residues of the coagulation factors. The mechanism of action of oral anticoagulants is the block of such metabolic cycle through the inhibition of the reduction reactions [1] and [3]. The cytosolic enzyme NAD(P)H: quinone oxidoreductase (NQOR), also termed DT-diaphorase, catalyses the two-electron reduction of many quinones, including menadione (vit. K3), to hydroquinones. The corresponding gene, NQO1, is polymorphic (NQO1*2 allele) because of a missense mutation (C609>T), which codes for a proline to serine change in the human protein. Subjects carrying a NQO1*2/*2 genotype (about 4% of Caucasians) lack any NQO1 activity because of an accelerated degradation of the mutant protein. The present study was carried out to investigate whether the NQO1 polymorphism can modify oral anticoagulant dose requirement; 214 subjects (133 males), aged 64+10 years on average, attending the Haemostasis Centre of the Parma University Hospital for oral anticoagulant therapy and not assuming any other drug known to interfere with oral anticoagulants, were enrolled into the study. Of these, 140 subjects were in treatment with acenocoumarol (mean dose 14.3±7 mg/week) and 74 received warfarin as their anticoagulant drug (mean dose 28.7±12 mg/week). The NQO1 polymorphism was characterized by an already published PCR-RFLP method on a venous blood sample taken during a periodic check. ANOVA demonstrated a significant interference on the dose requirement by both the NQO1 genotype (p=0.001) and the age of subjects (p<0.001). The 121 subjects carrying two wild type alleles (NQO1*1/*1) required significantly more drug than subjects carrying at least one defective allele (NQO1*1/*2 and NQO1*2/*2) (mean doses 15 mg/week and 11.8 mg/week, respectively, p<0.001). To our knowledge, this is the first time that an interference by the NQO1 genotype on oral anticoagulant therapy has been found. The biological plausibility of the observed findings could rely on the better bioactivating capacity of vitamin K, and hence *resistance* to oral anticoagulants, of NQO1*1/*1 subjects, compared to people bearing at least one defective NQO1*2 allele.

C122

THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR DEPENDENT INHIBITION OF FIBRINOLYSIS IS NOT AFFECTED BY HEPARIN

Colucci M, Pentimone A, Binetti BM,° Cramarossa M, Piro D, Semeraro N

Dipartimento di Scienze Biomediche, Sezione di Patologia Generale, Università di Bari, Italy

Thrombin activatable fibrinolysis inhibitor (TAFI) is a carboxypeptidase-B-like zymogen that, upon activation by thrombin and plasmin, inhibits fibrinolysis through the removal of the plasminogen binding sites from partially degraded fibrin. Heparin, by virtue of its anti-thrombin activity, has been proposed to enhance thrombolysis via inhibition of TAFIa generation. We evaluated the effect of heparin in an *in vitro* model consisting of a radiolabeled blood clot submerged in defibrinated plasma. Fibrinolysis was induced by adding t-PA (250 ng/mL), test material and calcium to the plasma bath. Thrombin generation occurring upon recalcification caused TAFI activation (about 4%) and inhibited clot lysis as indicated by the finding that omission of calcium, substitution of normal plasma with $BaSO_4$-adsorbed plasma (lacking vitamin K-dependent factors), or addition of a specific inhibitor of TAFIa (PTI, 50 ug/mL) enhanced fibrinolysis by more than 50%. Surprisingly, heparin (up to 1 U/mL) failed to enhance clot lysis despite complete inhibition of thrombin and TAFIa generation in the fluid phase. Assay of clot-bound thrombin by ^{125}I-fibrinogen uptake revealed that heparin was virtually unable to prevent thrombin generation on the fibrin surface. Moreover, when hippuryl-Arg was added to the clot lysis system, in order to detect TAFIa eventually associated with the clot, a weak TAFIa activity was generated in heparin-containing samples. This was further supported by the observation that the addition of PTI along with heparin enhanced clot lysis. Hirudin (10 μg/mL), at variance with heparin, inhibited clot-bound thrombin by > 60% and enhanced clot lysis (> 40%) via a TAFI-dependent mechanism. These data show that heparin is unable to stimulate fibrinolysis via TAFI, most likely because of its inefficiency in inhibiting thrombin generation on the clot surface. Moreover, they suggest that clot-bound thrombin plays a major role in TAFI-mediated inhibition of fibrinolysis through *localized* TAFIa generation.

C123

EFFECTS OF SOME PRE-ANALYTICAL CONDITIONS ON THE MEASUREMENT OF HOMOCYSTEINE AND CYSTEINE IN PLASMA

Zighetti ML, Lecchi A, Lombardi R, Cattaneo M

A. Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine IRCCS Ospedale Maggiore and University of Milan, Italy

The association of hyperhomocysteinemia with the risk of arterial and venous thrombosis is well documented. Recent studies suggest that high plasma levels of cysteine (tCys) are also associated with high cardiovascular risk. Standardized pre-analytical conditions are necessary for reliable measurement of plasma homocysteine (tHcy). The aim of the study was to evaluate the effects of pre-analytical conditions on the measurement of tHcy and tCys in plasma. Eleven healthy volunteers were enrolled in the study. For each subject, blood samples were collected in

8 tubes with EDTA and 8 tubes with ACD. Four tubes with EDTA and 4 with ACD were immediately placed on crushed ice, while the remaining tubes were stored at room temperature (RT). All tubes were centrifuged at 2200g (4°C, 20 min) at 15 min, 2, 4 and 6 hours after sampling. The supernatant platelet-poor plasma was frozen at – 20°C until assay. Plasma tHcy and tCys levels were measured with an HPLC method with fluorometric detection. The plasma concentrations of tHcy and tCys did not change over 6 hour in blood samples that had been kept on ice until centrifugation, independently of the anticoagulant used. In blood samples that had been kept at RT, the plasma concentration of tHcy tended to increase over time (38% increase in EDTA; 9.5% in ACD, after 6h storage), while that of tCy tended to decrease slightly in samples with EDTA (5% decrease after 2h storage). No changes in tCys concentration were observed in samples in ACD stored at RT. Therefore, the plasma concentrations of both tCys and tHcy remain stable for at least 6h, independently of the anticoagulant used, when blood samples are stored on ice. If blood samples must be kept at RT, ACD anticoagulant is preferable to EDTA.

C124

HYPERHOMOCYSTEINEMIA INHIBITS NITRIC OXIDE PRODUCTION BY HUMAN PLATELETS

Gresele P, Lucarelli G, Mezzasoma AM, Falcinelli E, Guglielmini G

Dept. of Internal Medicine, Sect. of Internal and Cardiovascular Medicine, University of Perugia, Italy

Elevated plasma homocysteine (HCy) is associated with an enhanced risk of atherothrombosis, however the exact causal mechanisms are not yet fully established. Elevated Hcy has been shown to suppress endothelial nitric oxide (NO) production. Platelets contain constitutive NO synthase and both soluble agonists and shear stress can activate platelet production of NO. Data are accumulating on a role of platelet-released NO in the protection against thrombosis. The aim of our study was to assess whether hyperHCy affects platelet NO production. Twenty-two subjects not taking drugs affecting platelets underwent an oral L-methionine load (0.1 g/kg), after a overnight fast. Blood was sampled before and six hours after the load. Collagen- (3 μg/mL) or ADP-(10 μM) induced platelet NO production was assessed using a NO sensitive electrode, and by the measurement of NO2/NO3 with a colorimetric (Griess) method. ADP- and collagen- induced platelet aggregation was also studied. NO production by Gel Filtered Platelets (GFP) stimulated with ADP decreased from 18.9±7 to 1.4±1.1 pmol/10^8 platelets (p<0.02) and with collagen from 24.9±9.2 to 7.6±3 pmol/10^8 platelets (p<0.02). Total NO_2/NO_3 in the supernatant of ADP-stimulated platelets decreased from 16±2.9 to 10.1±1.2 nmol/10^8 plts (p<0.04) and from 12.1±1.6 to 8.9±1.6 nmol/10^8 platelets with collagen (p<0.05). Shear-induced platelet activation was increased, with a shortening of filter closure time from 55±5.8 to 46.1±2.5 sec. (p<0,04) and an increase of retained platelets (20-40 sec) from 75±2.7% to 80±1.1% (p<0.05). Similarly, aggregation was enhanced from 56.6±11% to 75±9% for ADP (p<0.05) and from 28.3±8.5% to 53.7±9.5% for collagen (p<0.05). Finally, platelets preincubated with HCy (100 μM) for 1h and then gel filtered produced significantly less NO upon stimulation with ADP (10.5±5.5 vs 34.4±10.8 pmol/10^8 platelets, p< 0.05). Our data demonstrate that hyperHCy inhibit platelets NO production *ex vivo* and *in vitro* and enhances platelet reactivity to both soluble and shear stimuli. Inhibition of platelet NO represents a new mechanism of the prothrombotic effect of hyperHCy.

C125

ANTIOXIDANT TREATMENT DECREASES THE TITER OF CIRCULATING ANTICARDIOLIPIN ANTIBODIES

Ferro D, Valesini G,* Conti F,* Iuliano L, Saliola M, Violi F

*Institute of Clinical Medicine I, and *Division of Rheumathology, Rome, University "la Sapienza", Italy*

A close association between antiphospholipid antibodies (aPL) and *in vivo* markers of lipid peroxidation has been previously demonstrated. We undertook an interventional study to assess whether antioxidant treatment is able to affect the serum titer of anticardiolipin antibodies (aCL). We studied 14 consecutive aCL positive outpatients (12 women, 1 man; age 24 to 49), with a titer ranging from 14 to 120 GPL or MPL. Six of 14 aCL positive subjects were affected by primary antiphospholipid syndrome (PAPS). The remaining 8 patients suffered from systemic lupus erythematosus (SLE). Patients were randomly treated with (n=7) or without (n=7) antioxidant supplementation (vitamin E 900 IU/day, vitamin C at 2000 mg/day) for 4-6 weeks. In each patient, before and after the treatment, we evaluated aCL, lupus anticoagulant (LA), prothrombin fragment 1+2 (F1+2) (Behringwerke, Marburg, Germany), fibrinogen, tumor necrosis factor α (T cell Diagnostics Inc, Cambridge, MA, USA) and plasma vitamin E and C. Vitamin E (r=-0.63, p<0.02) and vitamin C (r=-0.65, p=0.01) were significantly inversely correlated with F1+2 levels. In the subjects not assigned to antioxidant treatment, no changes of clinical and laboratory variables were observed. Conversely, patients given antioxidants showed a significant decrease of aCL titer [32 (16-120) vs. 8 (3-100) GPL or MPL; p=0.016] and F1+2 (1.99±0.34 vs. 1.19±0.34 mM; p=0.016) and a significant increase of both vitamin E (p=0.016) and C (p=0.016) plasma levels. Analysis of data by ANOVA confirmed the results. In these patients, clinical characteristics, fibrinogen and tumor necrosis factor α circulating levels did not change after the treatment, so excluding modification of disease activity. The study shows that antioxidant treatment is able to decrease aCL titer, so supporting the hypothesis that oxidative stress plays a central role in the formation of aCL.

C126

A SIMPLE TEST TO DETECT β_2GPI-DEPENDENT LUPUS ANTICOAGULANTS

Biasiolo A, Filippi B, Pengo V

Clinical Cardiology, Thrombosis Centre, University of Padua, Italy

Among antiphospholipid antibodies, lupus anticoagulant (LA) is the most powerful marker of thromboembolic disorders. LA activity is explained by the formation of bivalent antibody-antigen (β_2GPI or prothrombin) complexes over the phospholipid surface, thus impeding normal assembling of coagulation factors. Recent data confirm the role of anti- β_2GPI monoclonal antibodies and β_2GPI deposition (clustering) on lipid vescicles and highlight the relationship between this phenomenon and calcium concentration in the system. To evaluate the role of calcium ions in LA screening tests, we have developed a new prothrombin time test (PT), in which human thromboplastin is adsorbed on a microtiter plate. This assay is comparable to a diluted PT (dPT), and the mean clotting time of LA positive plasmas (110 seconds) is significantly prolonged when compared to that of a control plasmas (62 seconds). We tested 14 patients' LA positive plasmas, 6 of which were positive in (medium-high titer) anti-human β_2GPI antibodies ELISA and we then tested the sensitivity of LA plasmas to variation in calcium concentration in this assay and observed a different behavior in relation to the presence or absence of anti-human β_2GPI antibodies. Reducing final calcium concentration from 0.01 M to 0.005 M, we observed an increase of mean clotting time ratio to 160% in the group of patients positive for anti- β_2GPI antibodies and no increase (107%) in the group of patients testing negative for anti- β_2GPI antibodies. Thus, a simple dilution of calcium ions in a modified PT assay, might be useful in identifying anti- β_2GPI-dependent LA. These patients are at high risk of thromboembolic complication and this is confirmed in our series in which 6 out 6 (100%) patients had thromboembolic complications which were only present in 2 out 8 patient with anti- β_2GPI-independent LA.

Oral Communications
von Willebrand factor/von Willebrand disease

C127

DISTRIBUTION OF VON WILLEBRAND'S DISEASE TYPES IN 316 PATIENTS FOLLOWED BY A SINGLE HEMOPHILIA CENTER SINCE 1992: A REAPPRAISAL OF TYPE 1 VERSUS TYPE 2M DIAGNOSIS AFTER TEN YEARS

Federici AB, Canciani MT, Baronciani L, Castaldo M, Cozzi G, Forza I, Dallagiovanna S, Burgo I, Mannucci PM

Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Maggiore Hospital and University of Milan, Italy

Type 1 von Willebrand's disease (VWD) is considered the most frequent form of VWD (60-80%): it is characterized by reduced plasma levels of von Willebrand factor (VWF) with normal multimeric pattern. Until 1992, the diagnosis of type 1 VWD was based on reduced plasma levels of ristocetin cofactor activity (VWF:RCo) and normal multimeric pattern, without considering the ratio between VWF:RCo and VWF antigen (VWF:Ag). In 1994, the SSC on VWF of the ISTH distinguished six different VWD types, defining type 2M VWD as *qualitative variants with decreased platelet dependent function not caused by the absence of high-molecular-weight multimers*. We have recently reconsidered the diagnosis of VWD types in a large number of patients (n=316) already diagnosed before January 1992. Laboratory diagnosis of VWD types was based not only on VWF:RCo, VWF:Ag levels, ristocetin induced platelet agglutination (RIPA), multimeric pattern and mutation screening but also on VWF:RCo/Ag ratio, meant to differentiate type 1 (ratio >0.7) from 2M (ratio <0.7) VWD; moreover, patients with *supranormal* multimers in plasma (former type 1 *Vicenza*) were classified type 2M *Vicenza*. The actual (January 2002) distribution of VWD types in the 316 cases studied on January 1992 is as follows (see Table below). Our data demonstrate that the diagnosis of type 3, 2A, 2B and 2N VWD remained identical after ten years. Conversely, type 1 VWD is less frequent than previously reported when more stringent diagnostic criteria are used, because most type 1 VWD cases are characterized by an abnormal VWF, in the presence of normal VWF multimeric pattern, and therefore must be classified as type 2M VWD.

Diagnosis of VWD types	1	2A	2B	2M	2N	3	Total
January 1992							
Case n. (%)	218 (69)	45 (14)	30 (9.5)	n.i.	1 (0.3)	22 (7)	316 (100)
Family n. (%)	123 (69)	23 (13)	14 (8)	n.i.	1 (0.5)	18 (10)	179 (100)
January 2002							
VWF:RCo/Ag (mean)	0.96	0.26	0.53	0.57	1.01	n.c.	n.c.
Mutation found (%)	10	90	94	46	100	91	53
Case n. (%)	108 (34)	45 (14)	35 (11)	105 (33)	1 (0.3)	22 (7)	316 (100)
Family n. (%)	85 (47)	23 (13)	15 (8)	37 (21)	1 (0.5)	18 (10)	179 (100)

C128

CLINICAL PRESENTATION OF TYPE 1 AND 3 VON WILLEBRAND'S DISEASE IN OBLIGATORY CARRIERS: RESULTS FROM A COLLABORATIVE, INTERNATIONAL, MULTICENTER STUDY

Rodeghiero F, Batlle J, Baudo F, Blömback M, Castillo R, De Bosch N, Eikenboom J, Federici AB, Lethagen S, Lusher J, Linari S, Monteagudo J, Nishino M, Srivastava A, Tosetto A, Castaman G

The International Multicenter Study Group on the Validation of the Diagnostic Criteria of type 1 and 3 VWD

Aim of the study. Multicenter, international study within the framework of SSC ISTH on von Willebrand factor, to investigate bleeding history in a sample of obligatory carriers of type 1 and 3 von Willebrand's disease (VWD). Bleeding history compared with that of affected members and healthy controls. *Methods.* Patients and obligatory carriers (OC) of type 1 VWD identified by the presence of parent, offspring or sibling with type 1 VWD; patients and obligatory carriers of type 3 VWD (parents or offspring) were enrolled with age and sex-matched control. The questionnaire evaluated each hemorrhagic symptom at presentation, using a score system ranging from 0 (no symptom) to 3 (hospitalization, replacement therapy, blood transfusion) to take into account the severity of bleeding. For each symptom, the highest score referred by the patient was evaluated. Multivariate analysis of data using logistic regression. *Results.* Three hundred and five subjects were available for analysis (32 type 1 OC, 67 type 3 OC, 63 type 1 patients (32 parents and 31 sibs), 34 type 3 patients and 106 controls). Hemorrhagic scores are reported in the table below.

	Epistaxis	Cut. bleed.	Tooth extraction	Surgery	Menorrhagia	Post-partum
Controls	0.17	0.05	0.19	0.14	0.58	0.06
Type 1 OC	0.87*	1.06*	1.45*	1.29*	1.52*	1.36*
Type 1 Aff.	0.73*	1.16*	1.16*	1.03*	1.53*	1.26*
Type 3 OC	0.34	0.31	0.08	0.46	0.39	0.34
Type 3 Aff.	0.55	1.61*	1.33	1.5	1.28	1.00

Significant differences. No control showed more than two separate bleeding symptoms. Logistic regression showed that the probability of being type 1 VWD in subjects with 1 or 2 symptoms was 1.9 and 14.2 fold higher than in controls (p=0.38 and 0.002, respectively). Bleeding after at least one tooth extraction multiplies this probability 9.3 times. *Conclusions.* An high penetrance of hemorrhagic symptoms was found in OC of type 1 VWD. Type 3 OC were substantially undistinguishable from controls. Three separate hemorrhagic symptoms are highly predictive of VWD carriership.

C129

MULTIETHNIC STUDY OF THE VON WILLEBRAND'S FACTOR GENE IN PATIENTS WITH TYPE 3 VON WILLEBRAND DISEASE: AN UPDATE

Baronciani L, Cozzi G, Federici AB, Canciani MT, Peyvandi F, Srivastava A,* Mannucci PM

*Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, IRCCS Maggiore Hospital and University of Milan, Italy; and *Department of Hematology, Christian Medical College Hospital, Vellore, India*

Severe deficiencies of VWF result in type 3 VWD, characterized by unmeasurable VWF antigen levels in plasma and platelets and severe hemorrhagic symptoms. Despite the large size of the gene, and the low prevalence of the disease (1-5 per million), about 55 distinct mutations have been so far reported. We have previously identified 20 novel mutations in a group of 21 patients (*Baronciani et al, Thromb Haemost 2000, 84*). We extended this study to a new group of 19 patients (6 Italians, 5 Iranians and 8 Indians), in order to identify the molecular defects and to evaluate genetic heterogeneity among these populations. All coding regions and intron/exon boundaries of the VWF gene were screened by SSCP analysis. Direct sequencing was performed for the exons in which bands of abnormal mobility were observed. Thirty distinct mutations were identified, 4/30 already reported. Nine were small deletions (526delT, 1038-1061del, 2266-69delCTCT, 2406delA, 2519-20delCT, 3904delC, 4338-39delAC, 7544-45delGT, 7931delT), 8 nonsense mutations (R365*, Y610*, W502*, W642*, Q1311*, E1981*, R2434*, Q2544*), 5 possible splice site mutations (IVS15(-4)c→t,IVS23(+5)g→a, IVS25(+1)g→a, IVS28(+1)g→a, IVS45(+7)c→t), 3 single nucleotide insertion (4664insC, 7375insC, 7387insT) and 5 candidate missense mutations (D47H, S85P D141Y, D141N, C1071F). All mutations, with the exceptions of the 5 missense mutations and one in-frame deletion, cause null alleles. This study confirms our previous report. Only one mutation (7375insC) was found more than once. Only 4 of the mutations found have been previously reported and 3 are *hotspot* mutations. Four of the 5 missense mutations are located in the propeptide supporting its important role in the VWF multimerization and formation of storage granules. Type 3 VWD molecular defects are heterogeneous, being characterized by a large number of different mutations arising within the entire VWF gene and often responsible for null alleles.

C130

IN VITRO EXPRESSION STUDY OF A NATURALLY OCCURRING MUTATION (C275S) ON THE VON WILLEBRAND'S FACTOR PROPEPTIDE CAUSING TYPE 3 VON WILLEBRAND DISEASE

Baronciani L, Peyvandi F, Jenkins VP, Cozzi G, Canciani MT, Federici AB, Mannucci PM

Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, IRCCS Maggiore Hospital and University of Milan, Italy

We report *in vitro* expression analysis of a missense mutation characterized by a T to A transversion at nucleotide 1073 of the von Willebrand factor (VWF) cDNA, predicting a substitution of cysteine by serine at amino acid position 275 (C275S) of the VWF

propetide (*Baronciani et al., Thromb Haemost 2000, 84*). A patient carrying this mutation had severe VWF deficiency (VWF:Ag levels of < 0.01 IU/mL) and was also a carrier of a nonsense mutation W222*. Her mother, carrier of C275S, did not have a bleeding tendency but showed a reduced VWF level in plasma (VWF:Ag 34 IU/mL, VWF:RCo 25 IU/mL). To determine whether the C275S mutation could be responsible for the patient's phenotype, the plasmid pSV-VWFH, containing cDNA of the human VWF, was used as a template to make, by the site direct mutagenesis method, vector pSV-VWFHC275S. Both expression vectors were used independently for transient transfection studies in COS-7 cells. Data of VWF:Ag and the collagen binding assay (VWF:CB) are reported as mean of four different transfections (n=4). In lysates of cells transfected with either pSV-VWF-WT or pSV-VWFC275S constructs, VWF:Ag levels were similar (0.1 IU/mL and 0.14 IU/mL). However, no VWF:Ag was secreted by cells transfected with pSV-VWFR275S, whereas that secreted by cells transfected with pSV-VWF-WT was measured to be 0.02 IU/mL. Multimer analysis of the recombinant wild type VWF in culture medium (VWF:CB/Ag 2.24) and cell lysates showed that all multimers were present: in cell lysates there was a high portion of low molecular size multimers (VWF:CB/Ag 0.18). Conversely, only low molecular size multimers were visualized in recombinant C275S VWF cell lysates (VWF:CB/Ag 0.14). In conclusion, these experiments showed that the mutation C275S of VWF gene results in a quantitative deficiency of VWF in plasma, due probably to a secretion pathway defect associated with partial intracellular degradation.

C131

TYPE 2 B (P1337L) VON WILLEBRAND'S DISEASE ASSOCIATED WITH HETEROZYGOUS DEFECT OF TYPE 1 (C275R) IN A PATIENT PREVIOUSLY DIAGNOSED AS TYPE 2 A: THE IMPORTANT ROLE OF MOLECULAR CHARACTERIZATION OF THE ENTIRE FAMILY

Canciani MT, Baronciani L, Forza I, Cozzi G, Siboni S, Federici AB

Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Maggiore Hospital and University of Milan, Italy

In a 50-year old man apparently carrying the phenotype of the 2A von Willebrand's disease (VWD) (mean values: FVIII = 32 U/dL,VWF:Ag = 7 U/dL,VWF:RCo = < 6 U/dL, VWF:CB = <1, RIPA ≥ 2.0 mg/mL, loss of high molecular weight multimers in plasma and low platelet VWF), a transient thrombocytopenia occurred after an infusion test with desmopressin. The propositus' brother showed similar but less severe laboratory data (mean values: FVIII = 36 U/dL, VWF:Ag = 17 U/dL, VWF:RCo = 6 U/dL, VWF:CB=1, RIPA = 1.2 mg/mL). To make a correct diagnosis in these two patients, eleven individuals of three generations of the family were studied and characterized by bleeding history as well as by all the clinical and laboratory parameters available in our laboratory. Molecular polymorphisms such as VNTR I and VNTR II were also used to perform linkage analysis. The search for mutations started by amplification of exon 28 but was also extended to the entire VWF gene. A total of 9/11 members were affected by gene VWF defects but phenotypic and genotypic data were very heterogeneous within the family. The two brothers were found to be compound heterozygotes for the already reported type 2B mutation (P1337L) and for a novel candidate defect C275R. Three family members showed a classical 2B phenotype (RIPA = 0.5 mg/mL, and normal platelet VWF) and were associated with the mutation P1337L, whereas four additional affected members characterized by type 1 VWD phenotype and low platelet VWF carried the novel defect C275R. VNTR analysis in all the members of the family confirmed the linkage of these two defects with the respective phenotypes. Type 2 VWD diagnosis can be very difficult especially when a double genetic defect is present in the same family. VWF molecular analysis should be carried out for a correct VWD diagnosis, when phenotypic tests are misleading.

C132

VON WILLEBRAND FACTOR CLEAVING PROTEASE IN THE HELLP SYNDROME

Lattuada A, Mannucci PM, Sacchi E, Masseroni C, Vaghi U, Carpani G, Rossi E

Hematology and Blood Transfusion Service L. Sacco Hospital, milan; A. Bianchi Bonomi Hemophilia and Thrombosis Center, University of Milan, IRCCS Maggiore Hospital, Milan; Department of Laboratory Medicine, S. Giuseppe Hospital, Milan; Blood Transfusion Service, S. Paolo Hospital, Milan, Italy

von Willebrand factor (VWF) is a multimeric glycoprotein that plays a central role in hemostasis by mediating adhesion of platelets to the exposed subendothelium. In normal plasma vWF undergoes proteolysis and recently a specific protease that cleaves VWF has been identified as a new member of the ADAMTS family of metalloproteinases. Deficiency of von Willebrand factor cleaning protease (VWF:CP) is associated with the circulation of unusually large VWF multimers in plasma. These extremely large multimers may aggregate platelets at sites with high levels of intravascular shear stress. A physiological condition with an increase thrombotic risk is pregnancy and a complication of pregnancy is the HELLP syndrome (HS). Patients with the HS have platelet consumption and erythrocyte disruption attributed to thrombotic occlusions in the microvasculature of various organs. These alterations present similarities with those of thrombotic thrombocytopenic purpura and other microangiopathies. The aim of our study was to evaluate whether the VWF:CP is altered in women with HS. We studied VWF:CP, VWF: antigen (VWF: Ag) and VWF collagen binding (VWF:CB) in plasma from women with HS, after six months (after) and we compared these values to those of normal pregnancy. Results are given as mean±SD with observed ranges between parentheses. *Results* (see Table below).

	VWF:CP %	VWF:Ag %	VWF:CB %
HELLP Syndrome	35±6 (26-43)	330±56 (234-422)	242±39 (188-332)
HELLP in remission	107±11 (105-130)	119+53 (60-217)	107±30 (71-152)
Normal pregnancy	72±15 (48-105)	189±68 (102-320)	148±34 (102-228)

Conclusions. Our results show that the VWF:CP is low in HS whereas there are high VWF levels. These alterations may play a role in the thrombotic microangiopathy typical of the HS.

Oral Communications
Recurrent Venous Thromboembolism

C133

MOLECULAR THROMBOPHILIC ABNORMALITIES AND RECURRENCE OF VENOUS THROMBOEMBOLISM: RESULTS FROM THE WODIT STUDY

Santamaria MG, Agnelli G, Prandoni P, Taliani MR, Bagatella P, Bazzan M, Moia M, Guazzaloca G, Bertoldi A, Tomasi C, Ambrosio G, Ageno W, Silingardi M, for the Warfarin Optimal Duration Italian Trial (WODIT) Investigators

Sezione di Medicina Interna e Cardiovascolare, Dipartimento di Medicina Interna, Università di Perugia, Italy

Background. It has been claimed that patients with hereditary or acquired thrombophilia have an increased risk of recurrence of venous thromboembolism (VTE). *Aim and Methods*. To assess the incidence of molecular thrombophilic abnormalities in patients with and without VTE recurrence. Database was from a multicenter randomized study aimed at evaluating the long-term clinical outcome of extending to one year the three-month oral anticoagulant treatment after a first episode of idiopathic proximal deep vein thrombosis. A screening for hereditary or acquired thrombophilia was performed after the completion of the study. A Cox proportional hazard model was used to evaluate the role of molecular thrombophilic abnormalities as predictors of VTE recurrence. *Results*. Among the 267 patients included in the study, 42 (15.7%) experienced a recurrence of VTE: 21 randomized to anticoagulation withdrawal and 21 to extended anticoagulation. All episodes of recurrent VTE were idiopathic and none was fatal. A screening for thrombophilia was performed in 184 patients (68.9%): 36 of the 42 patients (85.7%) with VTE recurrence and 148 of the 225 patients without recurrence (65.8%). Among the screened patients (105 males and 79 females; mean age 62 years, range 19-89), 52 (28.3%) had one or more molecular thrombophilic abnormalities. The mean age of thrombophilic patients was 57 years (range 20-88) with respect to 64 years (range 19-89) of the non-thrombophilic patients. Among the screened patients, 13 patients (36.1%) with recurrent VTE were found to have a thrombophilia as compared with 39 patients (26.4%) without VTE recurrence (p=ns). The following molecular abnormalities were found: FVR506Q mutation (21), Pt20210GA mutation (14), hyperhomocysteinemia (9), 6 antiphospholipid antibodies (6), protein C deficiency (5), antithrombin deficiency (3), protein S deficiencies (3). Eight patients had two or more abnormalities. *Conclusions*. The role of thrombophilia in the long-term management of VTE should be addressed in prospective clinical outcome studies.

C134

SEVERE THROMBOPHILIA-ASSOCIATED RISK FOR RECURRENT VENOUS THROMBOEMBOLISM

De Stefano V, Martinelli I, Legnani C, Rossi E, Grandone E, Castaman G, De Stefano V, Palareti G, Mannucci PM, Leone G

On behalf of the GIRTE (Gruppo Italiano per la Ricerca sulla Trombofilia Ereditaria)

In the frame of an Italian survey we collected the clinical data of 1322 index patients referred to specialized Thrombosis Centers for a history of objectively proven deep venous thrombosis (DVT) ; all patients were recruited according to the absence of an overt neoplasia, congenital deficiency of natural coagulation inhibitors, isolated heterozygosity for factor V Leiden (FV-GA) or prothrombin G20210A (PT-GA). A further exclusion criterion consisted of a time interval from the first DVT shorter than 1 year and/or a period of oral anticoagulation after the first DVT longer than 6 months, so that 843 patients were considered eligible to estimate the risk for recurrent venous thromboembolism (VTE): 741 had a normal genotype (FV-GG/PT-GG), 67 carried both FV-GA and PT-GA, 35 were homozygous for factor V Leiden (FV-AA), in 5 cases also carried PT-GA. The groups did not differ in sex distribution or in the rate of unprovoked first DVT; the observation time from the first DVT was longer among the 30 FV-AA/PT-GG patients (p= 0.034) (see Table below). The relative risk of recurrent VTE was calculated with the use of a proportional-hazards model. The hazard ratio for recurrent VTE in comparison with the patients with normal genotype was 1.7 (95% CI 1.0-2.8) among homozygous patients and 2.2 (95% CI 1.6-3.0) among double heterozygotes. In the case of unprovoked first DVT the risk for recurrent VTE was 2.0-fold increased (95% CI 1.0-4.2) among homozygotes and 3.1-fold increased (95% CI 1.9-5.1) among double heterozygotes; in the case of provoked first DVT the risk remained significantly higher only among double heterozygotes (1.8, 95% CI 1.1-2.8). In conclusion, double heterozygotes are at higher risk of recurrence after a first DVT. The increase in risk of recurrence among homozygotes was only of borderline significance in spite of a longer observation time; accordingly, it is doubtful if such patients should be treated after a first DVT differently than patients with a normal genotype.

	FV-AA + PT-GG	FV-GA + PT-GA	FV-GG + PT -GG
Sex (M/F)	17 / 13	31 / 36	332 / 409
Age at first thrombosis (median, range)	26 (16 – 70)	32 (6 – 74)	42 (6 – 86)
Interval from the first VTE (median, range – yr)	4.5 (1 – 27)	3 (1 – 40)	2 (1 – 43)
Unprovoked first DVT	12 (40%)	26 (39%)	317 (43%)
Recurrent VTE	15 (50%)	41 (61%)	181 (24%)

C135

HIGH PLASMA LEVELS OF FACTOR VIII AND RISK OF RECURRENCE OF VENOUS THROMBOEMBOLISM

Legnani C, Palareti G, Cosmi B, Cini M, Abate C, Fariselli S, Frascaro M, Poggi M, Coccheri S

Unità di Ricerca Clinica sulla Trombofilia "Marino Golinelli" – Dipartimento Cardiovascolare, UO Angiologia, Azienda Ospedaliera di Bologna, Policlinico S.Orsola-Malpighi, Bologna, Italy

Elevated factor VIII (FVIII) levels are reported as risk factor for venous thromboembolism (VTE) and for recurrence in patients with a first idiopathic VTE. We examined 529 patients, with a previous first VTE event, for a mean follow-up period of 18 months after oral anticoagulant (OC) withdrawal. Patients who had cancer (n=48) as well as patients in which a thrombophilic alteration was diagnosed were excluded (n=114; ATIII deficiency n=1, PC deficiency n=8; PS deficiency n=3; FV Leiden n=59; G20210A prothrombin n=32; LAC n=6, combined alterations n=5). The end point was an objectively documented, symptomatic recurrent VTE (DVT and/or PE). FVIII activity was measured 3-4 weeks after OC was stopped, by a chromogenic assay (Chromogenix). Among the 367 patients included in the study, 176 and 191 had a first idiopathic or secondary VTE event, respectively. FVIII levels were significantly higher in patients with idiopathic than secondary VTE (1.68±0.42 vs 1.43±0.45 IU/mL, p=0.0000). Recurrent VTE developed in 10/176 (5.7%) and in 7/191 (3.7%) patients with idiopathic or secondary VTE, respectively. Among patients with idiopathic VTE, the cumulative incidence of recurrences was 13.7% in those with FVIII level above the 90th percentile (FVIII >2.18 IU/mL), as compared with 6.2% in those with lower levels (Hazard ratio: 2.74; 95% CI: 0.42-43.2). Hazard ratio for recurrence in patients with secondary VTE and FVIII above the 90th percentile (FVIII >1.95 IU/mL) vs those with lower levels was 4.32 (95% CI: 0.94-191.7). The cumulative incidence of recurrence was 12.3% and 3.2% in cases with FVIII levels above or below the 90th percentile, respectively. The hazard ratio was 3.43 (95% CI: 1.34-42.8; p<0.0218) when all the 367 patients were considered. The risk of VTE recurrence is significantly higher in patients with high FVIII levels; no significant difference was present among patients whose first VTE was idiopathic or secondary.

C136

INCREASED LIPOPROTEIN (A) LEVELS AS AN INDEPENDENT RISK FACTOR FOR IDIOPATHIC AND RECURRENT DEEP VENOUS THROMBOSIS

Marcucci R, Alessandrello Liotta A, Fedi S, Rogolino A, Cellai AP, Pepe G, Gori AM, Attanasio M, Prisco D, Abbate R

Dipartimento Area Critica Medico Chirurgica, Università di Firenze; Centro Trombosi, Azienda Ospedaliera Careggi, Florence, Italy

Elevated lipoprotein (a) (Lp(a)) plasma levels are an established risk factor for arterial thrombotic disease. At variance, contrasting and scarce data are available on the role of increased Lp(a) plasma levels in venous thromboembolism (VTE). We studied 603 (209 M/394 F) consecutive unselected patients, with a history of VTE referred to our Thrombosis Center from January 2000 to January 2001, six months to one year after the acute event. Exclusion criteria were a history of arterial thromboembolism and the presence of a cancer. The control population was 300 healthy subjects (100 M/200 F) recruited from partners or friends of the patients. We determined on patients and controls: plasma levels of Lp(a); antithrombin, protein C and protein S; activated protein C resistance; FV Leiden; polymorphism G20210A of the prothrombin; fasting Hcy levels; antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibodies). Lp(a) levels were significantly higher in patients than in controls (124 (1-1497) mg/L vs 102 (9-695) mg/L; p<.05). Lp(a) levels above 300 mg/L were detected in 146/603 (24.2%) patients and in 41/300 (31.6%) controls (p<.005). At the multivariate analysis (adjusted for all acquired and hemostasis-related risk factors) the role of elevated Lp(a) levels as an independent risk factor for VTE was demonstrated (OR=2.1 (1.3-3.4); p<.001). In 368/603 (61%) patients no circumstantial risk factor was present: among these patients the role of elevated Lp(a) levels as independent risk factor was confirmed (OR=3.1 (1.3-3.4); p<.001) whereas in 235/603 (39%) patients with a secondary episode of VTE we were not able to document a role of elevated Lp(a) levels. Overall 136/603 (22.5%) patients had a recurrent VTE. Independent risk factors for recurrent VTE were: Lp(a) levels >300 mg/L (OR=5.1 (3.1-8.4); p<.001), hyperhomocysteinemia (OR=5.0 (3.0-8.4); p<.001) and the presence of both FV Leiden and FII polymorphisms (OR=3.7 (1.6-8.4); p<.001). These results suggest the possible utility of including Lp(a) determination in the evaluation of patients with a history of venous thrombosis and in particular in patients with idiopathic and recurrent VTE.

C137

D-DIMER TEST PERFORMED AFTER ORAL ANTICOAGULATION IS STOPPED HAS A HIGH NEGATIVE PREDICTIVE VALUE FOR RECURRENCE IN PATIENTS WITH THROMBOPHILIC ALTERATIONS AND PREVIOUS VENOUS THROMBOEMBOLISM

Palareti G, Legnani C, Cosmi B, Fortunato G, Lunghi B,* Bernardi F,* Bettini F, Coccheri S

*Unità di Ricerca Clinica sulla Trombofilia "Marino Golinelli" Dip. Cardiovascolare, U.O. Angiologia, Azienda Ospedaliera di Bologna, Policlinico S.Orsola-Malpighi, Bologna; and * Dipartimento di Biochimica e Biologia Molecolare, Università di Ferrara, Italy*

In a recent study we showed that a normal D-dimer test (VIDAS, bioMerieux) measured 3 months after OAT discontinuation in patients with a previous venous thromboembolism (VTE) has a very high negative predictive value (NPV) for recurrence, whereas the presence of increased D-dimer levels was associated with a significantly higher hazard ratio for VTE recurrence. The aim of the present study was to evaluate the predictive value of D-dimer for recurrence after OAT withdrawal in carriers of a thrombophilic alteration. Five-hundred and sixty patients (283 males) were screened for thrombophilic alterations (TA), including factor V Leiden (FVL) and prothrombin mutation (PM), antithrombin III, protein C and protein S deficiency, and presence of lupus anticoagulant phenomenon (LAC), after a first VTE episode. The presence of one (126 cases) or more (7) TA was detected in 133 (23.7%), FVL (76; 3 of whom homozygotes) and PM (43) being the two most prevalent. All patients were prospectively investigated and 52 VTE recurrences (9.3% of patients; 6.7% patient-years of follow-up) occurred during the 771.4 y follow up. D-dimer was measured on the day of OAT withdrawal and after 3-4 weeks and 3 months. Increased D-dimer at 3

months (or last available before a recurrence) was observed in 67/133 (50.4%) and in 198/427 (46.4%; n.s.) in patients with or without TA, respectively. Recurrences were 17 and 1 in carriers of thrombophilia with or without altered D-dimer, respectively. The hazard ratio for recurrence was significantly higher in subjects with abnormal versus normal D-dimer (21.2; 95% CI: 3.03-19.6; $p<0.0001$). The sensitivity, specificity and negative predictive values of D-dimer for VTE recurrence in subjects with thrombophilia were 94.4%, 56.5% and 98.5%, respectively. D-dimer assay performed after OAT withdrawal in thrombophilic subjects with a previous VTE has a very high NPV for recurrence. Specifically designed clinical studies are needed to assess whether the test can be used to optimize duration of anticoagulation in these patients.

C138

RESIDUAL VEIN THROMBOSIS AS A PREDICTIVE FACTOR OF RECURRENT VENOUS THROMBOEMBOLISM. A PROSPECTIVE COHORT STUDY

Prandoni P, Lensing AW, Prins MH, Simioni P, Bernardi E, Tormene D, Bagatella P, Frulla M, Mosena L, Marchiori A, Girolami A

Department of Medical and Surgical Sciences, 2nd Chair of Internal Medicine, University of Padua, Italy; Academic Medical Center, University of Amsterdam, The Netherlands

Among factors associated with an increased risk for recurrent venous thromboembolism (VTE) in patients with deep-vein thrombosis (DVT) of the lower extremity the persistence of vein thrombosis, as shown by repeated ultrasonography over time, has been advocated. To estimate the risk for recurrent VTE in patients with and without residual vein thrombosis, repeat ultrasound was performed over time in 313 consecutive patients with proximal DVT who were followed prospectively for up to six years after a 3 to 6-month period of anticoagulation. One hundred and twenty-four patients (39.6%) had an idiopathic DVT, 109 (35.2%) had a thrombosis associated with transient risk factors, and 80 (25.6%) were carriers of thrombophilic defects. Venous ultrasonography showed a full vein recanalization at three months in 61 (19.5%) patients. The cumulative incidence of normalized ultrasonography was 38.8% at 6 months, 58.1% at one year, 69.3% at two years, and 73.8% at three years. Of the 313 patients, 58 experienced a recurrent VTE, of whom 41 occurred while the patient still had residual thrombosis. Using a multivariate stepwise Cox proportional hazards model with persistent residual thrombosis as a time-dependent variable, the HR for a recurrent event was 2.4 (95% CI, 1.3 to 4.4) for persistent residual thrombosis, 2.5 (95% CI, 1.4 to 4.4) for idiopathic thrombosis, and 3.1 (95% CI, 1.8 to 5.2) for thrombophilia. In conclusion, persistent venous obstruction is a powerful and independent risk factor for recurrent VTE in patients with venous thrombosis.

Oral Communications
Risk Factors for Atherothrombosis

C139

RESISTANCE TO ACTIVATED PROTEIN C IS RELATED TO AN INCREASED INTIMA-MEDIA THICKNESS IN THE GENERAL POPULATION

Tosetto A,* Baracchini C,* Manara R,* Prati P,° Simioni M,* Dall'Oste C,* Rodeghiero F*

**Department of Hematology, S. Bortolo Hospital, Vicenza; °Department of Neurology, Gervasutta Hospital, Udine, Italy*

Background. There are conflicting reports on the relationship between resistance to activated protein C and development of atherosclerosis and arterial thrombosis. *Aim of the study*. To evaluate if resistance to activated protein C may be associated with increased intima-media thickness (IMT), a marker of pre-clinical atherosclerosis, in the general population. *Subjects and Methods*. We evaluated 2373 subjects (1304 females, 1069 males) previously enrolled in the VITA Project, with a median age of 54 years. In all subjects, IMT was measured in common carotid arteries using B-mode ultrasonography and digital acquisition/measurement software (M'ath, Metris, France). Blood sampling, plasma preparation and storage and measurement of APC resistance (as APC-SR) was performed using previously published methods (*Rodeghiero and Tosetto, Ann Intern Med, 1999*). A phenotypic resistance to APC was considered to be present when a subject had an APC-SR below the highest APC-SR of FV Leiden carriers (APC-SR 0.84). Logistic regression was used to model for the individual probability of having an IMT above the age-adjusted reference limit. *Results*. In a multivariate analysis that accounted for the effect of gender, cholesterol and smoking, subjects with an APC-SR below 0.84 confirmed in two VITA visits had a 2.48-fold increased probability of having an IMT above the upper limit (95% CI 1.25-4.90). Subjects with an APC-SR below 0.84 in only one VITA visit had a marginally increased probability (OR=1.12, 95% CI 0.63-1.97). Carriers of the FV Leiden mutation had a 2.5-fold increased probability (95% CI 1.05-6.15). After exclusion of FV Leiden carriers, there was a slight decrease of the association observed in those with a persistent APC-resistance phenotype (OR=2.1, 95% CI 0.9-5.2). *Conclusions*. A persistent APC resistance phenotype is associated with pre-clinical atherosclerosis. The effect is partly, but not completely, dependent on the presence of FV Leiden.

C140

CLINICAL DETERMINANTS OF INTIMA-MEDIA THICKNESS IN THE GENERAL POPULATION

Tosetto A,* Baracchini C,* Manara R,* Prati P, Rodeghiero F*

**Department of Hematology, S. Bortolo Hospital, Vicenza; °Department of Neurology, Gervasutta Hospital, Udine, Italy*

Background. Intima-media thickness (IMT) has been proposed as a quantitative index of atherosclerosis and as a surrogate end-point for pre-clinical lesions. *Aim of the study*. To evaluate

the determinants of IMT of a population at low risk of cardiovascular disease and establish a reference range. *Subjects and Methods*. From January 2000 to January 2002, we evaluated 2373 subjects (1304 females, 1069 males) previously enrolled in the VITA Project. The median age was 54 years (range 44-71). In all subjects, common carotid arteries (CCA), bifurcation, internal and external carotid arteries were investigated bilaterally using B-mode ultrasonography and digital acquisition/measurement software (M'ath, Metris, France). IMT measurements were obtained from CCA images with at least 10 mm of continuous boundary between intima-media and media-adventitia clearly distinguishable. Anthropometric and laboratory measurements were obtained as previously described (*Rodeghiero and Tosetto, Thromb Haemost 1993*). *Results*. The median IMT was 0.66 mm. There was an evident age-related increase of IMT, that resulted in significantly different reference ranges for age (upper 97.5 percentile and 90% CI: age 45-50: 0.76 (0.75-0.79); age 50-55: 0.82 (0.81-0.84); age 55-60: 0.88 (0.86-0.91); age >60: 0.89 (0.86-1.00). When considering the probability of having an IMT above the age adjusted reference limit, male gender, cholesterol above 200 mg%, HDL cholesterol below 50 mg%, smoking and a previous history of arterial disease were all strongly and independently associated with increased IMT. *Conclusions*. No major differences are apparent in median IMT thickness between our study and those reported on populations at higher cardiovascular risk. IMT is strongly related to major cardiovascular risk factors.

C141

RELEVANCE OF POST-METHIONINE HOMOCYSTEINE AND LIPOPROTEIN(A) DETERMINATION IN THE EVALUATION OF THE CARDIOVASCULAR RISK PROFILE IN YOUNG CORONARY ARTERY DISEASE PATIENTS

Marcucci R, Giusti B, Brunelli T, Attanasio M, Coppo M, Margheri M, Giglioli C, Falai M, Valente S, Gensini GF

Dipartimento Area Critica Medico-Chirurgica, Università di Firenze; Centro Trombosi, Azienda Ospedaliera Careggi, Florence, Italy

Aims. The aims of the study were: to evaluate the prevalence of high lipoprotein (a) (Lp(a)) and homocysteine (Hcy) levels - both in the fasting state and post-methionine- in a group of young CAD patients and to investigate the role of genetic and environmental factors for hyperhomocysteinemia. High Hcy and Lp(a) levels have been recognized as emerging cardiovascular risk factors but no study has investigated both Lp(a) and Hcy - fasting and post-methionine loading- in young patients. *Methods and Results.* We studied 142 patients with angiographically documented CAD (24 women ≤55 yrs and 118 men ≤50 years) and 140 healthy subjects as controls. Hyperhomocysteinemia was diagnosed in 118/140 patients (84.2%). Lp(a) levels were significantly higher in patients than in controls (200 (3-1486) mg/L vs 97 (10-412) mg/L). At the multivariate analysis, adjusted for the classical risk factors, the OR (95% CI) for CAD at young age significantly increased in the fourth quartile of the distribution of Hcy and Lp(a) levels (fasting Hcy: 15.6 (4.2-57.3)μmol/L, $p<0.0001$; post-methionine Hcy: 19.8 (4.4-89.1) μmol/L; $p<0.0001$; Lp(a): 20 (4.9-81): $p<0.0001$). Vitamin deficiencies was detected in 28/140 patients. The prevalence of the homozygous C677T ($^{+/+}$) methylenetetrahydrofolatereductase genotype was higher, but not significantly different, in patients (22.8%) than in controls (18.6%). The allele frequency of the 844ins68 insertion variant in the cystathionine β-synthase gene was 0.08 in the control group and 0.06 in patients. *Conclusions*: The results of the present study indicate the usefulness of including Hcy-both in the fasting state and post-methionine- and Lp(a) determination in the diagnostic panels of young CAD patients, in order to obtain a better assessment of their cardiovascular risk profile.

C142

DETERMINANTS OF CAROTID INTIMAL-MEDIAL THICKNESS IN A SOUTHERN-ITALY POPULATION OF PATIENTS WITH HYPERTENSION

Trotta R,*° Trimarco B,° De Luca N,° Rosiello G,° Iovino G,° Izzo R,° Di Castelnuovo A,* Donati MB,* Iacoviello L*

**"A. Valenti" Laboratory of Genetic and Environmental Risk factors for Arterial Thrombosis, Consorzio Mario Negri Sud, S. Maria Imbaro; °Dipartimento di Medicina Clinica Scienze Cardiovascolari ed Immunologiche, Università di Napoli Federico II", Italy*

Higher carotid intima-media thickness (IMT) is considered a marker of the total individual burden of arteriosclerosis, and is associated with a higher cardiovascular risk. The aim of the present study was to investigate the determinants of maximal IMT of carotid arteries in a population of out-patient hypertensive patients referred to our Hypertension Center. A cohort of 492 consecutive hypertensive patients (mean age 53.8±10.4 (SD) years, 296 males and 196 females) was studied. Systolic blood pressure (SBP) ranged between 227-105 mmHg and diastolic blood pressure (DBP) ranged between 144-60 mmHg. IMT was evaluated with a high-resolution echo-Doppler technique in the common carotid, in the carotid bifurcation, in the internal carotid artery. Left ventricular mass was measured according to the Penn Convention, by echocardiography. Furthermore, data on family history of hypertension, body mass index, diabetic state, smoking history, antihypertensive therapy, fundus oculi, blood pressure, total serum cholesterol, and triglycerides were collected. Subjects were stratified in three subgroups, according the increase of IMT (≤1 mm, 22.1%; >1 mm and ≤1.3 mm, 23.4%; >1.3 mm, 54.5%). In univariate analysis the increase in age, systolic blood pressure, cholesterol and triglyceride levels, diabetes, smoker status, antihypertensive therapy ($p=0.007$), and left ventricular hypertrophy were significantly associated with increased IMT. In multivariate analyses, however, only age (OR=11.5, CI95% 5.0-26.2), high systolic (OR=2.2, CI95% 1.03-4.6) and diastolic blood pressure (OR=3.4, CI95% 1.02-10.9) and smoker status (OR=3.4, CI95% 1.6-7.2) were significantly associated with high IMT (upper versus lower tertile). This study shows that in uncontrolled hypertensive patients age, smoking and high blood pressure are the major risk factors for increased IMT.

C143

LIPID AND PROTEIN OXIDATION CONTRIBUTE TO A PROTHROMBOTIC STATE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

De Cristofaro R,* Rocca B,* Vitacolonna E,° Marchesani P,° Ciabattoni G,° Landolfi R,* Patrono C,° Davì G

Center of Excellence on Aging and Departments of Medicine & Aging and Drug Sciences, "G. D'Annunzio" University School of Medicine and Pharmacy, Chieti, and Hemostasis Research Center, Department of Internal Medicine, Catholic University School of Medicine, Rome, Italy

Diabetes mellitus (DM) is associated with enhanced *in vivo* lipid peroxidation and persistent platelet activation. We tested the hypothesis that oxidant stress could affect circulating proteins and that it may be associated with coagulative dysfunction. Urine samples were obtained from 72 type 2 diabetes mellitus (T2DM) patients and 72 age- and gender-matched healthy subjects for measurement of immunoreactive 8-iso-prostaglandin (PG) F2α and 11-dehydro-thromboxane B2 (TXM), as *in vivo* indices of lipid peroxidation and platelet activation, respectively. Plasma samples were obtained from the same subjects for measurement of both procoagulant markers such as prothrombin fragment F1+2, and fibrinopeptide A, and anticoagulant markers, such as protein C zymogen, protein C activation peptide (PCP), activated protein C (APC) and soluble thrombomodulin (TM). The carbonyl content of plasma proteins was measured as a global index of protein oxidation. Urinary 8-iso-PGF2α excretion and plasma protein carbonyl groups were linearly correlated and both significantly higher in patients than in controls (323±179 vs. 208±92 pg/mg creatinine and 6.1±1.4 $\times 10^{-6}$ vs 4.6±1$\times 10^{-6}$ w:w, respectively). F1+2 levels were significantly higher in diabetics than in controls: 1.7±0.8 vs 1.1±0.8 nmol/L. By contrast, APC, PCP, and soluble TM levels were significantly lower in T2DM than in controls. In a multiple regression analysis only age (p=0.008) and 8-iso-PGF2α excretion (p=0.0013) were positively associated with F1+2 levels. We conclude that type 2 diabetes mellitus is associated with both lipid peroxidation and protein oxidation linked to coagulative dysfunction. Enhanced thrombin generation and a depressed anticoagulant TM/PC pathway, in concert with persistent platelet activation, may contribute to atherothrombosis in this setting. These results provide a rationale for a more aggressive antithrombotic strategy in diabetes mellitus.

C144

HEMOSTATIC EVALUATION OF CARDIOVASCULAR RISK IN HIV-INFECTED PATIENTS WITH DIFFERENT ANTIRETROVIRAL TREATMENTS

Testa S,* Pan A,° Quiros E,* Bassi L,* Carosi G,° Carnevale G,* Denti N,* Ferrari L,* Alatri A,* Maserati R,# Morstabilini G,* Seminari E,# Tinelli C,# Voltini G,° for the Master Study Group

**Istituti Ospitalieri di Cremona; °Università degli Studi, Brescia, #IRCCS Policlinico S. Matteo, Pavia, Italy*

Background. An increased risk of cardiovascular disease has been associated with the use of highly active antiretreoviral therapy, possibly due to the alteration in lipid and glucose metabolism, but also to HIV itself. The objective of this study was to evaluate the hemostatic parameters of cardiovascular risk in patients treated with different antiretroviral regimens. *Methods.* We evaluated the hemostatic markers of cardiovascular risk in HIV-positive patients: fibrinogen-FBG, FVIII, von Willebrand factor-VWF, F1+2, D-dimer-DD, as well as a marker of endothelial activation and integrity: thrombomodulin-TM. Thirty-seven consecutive HIV+ patients treated with PI containing regimens, age 40 or less, were compared with 15 patients treated with NNRTI based regimens, 25 patients with double NRTI regimen, 50 HIV+ patients naïve to antiretroviral therapy and with 45 HIV negative subjects. The patients in the treatment arms had been treated for at least 18 months. *Results.* The 5 groups were comparable for generic factors of cardiovascular disease (smoking, alcohol use, blood pressure). An increase in F1+2 was seen in all HIV$^+$ patients (p<0.0001), but increased DD was noted only in the PI treated group (p<0.01). FVIII was slightly increased in both PI and NNRTI pts. VWF was elevated in all HIV$^+$ groups, while TM was significantly elevated (p<0.01), particularly in the PI pts.

	HIV + PI	HIV+ NNRTI	HIV+ 2 NRTI	HIV+ Naive	HIV-
F1+2	4.67±9.08	6.03±1.88	4.4±2.92	3.7±3.36	0.32±0.1
DD	0.83±0.14	0.31±0.07	0.4±0.34	0.37±0.19	0.27±0.1
TM	55.2±52.7	27.1±4.9	30.6±12.8	38.9±17.1	27.2±8.3

Conclusions. Our data show a hemostatic activation in both treated and untreated HIV patients, particularly in the PI treated group. Increased levels of TM were also seen in the PI patients. Compared with NNRTI and double NRTI regimens, PI-based regimens seem to cause both endothelial perturbation and prothrombin activation, which could lead to an increased risk of major cardiovascular events.

Oral Communications
Platelet Disorders and Deficiencies

C145

CHARACTERIZATION OF DYSFUNCTIONAL P2Y12 RECEPTOR IN A PATIENT WITH CONGENITAL BLEEDING

Cattaneo M,*° Zighetti ML,* Lombardi R,* Martinez C,° Lecchi A,* Conley PB,# Ware J,° Ruggeri ZM°

**Dept Intern Med, Univ of Milano, Italy; °Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA; #COR Therapeutics Inc, South San Francisco, CA, USA*

The combined action of the two platelet ADP receptors, P2Y1 (coupled to Gq and PLCb) and P2Y12 (negatively coupled to adenylyl cyclase through Gi), is necessary for the full platelet aggregation response to ADP. Four patients with bleeding disorders associated with severe deficiency of P2Y12 have been described so far. Here, we describe a patient (AC) with a congenital bleeding disorder and a dysfunctional P2Y12. ADP, induced normal shape change and a slight and rapidly reversible aggregation of AC's platelets. The inhibition of PGE1-stimulated adenylyl cyclase by ADP in AC's platelets was severely impaired, while that by epinephrine was normal. However, the number of binding sites and affinity for [^{33}P]-2MeSADP was normal in AC's platelets, suggesting the presence of a functionally abnormal P2Y12 receptor. Therefore, we aimed our studies at the characterization of the gene encoding for P2Y12. We found AC to be a compound heterozygote, with one allele containing a G to A transition resulting in an Arg256 to Gln codon substitution (R256Q) and the other allele containing a C to T transition resulting in an Arg265 to Trp codon substitution (R265W). The two substitutions are located in TM6 and EL3 of the receptor. Stable CHO cell lines were established expressing either P2Y12WT, P2Y12R256Q or P2Y12R265W. Neither mutation blocked the ability of the P2Y12 receptor to translocate to the CHO cell surface. ADP dramatically inhibited the forskolin-induced increase of cAMP in CHO cells transfected with P2Y12WT, while it was only partially inhibitory in CHO cells transfected with either mutant protein. Thus, the molecular basis for AC's dysfunctional platelet phenotype is explained by missense mutations and the expression of a dysfunctional P2Y12 receptor. The localization of both mutations in TM6 and EL3, identifies this region of P2Y12 as a structurally and functionally critical region of the receptor.

C146

AN INHERITED THROMBOCYTOPENIA WITH DEFECTIVE PLATELET-COLLAGEN INTERACTION AND REDUCED GPIA-IIA EXPRESSION

Ceresa I, Noris P, Torti M,* Di Pumpo M, Gamba A, Canobbio I,* Balduini CL

*Department of Internal Medicine and *Department of Biochemistry, University of Pavia, IRCCS, San Matteo, Pavia, Italy*

A 65-year old thrombocytopenic woman - previously diagnosed as suffering from ITP and treated with a short course of prednisone without benefit - was referred to us because of persistent thrombocytopenia (77×10^9 plt/L at light microscopy in a Bürker counting chamber). On arrival the patient presented a cutaneous-mucosal bleeding tendency with an otherwise unremarkable physical examination (no splenomegaly). Bleeding time measured with a modified Ivy's technique was >30'; a blood smear revealed slight platelet macrocytosis (20% of platelets with a diameter larger than 4μ). *In vitro* platelet aggregation induced by collagen (4 and 20 μg/mL) was reduced. Routine flow cytometry showed a severe reduction of platelet GpIa-IIa content (32% of control). Similar clinical and laboratory features were also observed in the patient's daughter. It has been recently shown that the amount of GpIa-IIa expressed in platelets depends on three polymorphisms of the α-2 gene defining three alleles: allele 1 (807T-873A/837T/Br(e)b) associated with increased levels of GpIa-IIa; allele 2 (807C-873G/837T/Br(e)b) associated with lower levels; allele 3 (807C-873G/837C/Br(e)a) associated with intermediate levels. On this basis, we genotyped these polymorphisms in the patient and in her daughter, and both of them had alleles 1/2. For subsequent analyses, a control donor with the same genotype was used. Also when compared to this genotype-matched control, the GpIa-IIa content of the patient and her daughter was severely reduced both when expressed as absolute fluorescence value or GpIa-IIa/GpIIb-IIIa ratio (39% of control). The GpIa-IIa defect detected by flow cytometry was confirmed by SDS-PAGE and immunoblotting. The rate of platelet adhesion to collagen evaluated in a static system was also decreased (30% of control). We conclude that our patients were affected by a new inherited thrombocytopenia mainly definied by severe deficiency of GpIa-IIa and defective platelet-collagen interaction.

C147

GLANZMANN'S THROMBASTHENIA ITALIAN TEAM (GLATIT): A MULTICENTER ITALIAN STUDY FOR THE IDENTIFICATION OF CAUSAL MUTATIONS

D'Andrea G, Colaizzo D, Vecchione G, Grandone E, Di Minno G, Margaglione M,* on behalf Of The Glanzmann's Thrombasthenia Italian Team (GLATIT)

*Unita' di Aterosclerosi e Trombosi, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo; *Genetica Medica, Università di Foggia, Italy*

Glanzmann's thromboasthenia (GT) is a genetically heterogeneous autosomal recessive syndrome associated with a bleeding tendency. To elucidate the molecular basis of GT we have screened 30 GT patients for mutations. On the whole, 21 different candidate causal mutations, 17 in the αIIβ and 4 in the β3 gene have been found. Only two (αIIβ Pro145Ala and T4003del[13bp]) have been previously reported. Nine mutations (42.9%) were likely to produce truncated transcripts, whereas the remaining 12 were missense mutations that affected highly conserved residues in αIIβ and β3 genes. Six mutations were found in different patients suggesting a possible founder effect. In 5 GT patients no possible causative point mutation was found. The wide spectrum of expressivity, ranging from mild to severe also among patients carrying the same mutations, provided evidence for a role of different loci or circumstantial factors. In conclusion, we have identified a spectrum of unreported mutations that may be of value for unraveling the role of specific regions of αIIβ and β3 genes.

C148

A NEW NOSOGRAPHIC ENTITY: MYH9-RELATED DISORDERS

Pecci A,* Savoia A, Seri M,° Gresele P,* Mazzarino I, Noris P,* Ghiggeri G, Benazzo M,** Manfrin M,** Di Pumpo M,* Ceresa I,* Balduini CL*

**Depts. of Internal Medicine and **Otorhinolaryngology, IRCCS Policlinico San Matteo and University of Pavia ; ^Telethon Institute of Genetics and Medicine (TIGEM), Napoli; °Laboratory of Molecular Genetics and °°Nephrology, IRCCS G Gaslini, Genova , *Dept. of Internal Medicine, University of Perugia and §Pediatric Unit, SiIvestrini Hospital, Perugia, Italy*

May-Hegglin anomaly (MHA) is an inherited disorder characterized by thrombocytopenia, giant platelets and Dohle-like inclusions in leukocytes. This triad is present also in Fechtner's syndrome (FTNS), which presents the additional findings of nephritis, sensorineural hearing loss and cataracts. Although MHA and FTNS have been considered as different entities, it has been recently shown that both of them derive from heterozygous mutations in MYH9, the gene coding for the heavy chain of nonmuscle myosin IIA (NMMHC-IIA). Several different mutations of MYH9 have been reported in these disorders, but no genotype/phenotype correlation has been identified yet. To better define these disorders, we re-examined carefully 8 patients from 6 families with MHA and 10 patients from a single family with FTNS. Five MYH9 mutations have been identified in 5 MHA families (N93K, R702C, R1165C, E1842K, E1945X). The sixth family is actually under investigation. The D1424H mutation was detected in the FTNS family. All MHA and FTNS subjects had macrothrombocytopenia and leukocyte inclusions. In MHA, audiometry and auditory brainstem evoked responses identified sensorineural hearing loss in all adults and in one of two pediatric patients, while ophthalmoscopy identified cataracts in one child and one adult. Seven of 10 FTNS subjects had hearing loss and/or cataracts. Urinalysis revealed proteinuria and/or microhematuria in 6 MHA subjects and 5 FTNS patients. Chronic renal failure was observed in 2 FTNS cases. Immunocytochemical studies with a mAb against NMMHC-IIA identified a similar abnormality of myosin distribution in leukocytes and platelets from both MHA and FTNS patients: platelet myosin was clustered in few spots instead of being uniformely distributed, and leukocyte myosin was clustered within Döhle-like bodies. Based on these results, we suggest that MHA and FTNS are different names applied to the same illness that, besides platelet and leukocyte abnormalities, may present a variable spectrum of hearing, ocular and renal involvement.

C149

GENETIC APPROACH TO THE STUDY OF AUTOSOMAL DOMINANT THROMBOCYTOPENIA

Savoia A,* Balduini CL,° di Bari F,* Savino M,# Noris P,° Di Pumpo M,° Perotta S,@ Servedio V,^ Iolascon A^

**Telethon Institute of Genetics and Medicine, Naples; °Department of Internal Medicine, IRCCS San Matteo, University of Pavia; #Medical Genetics Service, IRCCS Hospital CSS, San Giovanni Rotondo, Foggia; @Department of Pediatrics, II University of Naples; ^Department of Pediatrics, University of Foggia, Italy*

The molecular defect of inherited thrombocytopenias, which are clinically and genetically heterogeneous disorders, is often unknown. To study these forms, we have been collecting families in the attempt to identify the genes by positional cloning. We report here the results obtained in two poorly defined autosomal dominant disorders: Mediterranean macrothrombocytopenia, the most frequent inherited thrombocytopenia in our country, and an undefined form of thrombocytopenia with normal platelet volume. Linkage analysis in two large families affected by Mediterranean macrothrombocytopenia localized the gene to chromosome 17p, in an interval containing the GPIbα gene, which is altered in Bernard-Soulier syndrome (BSS). A heterozygous Ala156Val missense substitution (Bolzano variant) was identified in all patients of the two families and in another eight additional macrothrombocytopenic pedigrees. Since all patients with Bolzano mutation had reduced amount of platelet GPIb/IX/V complex, we concluded that they were affected by heterozygous BSS. Platelet membrane GP studies were also performed on families characterized by macrothrombocytopenia without the Bolzano variant. The analysis distinguished two groups: 1) patients with the GPIb/IX/V complex normally distributed on the surface of their platelets. We called this form *true* Mediterranean macrotrombocytopenia; 2) patients with a reduction of GPs comparable to that found in the BSS heterozygotes. Screening for mutations of all the GPIb/IX/V genes in patients of the second group excluded the presence of mutations. These results suggest that there is at least another gene, not assembled in the complex, responsible for the BSS heterozygous phenotype. We have also studied seventeen individuals from a large Italian family affected by autosomal dominant thrombocytopenia with normal MPV. The family was large enough to map the corresponding gene, THC2, on chromosome 10p12.1. We found that genetic heterogeneity characterizes also thrombocytopenia with normal MPV. In fact, there are families unlinked to 10p12.1, suggesting that at least another defective gene affects normal platelet production.

C150

LONG-TERM FOLLOW-UP OF PATIENTS AFFECTED BY ESSENTIAL THROMBOCYTHEMIA TREATED WITH ANAGRELIDE

Mazzucconi MG,* Redi R,* Bernasconi S,* Bizzoni L,* Latagliata R,* Santoro C,* Mandelli F*

**Ematologia, Dipartimento Biotecnologie Cellulari ed Ematologia, Università "La Sapienza", Rome, Italy*

We report our experience of long-term follow-up of essential thrombocytopenia (ET) patients treated with anagrelide (imidazoquinazoline compound which lowers platelet number). Forty patients were enrolled between 1989-1996 (M 17; F 23; median age 33 yrs; 25 previously untreated;19 with symptoms related to ET). Therapy schedule was as follows: 0.5 mg every 12 hours for 7 days; subsequently the daily dose was increased by 0.5 mg/day every week until response was obtained [decrease of platelet count $<500\times10^9$/L: complete response (C.R.); $<600\times10^9$/L: partial response (PR)], lasting over 1 month. Thirty-five of 40 patients were evaluable for response [5 stopped therapy in less than 1 month: for personal choice (3), for side-effects (2)]. Response was observed in 33/35 patients (94.28%). [CR: 20/35 (57.14%); PR: 13/35 (37.14%)]. Response was reached in a median time of 120 days (7–450) with a mean daily dose of 2 mg (1-3). Maintenance therapy was given in all responders (mean daily dose: 1.89 mg (1–3.5). Side-effects (mainly tachycardia, anemia, gastric distress) were recorded in 17/40 patients (42.50%). (total episodes: 28, 14 during initial therapy course, 14 during maintenance), thus, treatment was discontinued in 2 patients during the initial therapy, in 6 during maintenance. Adverse events (myocardial infarction, myocardial ischemia, TIA) were recorded in 4 patients during maintenance, therefore, in 3, treatment was discontinued. No hemorrhagic events, no evolution into leukemia occurred. Four patients chose to discontinue therapy during maintenance. At present, 20 responders are on maintenance [median follow-up: 97 months (50-136), mean platelet count: 506×10^9/L (275-774), mean daily maintenance dose: 1.78 mg (0.5-3.5)]. The response rate to therapy with anagrelide is comparable to that with conventional drugs (about 90%), but maintenance therapy is always necessary. Side-effects (especially tachycardia, anaemia, gastric distress) must be seriously considered. Heart function monitoring is imperative. Four adverse thrombotic events occurred. However, no case of leukemic evolution was recorded.

Oral Communications
Antiphospholipid Antibodies

C151

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

Finazzi G, Marchioli R, Barbui T, for the 'ad Hoc' Study Group

Divisione di Ematologia, Ospedali Riuniti, Bergamo e Laboratorio di Epidemiologia Clinica, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy

The WAPS study is a multicenter, randomized clinical trial with two objectives: to compare high-dose warfarin (PT INR 3.0-4.0) vs. conventional treatment (warfarin with PT INR 2.0-3.0 for venous and aspirin for arterial thrombosis) in patients with the antiphopholipid syndrome; b) to evaluate the clinical outcome of non-randomized patients in a parallel observational arm of the study. At June 2001, 454 consecutive patients (M/F 125/329; median age 41 years, range 15-82) with lupus anticoagulant or moderate to high titers of anticardiolipin antibodies had been enrolled. Of these, 112 (25%) were eligible for randomization, whereas 342 were included in the observational arm because of: no symtoms, (130, 29%), excessive bleeding risk (71, 15%), absolute need for high-dose warfarin (55, 12%), or patient's unwillingness to participate (86, 19%). Median follow-up in both groups was 36 months (range 5-48). Actual PT INR of the 112 randomized patients was assessed at 3, 6, 12, 24 and 36 months. Median PT INR values of patients randomized in the high-dose group (n=56) was 3.1, 3.2, 3.3, 3.2 and 3.3, whereas in the conventional group (n=56) it was 2.3, 2.5, 2.6, 2.5 and 2.1, respectively. For safety reason, an interim analysis of the main end points of the study was carried out in the total population, divided into randomized (n=112) and observational (n=342) patients. All cause mortality (2.7% vs. 2.3%) and thrombotic events (7.1% vs. 8.2%) were similar between the two groups. However, both major (4.5% vs. 0.9%, p=0.01) and minor bleeding (12.5% vs. 5.3%, p=0.009) were significantly more frequent in the randomized patients. The WAPS study was concluded at 31/12/2001 and the final analysis of results is currently ongoing.

C152

ENHANCED TISSUE FACTOR EXPRESSION BY OXIDATIVE STRESS IN PATIENTS WITH ANTIPHOSPHOLIPID ANTIBODIES

Ferro D, Iuliano L, Loffredo L, Praticò D,** Valesini G,* Conti F,* Fitzgerald G,° Violi F

*Institute of Clinical Medicine I, University "La Sapienza" Rome; *Division of Rheumathology, Rome university "La Sapienza", Italy. °Center for Experimental Therapeutics University Of Pennsylvania, Philadelphia PA, USA*

In a recent paper, we demonstrated that in antiphospholipid antibodies (aPL) positive patients the clotting system activation could be mediated by increased lipid peroxidation. The aim of

this study was to evaluate the relationship between lipid peroxidation and monocyte TF expression in aPL (+) patients. We studied 11 consecutive aPL positive out-patients (10 women, 1 man; 24 to 51 years) Five of 11 aPL (+) ones were affected by primary antiphospholipid syndrome (PAPS), while the remaining 6 subjects suffered from systemic lupus erythematosus (SLE). In the same period, we selected 13 aPL (-) patients (12 women,1 man; 18 to 49 years) suffering from SLE. In a first study, we measured the urinary excretion of isoprostane-F-2-α-III (IPF-2-α-III) and Isoprostane-F-2-α-VI (IPF-α-VI), two markers of *in vivo* lipid peroxidation, and monocyte TF antigen and activity, in aPL (+) and aPL (-) patients. In a second study we sought to investigate whether antioxidant treatment affected tissue factor expression, as well as the Isoprostane levels. To this purpose, 11 aPL (+) patients were randomly treated with (n=6) or without (n=5) antioxidant supplementation (vitamin E at 900 IU/day, vitamin C at 2,000 mg/day) for 4 weeks. APL (+) patients showed higher IPF-III [228 (80-386) vs 152 (76-218) pg/mg creatinine; $p<0.001$], isoprostane-F-2-α-VI [1674 (632-2424) vs 1023 (426-1780) pg/mg creatinine; $p<0.008$] monocyte TF antigen [52 (20-78) vs 18 (10-28) pg/200,000 monocytes; $p<0.0001$] and activity [35 (20-48) vs 12 (6-20) U/2×10^5 monocytes; $p<0.001$] than aPL (-) subjects. Monocyte TF antigen correlated with isoprostane-F-2-α-III (r:0.87; $p<0.0001$) and isoprostane-F-2-α-VI (r:0.79; $p<0.003$) in aPL (+) subjects. After supplementation with antioxidant vitamins, we found a significant decrease in monocyte TF antigen ($p<0.005$) and activity ($p<0.01$), concomitantly with a reduction of both Isoprostanes. The study suggests that lipid peroxidation might contribute to the enhanced monocyte TF expression in aPL (+) patients.

C153

ANTI β2 GLYCOPROTEIN 1 ANTIBODIES INDUCE *IN VITRO* MONOCYTE TISSUE FACTOR EXPRESSION AND SUPEROXIDE ANION RELEASE, EFFECT OF VITAMIN E

Saliola M, Ferro D, Caroselli C, Merulli C,* Meroni P, Violi F

*Institute of Clinical Medicine I University of Rome "La Sapienza"; *Allergy and Clinical Immunology Unit, IRCCS Istituto Auxologico Italiano, Department of Internal Medicine, University of Milan, Italy*

A previous study showed that anti-β2 glycoprotein 1 antibodies stimulated tissue factor release by human endothelial cells. In this study we investigated the effect of scalar concentrations of anti-β2 glycoprotein 1 (50, 100, 200 μg/mL) on monocyte (200,000 cells) tissue factor expression (incubation time: 3 hours at 37°C, 5% CO2) and superoxide anion (incubation time 30 minutes at 37°C, 5% CO2) production. The same experiment was carried out by using similar concentrations of human IgG, as control. Furthermore, we evaluated the influence of co-incubation of scalar concentrations of vitamin E (50, 100 μM) on monocyte superoxide anion release and monocyte tissue factor expression. Human monocytes were obtained from healthy volunteers who gave informed consent. Compared to normal IgG, anti-β2-glycoprotein 1 antibodies induced a dose-dependent increase of monocyte tissue factor antigen (ANOVA F: 354, p=0,0001), tissue factor activity (ANOVA F: 75, p=0.0001) and superoxide anion release (ANOVA F: 242, p=0.0001). Monocyte superoxide anion production was significantly correlated with tissue factor antigen (p=0.0001) and activity (p=0.0001). The co-incubation with vitamin E induced a dose-dependent decrease of tissue factor antigen (ANOVA F: 101, p=0.0001), tissue factor activity (ANOVA F:148, p=0.0001) and superoxide anion release (ANOVA F:175, p=0.0001) by monocytes stimulated with anti-β2 glycoprotein 1 antibodies (200 μg/mL). Coincubation of monocytes with anti-β2 glycoprotein 1 (200 μg/mL) and superoxide dismutase (300 U/mL) decreased superoxide anion production by 70%. This study suggests that anti-β2 glycoprotein 1 antibodies increase monocyte tissue factor expression likely throughout a pro-oxidant mechanism. In fact, superoxide anion release and tissue factor expressed by monocytes stimulated with anti-β2 glycoprotein 1 antibodies, were significantly correlated. Moreover vitamin E was able to reduce anti-β2 glycoprotein 1-induced monocyte tissue factor expression, by inhibiting monocyte superoxide anion release.

C154

LUPUS ANTICOAGULANT AND INCREASED ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS WITH HEPATIC ARTERY THROMBOSIS AFTER ORTHOTOPIC LIVER TRANSPLANTATION

La Barba G, Vivarelli M, Legnani C,* Palareti G,* Bellusci R, Abdiueli Aden A, Cucchetti A, Coccheri S,* Cavallari A

*Dip. Discipline Chirurgiche, Rianimatorie e dei Trapianti, Chirurgia Generale e *Dip. Cardiovascolare, U.O. Angiologia, Azienda Ospedaliera di Bologna, Policlinico S.Orsola-Malpighi, Bologna, Italy*

Hepatic artery thrombosis (HAT) remains a devastating complication after orthotopic liver transplantation (OLT) and almost always results in graft loss. Several factors, such as technical problems, size of the recipient hepatic artery, and acute rejection, have been reported to be implicated in HAT, whose etiology often still remains undetermined. The presence of lupus anticoagulant (LAC) and/or increased levels of antiphospholipid antibodies (APA), alterations that are strongly associated with both arterial and venous thrombosis, has been suggested to contribute factor to the pathogenesis of HAT. We studied 3 groups of subjects: group A= 24 patients (mean age 50.1y, 25-66y; 17 males) who developed HAT (15 cases) or hepatic artery stenosis (9) after OLT, group B= 14 patients who underwent uncomplicated OLT (mean age 46.1y, 36-62y; 10 males, matched for age, sex and OLT indication); group C= 38 healthy subjects (mean age 53.8 y, 30-71 y; 30 males). LAC was assessed according to the criteria of the ISTH: a) diluted aPTT (1:15 PTT LA, Diagnostica Stago) and b) diluted Russel's viper venom time (LA-Test, Organon Teknika); both tests were also performed after mixing with normal pool plasma (1:1) and repeated using higher phospholipid concentration (Platelet Extract Reagent, Bio-Data; LA-Check, Organon Teknika). Anticardiolipin (IgG and IgM) and anti β2 glycoprotein I (IgG and IgM) levels were measured by ELISA assays (BEIA Bouty). LAC phenomenon and/or elevated APA levels were recorded in: 9/24 (37.5%), 2/14 (14.3%) and 3/38 (7.9%) in groups A, B and C, respectively (A vs B: p=0.256; A vs C: p=0.0115). Our data show a significantly higher rate of LAC/APA among subjects who had HAT (or hepatic artery stenosis) after OLT than in normal subjects. The comparison between patients with or without thrombotic complications after OLT did not reach statistical significance; this was probably due to the insufficient size of two groups.

C155

ASPIRIN AND CALCIUM HEPARIN: A COMPARATIVE EVALUATION OF BOTH TREATMENTS IN ANTIPHOSPHOLIPID ANTIBODIES POSITIVE WOMEN WITH RECURRENT PREGNANCY LOSS

Ciampa A,* Manzo A,* Masucci R,^ Volpe E*

**"G. Moscati" Hospital, Hematology Department; ^Gynecology Department, Avellino, Italy*

Objectives. Our goals were: 1) to detect the incident role of LA and ACA in early miscarriages (< 13 weeks gestations); 2) to evaluate the effectiveness of aspirin treatment and calcium heparin treatment; 3) to compare the obtained results. *Study design*. Four-hundred healthy women, mean age 30 years, with a history of fetal loss and with no evidence of any underlying connected disease were studied. Healthy controls were 50 women, mean age 29 years, recruited among LA and/or ACA-subjects. IgG and IgM ACA isotypes were measured using a standardized ELISA method. LA activity was diagnosed on criteria according to ISTH-recommendations. *Results*. Of the 400 patients tested, 256 (64%) were aPL^-. Of the remaining 144 pts (36 %) who were aPL^+, 9 were LA^+, 111 were ACA^+ and 24 were LA^+ ACA^+. 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 every 4 weeks. Following treatment, we found a downward trend for IgG and IgM ACA levels and, subsequently, 68 women became pregnant. They were divided into two different treatment groups: 22 patients were given 5000 IU calcium heparin subcutaneously twice daily; 39 pts were given aspirin 100 mg per day (7 pts of the 68 women withdrew from the study). Pts were followed up with repeated routine coagulation examinations. In the first trimester β-HCG too was monitored fortnightly. As to the results there were 22 live births in the calcium heparin treatment group and 36 in the aspirin treatment group. The 7 women who decided not to participate in the study any longer had mid-trimester losses. The remaining 10 pregnancies are still ongoing. *Conclusions.* Our results suggest the significant effectiveness of both treatments.

C156

ANTIBODIES TO TISSUE-TYPE PLASMINOGEN ACTIVATOR AS A POSSIBLE CAUSE OF THROMBOSIS IN PATIENTS WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME

Cugno M,* Galli M,° Caccia S,** Perrella M,** Bottasso B,* Agostoni A*

**Department of Internal Medicine, University of Milan, IRCCS Maggiore Hospital, Milan, °Department of Haematology, Ospedali Riuniti, Bergamo and **Department of Biomedical Sciences and Technologies, University of Milan, Italy*

Background. The cause of thrombosis in primary antiphospholipid syndrome (PAPS) is still unknown, although several hypotheses have been proposed and hypofibrinolysis has been described. Anti-tissue-type plasminogen activator (t-PA) antibodies could potentially induce fibrinolytic defects and our preliminary data indicate an association with thrombotic events in patients with PAPS. *Aims*. The aims of this study were the isolation and characterization of plasma anti-t-PA antibodies and the evaluation of the interaction of these antibodies with the catalytic domain of the t-PA molecule. *Patients*. We studied two female patients (46 and 36 years old) with PAPS, anti-cardiolipin antibodies and lupus anticoagulants. Both had high plasma levels of anti-t-PA antibodies. *Methods*. Anti-t-PA antibodies were measured in plasma by an immunoenzymatic assay. The method detects, by monoclonal antibodies, human immunoglobulins which interact with recombinant t-PA previously immobilized on microplates. The immunoglobulin G fractions containing anti-t-PA antibodies were isolated from plasma by adsorption on a column of protein-G-agarose. We identified the immunoglobulin subclasses of the anti-t-PA antibodies by specific monoclonal antibodies and evaluated the interaction of anti-t-PA antibodies with the recombinant catalytic domain of the t-PA molecule immobilized on microplates. We tested the inhibition of anti-t-PA antibodies binding to immobilized t-PA by adding to plasma increasing amounts of purified prothrombin and β-2-glycoprotein-I. *Results*. Plasma levels of anti-t-PA antibodies were high in both patients (100 U/mL and 130 U/mL; normal range 0-11 U/mL). Anti-t-PA antibodies were of subclass IgG3 in one patient and IgG1 in the other. Both recognized human melanoma t-PA, recombinant t-PA and the recombinant molecule consisting of the t-PA catalytic domain and did not recognize prothrombin and β-2-glycoprotein-I. *Conclusions*. Our data indicate that anti-t-PA antibodies that interact specifically with the catalytic domain of the t-PA molecule can be found in patients with PAPS.

Oral Communications
Diagnosis and Treatment of Atherothrombosis II

C157

EFFECT OF THE COMBINATION OF NCX 4016, ASPIRIN AND CLOPIDOGREL IN A MODEL OF PULMONARY THROMBOEMBOLISM IN MICE

Momi S,° Mezzasoma AM,° Leone M,° Del Soldato P,* Gresele P°

°Dept. of Internal Medicine, Sect. of Internal and Cardiovascular Medicine, University of Perugia; NicOx Research Institute, Milan, Italy*

The combination between a thienopyridine and aspirin (ASA) represents the treatment or choice for patients undergoing coronary angioplasty with stent implantation. Moreover, recent trial results have shown that the combination of clopidogrel and ASA in unstable angina is superior to aspirin alone, however at the price of enhanced bleeding. NCX 4016, an aspirin derivative releasing nitric oxide, exerts an antithrombotic activity superior to aspirin in some animal models and reduces the degree of restenosis after arterial injury in hypercholesterolemic mice. We have compared the effect of ASA, NCX 4016, clopidogrel and various combinations of them on platelet pulmonary thromboembolism and on bleeding in mice. Drugs were administered orally once a day for five days. The studies were carried out one hour after the last oral administration. Results are reported in the Table below.

	Mortality (%)	Bleeding time (sec)	Plasma NO2/NO3 (μM)	Serum TxB2 (ng/ml)
Control mice	80	202±10.5	14±2.5	101±19.7
ASA 30 mg/kg	75	410±114*	10.6±4	8±2.9*
NCX 4016 60 mg/kg	37*	415±95*	36.7±3.27*	28.6±12*
Clopidogrel 0.5 mg/kg	78	451.2±72*	17.8±6	–
ASA + Clopidogrel	53	800±68*	–	5.25±2*
NCX + Clopidogrel	41*	484±90*#	32±8.5*	19.6±9.2*
ASA+NCX+Clopidogrel	40*	733±87*	36.2±13.3*	5.8±2.5*

*p>0.05 vs control mice; #p<0.05 vs ASA+Clopidogrel

The combination of aspirin and clopidogrel exerts a stronger antithrombotic protection as compared with the single drugs, although associated with a striking prolongation of the bleeding time. On the other hand, the combination of NCX 4016 and clopidogrel exerts an even greater protection against collagen + epinephrine induced thromboembolism associated with a lesser prolongation of the bleeding time. Addition of NCX 4016 to the combination ASA+clopidogrel does not further increase antithrombotic protection but it does not enhance bleeding either. In therapeutic procedures of revascularization associated with a risk of restenosis in which ASA + clopidogrel is the first choice therapy, such as angioplasty with stenting, an associate use of NCX 4016 could be hypothesized.

C158

HUMAN ENDOTHELIAL CELL TISSUE FACTOR SYNTHESIS IS DOWNREGULATED BY ANTAGONISTS OF THE RENIN-ANGIOTENSIN SYSTEM

Napoleone E, Di Santo A, Donati MB, Lorenzet R

Istituto di Ricerche Farmacologiche Mario Negri, "Antonio Taticchi" Laboratory for Atherosclerosis and Thrombosis, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy

The renin-angiotensin system (RAS), through the action of its effectors, plays an important role in cardiovascular function, and inhibition of angiotensin II generation reduces the rate of myocardial infarction and stroke in patients at risk for cardiovascular disease. Tissue factor (TF), the cellular receptor for factor VIIa which triggers blood coagulation, has a direct role in the pathogenesis of atherosclerosis, since its concentration in atherosclerotic plaques is thought to determine their thrombogenicity Since we have shown that ACE inhibitors downregulate TF synthesis in monocytes, and since a local RAS is present at the level of vascular endothelium, we decided to determine whether blockade of the RAS could modulate TF expression also in endothelial cells. HUVECs were incubated with LPS, IL-1β, and TNF-α, in the presence of fosinopril and idrapril. Cells were then disrupted and procoagulant activity was measured by one stage clotting time. The different agonists induced a strong TF expression that was significantly inhibited in a dose-dependent fashion by both compounds. Since ACE converts angiotensin I in angiotensin II and the latter binds to the AT1 receptor, found on activated endothelial cells, we investigated whether blocking the receptor could affect TF production. Losartan, a competitive inhibitor of AT-1, reduced TF activity in stimulated HUVECs in a dose-dependent way to a degree similar to that caused by ACE inhibitors. The same effect was observed when an anti-AT1 antibody was substituted for losartan. Northern blot experiments showed that Losartan reduced TF mRNA synthesis. Moreover, the compound inhibited the translocation of cRel/p65 oligonucleotides that was induced by the different agonists, as determined by electromobility shift assay. Western blot analysis showed that inhibition of translocation of c-Rel/p65 was caused by a diminished degradation of its inhibitor protein IκBα. These data suggest an additional mechanism by which these drugs exert their cardioprotective effect.

C159

EFFECT OF NITRIC OXIDE -DONATING AGENTS ON MONOCYTE CYCLO-OXIGENASE-2

Leone M, Corazzi T, Del Soldato P,* Gresele P

Department of Internal Medicine, Section of Internal and Cardiovascular Medicine, University of Perugia, Italy, and NicOx S.p.A, Milan, Italy*

Two cyclo-oxigenase (COX) isoforms exist: COX-1 (constitutive) and COX-2 (inducibile). COX-2 is involved in inflammation but has been implicated in ischemic cardiovascular disease too. Aspirin is only a weak COX-2 inhibitor. As nitric oxide (NO) regulates COX activity in various cell systems, we investigated the effect of the novel NO-donating aspirin NCX4016, and other NO-donors, on monocyte COX-2. Heparinized human whole blood was incubated with LPS for 24 hours at 37°C and PGE2

was measured in the supernatant plasma as an index of monocyte COX-2 activity. Serum TxB2 was also assessed as an index of platelet COX-1 activity. The selective COX-2 inhibitor DUP697 (0.05-0.25 μmolar) dose-dependently reduced PGE2 production (85% maximal inhibition) and dexamethasone (10 μmolar) totally suppressed it, whereas aspirin (10-300 μmolar) was almost ineffective, producing only 15% inhibition at 300 μmolar. NCX4016 (50-300 μmolar) inhibited dose-dependently, though only partially, PGE2 production (50 μmolar = 19% inhibition, p =0.01; 300 μmolar= 36% inhibition, p<0.001). Among the NO-donors, SNP (0.1-1 mM) inhibited PGE2 dose-dependently (80% maximal inhibition, p< 0.05) and DetaNONOate (10 mM) completely suppressed it, whereas GSNO (0.1-1 mM) and SNAP (0.1 mM) were ineffective. NCX4016 and aspirin inhibited platelet COX-1 with comparable activity (IC_{50} 0.02 and 0.01 μmolar, respectively) while DUP697 and SNP were ineffective. Under the same experimental conditions COX-2 expression, measured by Western blot, was completely suppressed by dexamethasone while it was unaffected by aspirin, DUP697, NCX4016, and SNP. Finally, the role of NO-stimulated guanylyl-cyclase in the inhibitory effect of the drugs tested was assessed by the guanylyl-cyclase inhibitor ODQ (1 mM). A significant reduction of PGE2 inhibition by ODQ was observed for NCX4016 (-28%, p<0.05) and SNP(-38%, p<0.05), suggesting a partly GC-dependent mechanism. In conclusion, nitroaspirin, as well as other, but not all, NO-donors, inhibits monocyte COX-2 activity. This might represent an advantage over aspirin, given the possible detrimental role of COX-2 in cardiovascular disease.

C160

NOCTURNAL INCREASE IN THROMBOXANE B2 PRODUCTION IN CORONARY ARTERY DISEASE IS ONLY PARTLY COUNTERACTED BY LOW-DOSE ASPIRIN

Fimognari FL, Loffredo L, Gambuti F, Milite MT, Piccheri C, Monteleone G,* Violi F

*Institute of Clinical Medicine I, University "La Sapienza", Rome; *Laboratory of Clinical Pathology, Del Balzo-Squillacioti Hospital, Locri, Italy*

Previous studies have shown a peak of *in vitro* platelet aggregability in the early morning when an increased incidence of acute atherothrombotic events has also been observed. However, there is no evidence that this really occurs *in vivo*. Therefore, we measured urinary 11-dehydro-thromboxane B2 (TxB2) by immunoassay in 14 patients with angiographically documented, stable, coronary artery disease (CAD) and 12 normal controls matched for age and cardiovascular risk factors. None had been taking aspirin in the previous 15 days. These volunteers collected their 24-hour urine at intervals of 4 hours, as follows: 12-16, 16-20, 20-24, 24-4, 4-8, 8-12. In the healthy subjects a significantly increased 11-d-TxB2 was observed in samples 4-8 and 8-12, if compared with samples 16-20 and 20-24 (p< 0.05 with Wilcoxon's test). In CAD patients 11-d-TxB2 measured in samples 24-4 and 4-8 was significantly higher than in the other samples (p< 0.05 with Wilcoxon's test). In order to assess whether such increases could be due to platelet Tx production, 40 mg/day of aspirin, a dose well known to exert a nearly complete suppression of platelet-dependent thromboxane B2, were given to a subgroup of 4 CAD subjects, representative of the total group (Mann-Whitney U test) for 7 days, after which the urine collection was repeated. The study showed a mean decrease in thromboxane biosynthesis of 47%, as follows: 24-4: 40%; 4-8: 66%; 8-12: 61%; 12-16:13%; 16-20: 49%; 20-24: 55%. These results confim that in healthy subjects there is a circadian variation of platelet activation with a peak between 4 a.m. and 12 a.m. Results from CAD patients showed different degrees of aspirin-insensitive Tx biosynthesis in the six intervals of the 24 hours, with an early nocturnal increase which only in part was depending on platelet production, since the peak observed between 24 and 4 a.m. was poorly modified by aspirin in these patients. These data suggest an extraplatelet source in the early peak of Tx production in CAD patients.

C161

CHARACTERIZATION OF ENDOGENOUS PROTEOLYTIC ACTIVITY IN A RAT MODEL OF SPONTANEOUS CEREBRAL STROKE

Sironi L, Calvio AM, Bellosta S, Lodetti B, Guerrini U, Monetti M, Tremoli E, Mussoni L

Dept. of Pharmacological Sciences, University of Milan, Italy

Research carried out in the last few years has given new insights into the role of extracellular proteases, i.e. plasminogen activator (PA)/plasmin and matrix metalloproteinases (MMP), in cerebral ischemia. We thus evaluated the expression of these two enzymatic systems in stroke-prone spontaneously hypertensive rats (SHR-SP), an inbred animal model of cerebro-vascular pathology resembling in many aspects the human disease. This study was carried out using magnetic resonance imaging (MRI) measurements in combination with zymographic analysis in SHR-SP rats fed with a diet high in sodium to accelerate the onset of brain damage. Cerebral lesions, that appear as an hyperintense area, were localized in individual animals and followed by T2 weighted MRI. All animals developed brain abnormalities in 42±3 days (n=30) and were sacrificed three days after brain damage was detected. *In situ* zymography of brain sections showed an increase of PA/plasmin activity that co-localized with cerebral damage, as detected by MRI. This activity was inhibited by amiloride (2 mM), a urokinase specific inhibitor, but not by a monoclonal antibody against rat tPA. SDS-PAGE zymography of brain extracts revealed the presence of plasminogen-dependent lysis areas of 58 kDa in the ischemic and non-ischemic tissues and a 33 kDa lysis area in ischemic tissue only. An antibody against tPA inhibited the former, whereas the latter was inhibited by amiloride. The specific induction of uPA in the damaged tissue was further confirmed by the observation that both uPA protein mass, detected by Western blot, and mRNA, assessed by RT-PCR, were markedly increased in damaged cerebral areas. Concomitantly an activation of MMP2 was observed in the damaged area only. These data suggest that uPA selectively catalyzed proteolysis in the area of brain that developed a damage and this proteolytic cascade may contribute to tissue injury in this animal model.

C162

EFFECTS OF PROSTACYCLIN ANALOG ON ICAM-1 AND F1+2 LEVELS IN PERIPHERAL ISCHEMIA IN HUMANS

Mazzone A, Faggioli P, Cusa C, Rondena M, Morelli B*

*U.O. Internal Medicine and Oncology, *Laboratory of Hematology, Ospedale Civile di Legnano, (MI)*

Background. Iloprost has beneficial effects on microcirculation preventing the reciprocal potentiation of platelet and leukocyte activation, leading to vascular endothelial damage and to acute thrombosis. The mechanisms of actions are not completely known yet. Previous works showed that one day of therapy significantly reduces the αMβ2 integrin expression (ICAM-1) on phagocytes. *Aim of the study*. This study was designed to evaluate the effects of one course of treatment on the adhesion molecule ICAM-1 and on the marker of coagulation cascade F1+2, in patients suffering from systemic sclerosis (SS) and peripheral artery occlusive disease (PAOD). *Patients and Methods*. Forty patients were enrolled in the study. 29 with SS; 11 with PAOD. Iloprost was administered for 5 days in SS and for 21 days in PAOD. The plasma concentrations of ICAM-1 and F1+2 were detected on the first day and at the end of the first course of therapy. *Results*. In all of the patients a significant reduction in the plasma levels of ICAM–1 and F1+2 was observed. In SS patients the ICAM-1 level was 850.27±58.9 at baseline and 757.96±48.2 after 21 days (p<0.005); the F1+2 level was 0.985±0.05 vs 0.875±0.04 (p<0.0005). In PAOD patients the ICAM-1 level was 758.27±28.50 at baseline and 667.27±29.17 after 5 days (p<0.05); the F1+2 level was 0.818±0.10 vs 0.681±0.09 (p<0.005). *Conclusions*. The study provides further evidence that iloprost reducs inflammatory and coagulation cascade activation and confirms that the long term clinical benefit observed in patients with critical leg ischemia, may depend, among other things, on microvascular functional capacity improvements. Moreover, the data show that the effects are significantly greater in patients with SS vs PAOD whilst they are not dependent on the duration of therapy.

Oral Communications
Genetic Determinants of Atherothrombosis II

C163

ACE AND ENOS POLYMORPHISMS IN PATIENTS WITH ATRIAL FIBRILLATION

Gensini F,* Michelucci A, Fatini C, Sticchi E,* Pieragnoli P, Colella A, Musilli N, Falciani M, Coppo M, Padeletti L, Abbate R, Gensini GF

*Dipartimento Area Critica Medico Chirurgica; *Dipartimento di Fisiopatologia Clinica, Unità di Genetica Medica; Università di Firenze, Centro Trombosi, A.O. Careggi, Florence, Italy*

Recent reports suggest that atrial fibrillation (AF) is associated with the activation of the atrial angiotensin system; in atrial tissue of patients with AF, a significant increase of angiotensin converting enzyme (ACE) expression has been observed. Moreover it has been demonstrated that the ACE inhibitor, trandolapril, reduces the incidence of AF after acute myocardial infarction in patients with left ventricular dysfunction. ACE, by bradykinin degradation, may regulate endothelial nitric oxide (NO) synthesis, which modulates the activity of the autonomic nervous system that is involved in the development of AF. The aim of our study was to evaluate the prevalence of ACE insertion/deletion (I/D) and endothelial nitric oxide synthase (eNOS) T-786C and G894T polymorphisms in patients with AF. Forty-six patients (34 males and 12 females) with persistent AF and 112 control subjects were studied. ACE and eNOS polymorphisms were analyzed by molecular biology techniques (PCR-RFLP). ACE I/D polymorphism genotype distribution and allele frequency were significantly different between patients and controls (χ^2)= 10.78, p=0.005 and χ^2=12.25, p=0.0005, respectively). At univariate analysis the ACE DD genotype was significantly associated with the risk of AF (OR DD/ID+II=2.61, p=0.007). The analysis of eNOS polymorphisms did not show a significant difference in genotype distribution and allele frequency between patients and controls. Our results suggest a possible role of ACE DD genotype as a predisposing factor to AF and prompt us to increase the sample size to make a better evaluation of the genetic impact on the etiopathogenesis of the disease.

C164

ASSOCIATION OF APOLIPOPROTEIN E POLYMORPHISM WITH LIPID LEVELS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA AND FAMILIAL DEFECTIVE APOLIPOPROTEIN B-100

Vohnout B,[1,9] Raslová K,[1] Gasparovic J,[1] Franeková J,[2] Fábryová L,[3] Belosovicová M,[4] Kovác G,[5] Sebová C,[5] Rajecová E,[6] Stavny J,[7] Babjak M,[8] Donati MB,[9] Iacoviello L[9]

[1]Institute of Preventive and Clinical Medicine, Limbova 14, 83301 Bratislava, Slovakia; [2]OKB NsP Zilina; [3]NsP Milosrdní bratia, Bratislava; [4]DFNsP Bratislava; [5]FNsP aDerera Bratislava; [6]UTaRCH Bratislava; [7]OKB NsP Poprad; [8]OKB NsP Humenne, Slovakia; [9]"Angela Valenti" Laboratory of Genetic and Environmental Risk Factors for Thrombotic Disease, Department of Vascular Medicine and Pharmacology, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy

Familial hypercholesterolemia (FH) and familial defective apolipoprotein B-100 (FDB) are two of the most common genetic diseases. Both are characterized by elevated levels of LDL cholesterol and premature coronary heart disease. Apo E serves as a ligand for the apoB/E receptor and it plays a key role in lipoprotein metabolism and cholesterol homeostasis. Therefore the polymorphism of apoE may at least partially explain a diversity in the clinical expression of FH. In a cohort of 529 individuals from 165 families examined for possible FH we identified 35 families with a total of 122 patients who fulfilled clinical criteria of FH and, using PCR diagnosis, 12 families with a total of 31 patients with FDB. One hundred and two normolipidemic individuals (NL) from the cohort served as control group. As expected, patients with FH had the highest levels of TC, LDL-C and apoB, followed by the FDB patients and normolipidemic relatives with the lowest levels ($p<0.0001$ for all parameters). There were no differences between FH, FDB and normolipidemic groups in the frequency of apoE genotypes and alleles. No effect of apoE genotypes on lipid levels in NL or FH group was found. However, higher levels of total and LDL cholesterol in FDB carriers of _4 allele may suggest a possible role of apoE genotype in phenotypic expression of FDB patients.

C165

ANGIOTENSIN CONVERTING ENZYME I/D POLYMORPHISM, PLACENTAL VASCULAR IMPEDANCE AND PREGNANCY OUTCOME

Fatini C, Parretti E,* Gensini F,° Sticchi E,° Gazzini A, Cioni R,* Mecacci F,* Mello G,* Abbate R

*Dipartimento Area Critica Medico Chirurgica; *Dipartimento di Ginecologia, Perinatologia e Riproduzione Umana; °Dipartimento di Fisiopatologia Clinica, Unità di Genetica Medica; Università di Firenze; Centro Trombosi A.O. Careggi, Florence, Italy*

Pregnancy complications such as pre-eclampsia and fetal growth restriction (FGR) may stem from impaired placentation in early gestation, and have been associated with thrombophilia. Doppler ultrasonographic studies of uteroplacental and fetal umbilical circulations have shown that high impedance to flow is associated with subsequent pre-eclampsia, FGR and related complications. The renin angiotensin system plays a role in modulating vascular tone and recently effects on platelet, coagulation and fibrinolytic functions have been demonstrated. The aim of this study was to investigate the role of angiotensin converting enzyme (ACE) I/D polymorphism on uteroplacental and umbilical blood flow and pregnancy outcome in women with a history of pre-eclampsia. One hundred and six women with no congenital or acquired thrombophilic factors were enrolled in the study. All the women underwent Doppler investigation of uterine arteries resistance index (RI) and umbilical artery pulsatility index (PI) at 16, 20 and 24 weeks' gestation. ACE I/D polymorphism was analyzed by polymerase chain reaction and electrophoresis on agarose gel. Thirty-seven (35%) DD homozygous, 46 (43%) ID heterozygous, and 23 (22%) II homozygous subjects were found; ACE D allele frequency was 0.57. At 16, 20 and 24 weeks, values of uterine artery RI were significantly different among genotypes with lower values in II, higher in DD and intermediate in ID genotype carriers. At 16, 20 and 24 weeks the umbilical artery PI values were significantly higher in the DD group in comparison to ID and II genotypes. Interestingly, women with DD genotype had a significantly increased incidence of pre-eclampsia (24.3%) and fetal growth restriction (42.2%) as compared with the ID group (15.2% and 28.3%), which in turn had a significantly higher rate of these complications in comparison to the II group (4.3% and 8.7%). Our results suggest the relevance of ACE D allele in the modulation of impedance to flow, in uterine and umbilical arteries, and in the risk of recurrence of pre-eclampsia and fetal growth restriction.

C166

IDENTIFICATION OF A NOVEL (-748G→A) POLYMORPHISM IN THE PROMOTER OF THE GENE FOR CRP: LACK OF ASSOCIATION WITH C-REACTIVE PROTEIN LEVELS IN HEALTHY MEN

Zito F,* Hingorani A,° Hawe E,* Miller G,# Humphries SE*

**Centre for Cardiovascular Genetics; °Centre for Clinical Pharmacology, BHF Labs, RF and UCL Medical School, London WC1E 6JJ; #MRC Epidemiology and Medical Care Unit, Wolfson Institute St. Bartholomew's Hospital, London, UK*

Inflammation is a key component of coronary heart disease (CHD), and genes coding for cytokines and acute-phase reactants are candidates for predisposing to CHD risk. We examined the effect of two variants (-748G→A and 1059G→C), respectively in the promoter and exon 2 of C-reactive protein (CRP) gene, on CRP levels in 368 middle-aged healthy UK men. Genotype and CRP measurements were performed at baseline in 368 healthy men included in the prospective Northwick Park Heart Study-2 (NPHS-2). Subjects were aged 50-61 years, and free of evidence of CHD. The novel -748G→A variant was identified using SSCP of the region +12 to -1648, inverse PCR and DNA sequencing. Genotyping for this variation was carried out by PCR and SacII restriction endonuclease. The1059G→C variant was detected by PCR and Fnu4H restriction endonuclease. The MADGE high throughput method was used for all genotyping. C-reactive protein was measured using the CRP EIA HS kit from Kordia (UK), with an inter-assay CV of 9.7% and an intra assay CV of 8.5%. The two variants of the CRP gene were not in linkage disequilibrium. The frequency of the -748A allele was 0.27 (95% CI 0.25-0.30). There was a modest trend to lower mean (±SD) CRP levels in the A-allele carriers but this was not statistically significant (GG vs GA+AA: 1.30±1.40 vs 1.25±1.38 mg/L, $p=0.8$). The 1059C allele frequency was 0.07 (95%CI 0.05-0.08). No CC homozygous subjects were found. Also this variant was not associated with a significant effect on CRP levels (GG vs GC: 1.35±1.47 vs 1.08±1.13 mg/L $p=0.3$). Neither the previously reported exon 2 variant nor the novel promoter variant contributes to differences in CRP levels in healthy men. This suggests that they are unlikely to be functional or to determine differences in CHD risk. Association studies with different impact of CRP polymorphisms during an acute-phase could be appropriate.

C167

ASSOCIATION BETWEEN FIBRINOGEN LEVELS AND FIBRINOGEN G-455A POLYMORPHISM AND INCREASED INTIMA-MEDIA THICKNESS IN THE GENERAL POPULATION

Tosetto A,* Baracchini C,* Manara R,* Prati P,° Simioni M,* Dall'Oste C,* Rodeghiero F*

**Department of Hematology, S. Bortolo Hospital, Vicenza; °Department of Neurology, Gervasutta Hospital, Udine, Italy*

Background. Several prospective studies have demonstrated that plasma fibrinogen is a risk factor for incident arterial thrombosis. Its association with the development of atherosclerosis is however uncertain. *Aim of the study*. To evaluate whether increased fibrinogen level and a polymorphism in the β fibrinogen region (G-455A) is associated with increased intima-media thickness (TIM), a marker of pre-clinical atherosclerosis, in the general population. *Subjects and Methods*. We evaluated 2373 subjects (1304 females, 1069 males) previously enrolled in the VITA Project, with a median age of 54 years. In all subjects, IMT was measured in common carotid arteries using B-mode ultrasonography and digital acquisition/measurement software (M'ath, Metris, France). Blood sampling, plasma preparation and storage and measurement of fibrinogen were performed using previously published methods (*Rodeghiero and Tosetto, Thromb Haemost, 1993*). Fibrinogen was measured against a plasma calibrated by the clot-weight method. G-455A polymorphism was determined by PCR analysis. Logistic regression was used to model for the individual probability of having a IMT above the age-adjusted reference limit. *Results*. In a multivariate analysis that accounted for the effect of gender, cholesterol and smoking, subjects with a fibrinogen level above the third tertile at the first VITA visit had a 2.2-fold increased probability of having an IMT above the upper limit (95% CI 1.31-3.82). Subjects with a fibrinogen level above the third tertile in only one VITA visit had a lower probability (OR=1.68, 95% CI 1.02 – 2.81), whereas subjects with fibrinogen level above the third tertile (above 308 mg%) in both VITA visits had a higher probability (OR=2.61, 95% CI 1.53-4.47). The G-455A polymorphism had a clear effect on fibrinogen levels, but the effect on IMT was only marginal and observed only in H2/H2 carriers (mean IMT 0.68 vs. 0.66, p=0.05). *Conclusions*. Increased fibrinogen levels are associated with pre-clinical atherosclerosis. The effect seems largely independent of the G-455A polymorphism.

C168

EFFECT OF FACTOR XIII VAL34LEU POLYMORPHISM ON PLASMA CLOT FORMATION: CROSSLINKING FUNCTIONS AND CLOT LONGEVITY PROPERTIES

Gemmati D, Serino ML, Tognazzo S, Ongaro A, Gamba E, Mari R, Moratelli S, Scapoli GL

Centre for the Study of Hemostasis and Thrombosis, University of Ferrara, Italy

A common polymorphism in the coagulation factor XIIIA-subunit gene (FXIIIVal34Leu) is located close to the thrombin activation site. The substitution has the quality of protecting against thrombosis. Direct evidence fully explaining its protective role has not been obtained yet. An earlier thrombin-mediated activation of the Leu34 allele, with possible consequent premature depletion from the circulation, seems to be the key element. In the present study calcium-mediated activation of coagulation was utilized to endogenously transform plasma FXIII zymogen to the active enzyme. In two plasma pools of healthy subjects with opposite FXIII genotype (Val/Val, n=20; Leu/Leu, n=20), fibrin cross-linking was followed by SDS-PAGE, clot formation was monitored by thrombelastography, and the appearance of activated FXIII-A was confirmed by Western-blotting. The outcomes were unambiguously in favor of an early appearance of truncated FXIII-A and a higher rate of fibrin polymerization/crosslinking in homozygous Leu variant than in wild type. These results were in contrast with the associated protection against thrombosis, therefore, the protective effect could be better investigated during the fibrinolysis steps. Fully stabilized clots were lysed by rtPA and the reduction in turbidity was recorded every 5 minutes at 405 nm for 120 minutes. Clot lysis time was longer for Leu than for Val cross-linked fibrin. These results did not explain the protective effect of the FXIII variant, therefore we attempted clot longevity experiments in samples simultaneously containing thrombin/Ca2+/rtPA. Clot longevity, defined as the difference between time to lysis and time to clot formation, was longer in the Leu than in the Val sample also because the Leu sample had a shorter time to clot. These data, apparently at odds with the protective effect against thrombosis, strengthen the hypothesis that an earlier and wasteful activation of FXIIILeu34, leading to a premature depletion from circulation, is the main protection against cardiovascular disease.

Posters
Genetic Determinants of Vascular Risk

P001

GENETICS OF HUMAN CATHEPSIN G AND RISK OF MYOCARDIAL INFARCTION AT YOUNG AGE

D'Orazio A, Di Castelnuovo A, Zito F, Donati MB, Iacoviello L

"Angela Valenti" Laboratory of Genetic and Environmental Risk Factors for Thrombotic Disease, Department of Vascular Medicine and Pharmacology, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy

Human cathepsin G (h-CG) gene spans 2.7 kilobase pairs on chromosome 14q11.2 and consist of 5 exons and 4 introns. The promoter region of h-CG was screened by SSCP and three new single mutations were found: a T to C transition at the-227 position, a C to T transition at the-151 position and a C to T transition at the-132 position. The mutated allele at position -227 introduces a new binding site for the nuclear factor NF-κ. This transcription factor is involved in the regulation of many inflammatory and hemostatic genes. The presence of the new binding site for NF-κ-B was confirmed by electrophoretic mobility shift assay (EMSA). Labeled wild type and mutated -227CG promoter probes were incubated with and without a cold control probe (100 times more the labeled probes) with purified NF-κ-B protein (p50, human), to evaluate the presence and the specificity of the binding. The results showed the presence of a band in the presence of the mutated but not of the wild type probe. However, addition of a cold probe inhibited the band formation. Allele frequencies of -227CG polymorphism were studied in a population of 305 healthy Italian subjects (208 males and 114 females aged 20-78 years). The allelic frequency of -227C was 0.042 (95% IC 0.026-0.057). The genotypes were all in Hardy Weinberg equilibrium. Patients with myocardial infarction (MI) at young age (234) matched by age and sex with healthy subject were studied. The frequency of -277C allele was 0.038 (95% IC 0.021-0.055) in controls and 0.037 (95% IC 0.020-0.054) in cases. Thus, a polymorphysm of CG promoter introduces a new binding site for NF-κ-β. It does not, however, affect the risk of MI at young age.

P002

CONSTRUCTION AND SETTING-UP OF HUMAN DNA-MICROARRAYS TECHNOLOGY FOR THE EVALUATION OF GENE EXPRESSION AND MUTATION DETECTION PROFILING IN VASCULAR DISEASES

Giusti B, Rossi L, Poggi F, Gensini GF, Abbate R, Pepe G

Dipartimento Area Critica Medico-Chirurgica, Università degli Studi di Firenze; Centro Trombosi, Azienda Ospedaliera Careggi, Florence, Italy

Clinical and experimental evidence demonstrate an association between atherosclerotic lesions and inflammation. Vascular disease results from complex mechanisms involving environmental and genetic factors. Clinical expressions of atherosclerosis are dilatations and aneurysms resulting from chronic inflammation and extracellular matrix degradation. Although these processes are considered critical in the pathophysiology of aneurysmal degeneration, the exact causes of aneurysm growth and rupture are still unknown. In recent years, the development of microarray technology has made it possible to examine the simultaneous expression of multiple (thousand) gene products in the same experiment. These methods greatly facilitate the identification of altered patterns of gene expression including detection of unanticipated changes. In order to obtain a global portrait of gene expression in syndromic (Marfan's syndrome) and non-syndromic abdominal and thoracic aortic aneurysms, we decided to design and set-up experiments using DNA (70-base oligonucleotides)-microarray technology. We acquired a human oligonucleotide gene bank of 14,000 genes and constructed and validated 2 arrays of 7,000 genes plus controls with 1 replication/slide. Full-thickness aortic wall specimens, fibroblast cultures from skin biopsy and blood samples were obtained from 30 patients undergoing elective surgical repair (10 Marfan, 10 abdominal and 10 thoracic aneurysms). Total RNA was isolated with RNeasy Kit (QIAGEN). Pooling of total RNA from patients and from controls was performed in order to reduce individual variability. The simultaneous evaluation of the mRNA expression in patients and controls on the two arrays will give us the possibility of a better understanding of molecular mechanisms and development of new therapeutic strategies for aortic aneurysms. All these patients and another 70 subjects affected by thrombophilia were characterized for 35 genetic variants associated with thrombophilia. On these patients we are setting-up and comparing two alternative and innovative microarray technologies: minisequencing-based assay and a newly commercialized NanoChip platform. Our data will permit us to evaluate whether these two new technologies can be applied for profiling thrombophilic risk and diagnostic purposes.

P003

GENIMA STUDY: GENETIC POLYMORPHYSMS AND MYOCARDIAL INFARCTION AMONG ITALIAN YOUNG ADULTS

Ghirarduzzi A, Tortorella G, Iorio A, Casali B, Bellesia E, Silingardi M, Nicolini A, Guiducci U, Iori I

Thrombosis Center, Internal Medicine I, Cardiology, Molecular Biology, Clin Chemistry Depts. Reggio Emilia, Italy

Background. The potential role in the risk of AMI of some common polymorphisms in genes for platelet receptors, hemostatic factors, and endothelial cell functions has been suggested from family studies but has not yet been fully clarified. *Aim of the study.* To assess the relationship between some genetic polymorphisms and AMI in young adults. *Methods.* Consecutive patients admitted to the Intensive Cardiology Care Unit of Reggio Emilia between January 1998 and September 2000 with a diagnosis of AMI were prospectively recruited and compared to a cohort of 104 healthy blood donors. Cardiovascular risk factors were recorded at recruitment. Blood was drawn for molecular biology, biochemistry and coagulation studies. Standard PCR assays were performed for R506Q factor V Leiden, G20210A prothrombin, C677T MTHFR, platelet GP IIb/IIIa polymorphism PLA1/PLA2, and two endothelial cell nitric oxide synthase (ecNOS) gene polymorphisms, G894T and VNTR intron 4. Fasting and post methionine load plasma homocyst(e)ine (HCY) were measured in patient three months after AMI. A forward stepwise

logistic regression model was used to evaluate the independent and cumulative effect of the gene polymorphysms on the risk of AMI. *Results*. One hundred and twenty AMI patients (95/25 males/females, mean age 48 yrs, range 20-60) and 104 controls (59/45 males/females, mean age 49 yrs, range 22-69) were evaluated. The prevalence in the genotype distributions are reported as percentages in the table below.

Gene Polymorphysms	Patients (%)			Controls (%)		
	+/+	+/-	-/-	+/+	+/-	-/-
Factor V Leiden	99.0	1.0	-	95.4	4.6	-
G20210A Prothrombin	94.6	5.4	-	97.7	2.3	-
MTHFR C677T	24.4	31.4	24.4	50.6	35.3	14.1
ecNOS G894T	57.8	30.1	12.0	46.4	42.9	10.7
ecNOS VNTR-intron 4	63.4	32.9	3.7	66.7	31.0	2.3
GP IIb/IIIa PLA1/PLA2	57.6	41.2	1.2	65.5	32.2	2.3

The logistic regression model was highly significant ($p < .00001$) and included as explanatory variables smoking, gender, fibrinogen (373 vs 312 mg/mL patients vs controls) and fasting HCY (15 vs 9 mmol/L patients vs controls). *Conclusions*. Our study showed that there is not a statistically significant association between the hemostatic and endothelial function gene polymorphisms studied and AMI. Raised levels of HCY, but not its main genetic determinant (homozygous MTHFR gene mutation), resulted an important risk factor.

P004

HIGH PREVALENCE OF G894T, BUT NOT T-786C, ENOS POLYMORPHISM IN CAROTID ATHEROSCLEROSIS PATIENTS

Pratesi G, Gensini F,* Sticchi E,* Fatini C, Dorigo W, Rostagno C, Sofi F, Pratesi C, Gensini GF, Abbate R

*Dipartimento Area Critica Medico Chirurgica; *Dipartimento di Fisiopatologia Clinica, Unità di Genetica Medica; Università di Firenze; Centro Trombosi A.O. Careggi, Florence, Italy*

Nitric oxide synthesized by endothelial nitric oxide synthase (eNOS) plays a key role in vascular tone regulation and atherosclerosis. Recently a Glu298Asp (G894T) and a T-786C polymorphism in exon 7 and in the promoter region of the eNOS gene have been identified; these polymorphisms may modulate eNOS activity and NO production. The aim of this study was to investigate their prevalence in patients with carotid atherosclerosis, detected with Duplex ultrasound and magnetic resonance angiography and/or angiography. All patients underwent carotid endarterectomy. Sixty-nine patients (46 males and 23 females) and 81 control subjects (41 males and 40 females) were investigated. eNOS polymorphisms were studied by PCR-RFLP analysis. The T-786C polymorphism genotype distribution and allele frequency were comparable between patients and controls (p=0.4 and p=0.35). No association was found between –786C variant and carotid atherosclerosis. As far as concerns G894T polymorphism, a significant difference in allele frequency between patients and controls was observed (p=0.01). At univariate analysis 894T allele was significantly asoociated with the risk of disease. Our data show that G894T polymorphism, modulating eNOS activity, may influence mechanisms responsible for the pathogenesis of carotid atherosclerosis.

P005

HIGH PREVALENCE OF ANGIOTENSIN-CONVERTING ENZYME I/D AND ENDOTHELIAL NITRIC OXIDE SYNTHASE GLU298ASP POLYMORPHISMS IN SYSTEMIC SCLEROSIS PATIENTS

Fatini C, Gensini F,* Sticchi E,* Lapini I, Evangelisti L, Angotti C,° Fedi S, Abbate R, Matucci Cerinic M°

*Dipartimento Area Critica Medico Chirurgica; *Dipartimento di Fisiopatologia Clinica, Unità di Genetica Medica, °Dipartimento di Medicina Interna, Sezione di Reumatologia; Università di Firenze; Centro Trombosi A.O. Careggi, Florence, Italy*

Systemic sclerosis is characterized by progressive microvascular occlusion and fibrosis and by an imbalance of the fibrinolytic system. *In vivo* and *in vitro* studies suggest a role for the renin angiotensin system in the regulation of vascular fibrinolytic balance. Angiotensin II increases the production and secretion of plasminogen activator inhibitor-1 and the angiotensin converting enzyme (ACE) contributes to reduced production of tissue-plasminogen activator and to endothelial nitric oxide synthesis by bradykinin degradation. The aim of our study was to investigate the role of ACE insertion/deletion (I/D) and endothelial nitric oxide synthase (eNOS) Glu298Asp (G894T) polymorphisms in systemic sclerosis patients and to evaluate their association with the clinical features of the disease. Seventy-three consecutive patients (47 with limited and 26 with diffuse cutaneous systemic sclerosis) and 112 control subjects were studied. ACE I/D and eNOS G894T polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism analysis. A significant difference in ACE I/D and eNOS G894T polymorphism genotype distribution and allele frequency between patients and controls was observed. A significant association between ACE D and eNOS 894T allele and systemic sclerosis was found (ACE: OR=3.4 p=0.003 and eNOS: OR=1.9 p=0.04). The contemporary presence of ACE D and eNOS 894T allele increased the risk for systemic sclerosis (OR=5.9 p<0.0001). *Conclusions*. Our findings of an increased risk of systemic sclerosis in ACE D and eNOS 894T allele carriers suggest a contribution of these polymorphisms to the pathogenesis of the disease.

P006

ASSOCIATION OF PLA1/A2 POLYMORPHISM OF ITGB3 GENE WITH EARLY FETAL LOSS

Ruzzi L,° Abeni D,* Silvestri L,° Fusco A,° Semeraro ML,° Ciarafoni I°

*°Clinical Pathology, *Clinical Epidemiology Unit, Istituto Dermopatico dell'Immacolata IDI, IRCCS, Rome, Italy*

The platelet-specific antigen PLA1 is an epitope of glycoprotein-IIIa (GPIIIa) which forms, with glycoprotein-IIb (GPIIb), the fibrinogen receptor on the platelet's surface. Recent data suggest that PLA2 polymorphism of GPIIIa may be associated with increased platelet aggregability and risk of cardiovascular disease. Since pregnancy may be compromised by disorders of hemostasis associated with arterial thrombosis, and to investigate the relation between early miscarriages and the C1565T substitution in exon 2 of the GPIIIa coding gene, we introduced PLA1/A2 polymorphism analysis in the molecular screening for the diagnosis of inherited thrombophilia. We considered 34 consecutive case patients who had fetal loss during the first or sec-

ond trimester of gestation referred to our laboratory to evaluate the presence of factor V G1691A and prothrombin G20210A mutations and 31 control women who had normal pregnancies without thromboembolic complications. Of the 34 cases, 9 have been excluded because they were lupus anticoagulant and/or autoantibodies positive and 1 for the presence of endometriosis. Other causes of hereditary thrombophilia were not evident. Among cases, 12 women were A1A1 homozygous (48%), 9 were A1A2 heterozygous and 3 were A2A2 homozygous, while among controls these figures were 26 (83%), 5, and 0, respectively. We estimated the association between fetal loss and HPA1/A2 polymorphism of GPIIIa and the resulting odds ratio was 4.2 (95% confidence interval 1.1 to 16.6, p=0.016). No association was observed for factor V and prothrombin mutations. Although we studied a small sample of cases and controls, we observed a strong and statistically significant association between C1565T polymorphism of ITGB3 gene and early miscarriages. To the best of our knowledge, this is the first report of such an association. Although these results should be confirmed with larger studies, this could be a promising diagnostic and/or prognostic factor for fetal loss.

P007

ANALYSIS OF T-786C AND GLU298ASP POLYMORPHISMS ON THE ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE IN GLAUCOMA PATIENTS

Sticchi E,* Renieri G,° Fatini C, Gensini F,* Fedi S, Ucci F,° Vannozzi L,° Galassi F,° Abbate R

*Dipartimento Area Critica Medico Chirurgica; *Dipartimento di Fisiopatologia Clinica, Unità di Genetica Medica; °Dipartimento di Scienze Oto-Neuro-Oftalmologiche; Università di Firenze; Centro Trombosi, A.O. Careggi, Florence, Italy*

Endothelium-derived substances are important regulators of the microvasculature; nitric oxide (NO), which is synthesized by endothelial nitric oxide synthase (eNOS), is a potent modulator of vascular tone in the human ophthalmic artery, which is normally in a state of constant vasodilatation due to the actions of NO. NO is an important mediator of homeostatic processes in the eye, such as regulation of aqueous human dynamics, retinal neurotransmission and phototransduction. Changes in its generation or actions could contribute to pathological states such as degenerative disease glaucoma. In the human eNOS gene many polymorphisms have been identified; a T/C point mutation at position –786 in the promoter region, suppressing eNOS transcription, has been recently described in association with coronary spasm. Moreover, a Glu298Asp amino acid substitution in exon 7 (G894T) has been associated with the risk of coronary artery disease. In this study we investigated the role of these polymorphisms in 53 glaucoma patients (30 males and 23 females) matched with 60 control subjects (35 males and 25 females). eNOS polymorphisms were examined by RFLP analysis. Our data document no difference in genotype distribution and allele frequency between patients and controls when both polymorphisms are considered (T-786C: p=0.25 and p=0.53; G894T: p=0.3 and p=0.15). These preliminary results show no role of these polymorphisms in glaucoma, but the increase of the sample size will permit a better evaluation of their impact on the pathogenesis of the disease.

P007a

IN VIVO PLATELET ACTIVATION IN SUBJECTS WITH MUTATIONS IN THE GENES OF FACTOR V AND PROTHROMBIN

Margaglione M,° Dragani A,* Grandone E,+ Falco A,^ Ciabattoni G, Di Minno G, Davì G^

Center of Excellence and Dept of Medicine and Aging, University of Chieti;^ Servizio di Prevenzione e Cure delle Malattie Emorragiche e Trombotiche, Ospedale Civile di Pescara; Genetica Medica, University of Foggia;° IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo,+ Italy*

A state of hypercoagulability, showed by markers of heightened thrombin formation such as F 1+2 and TAT complexes, is present in a large part of carriers of the factor V Leiden or prothrombin A20210 mutation. To gain insight into mechanisms responsible for the high risk of vein thromboembolism in carriers of FV Leiden or FII A20210 gene mutation, we investigated whether increased thrombin formation is related to thromboxane-dependent platelet activation in these settings. A population of 54 subjects, referred to two thrombosis centres located in the south of Italy (Pescara and San Giovanni Rotondo) to be investigated for thrombophilia and were diagnosed as carriers of the FVArg506Gln mutation (22 heterozygotes, 2 homozygotes), of the prothrombin G20210A mutation (25 heterozygotes, 2 homozygotes), and of both mutations (3 double heterozygotes), were investigated. Twenty-seven, age and sex-matched, healthy subjects were also studied. None of the patients was on anticoagulant or antiplatelet treatment at the time of blood sampling. Carriers of FII A20210 and FV Leiden mutations had significantly higher F1+2 levels, 1.51±1.10 nM (range: 0.4-8.1), than age- and sex-matched healthy controls, 0.92±0.20 nM (range: 0.5-1.3) (p<0.001). Urinary 11-dehydro-TXB2 levels in heterozygous and homozygous carriers of both mutations were significantly higher, 611± 364 (range: 210-1447) pg/mg creatinine, than in non-carriers, 339±67 (range: 250-510), (p<0.01). A positive correlation between plasma F1+2 and urinary 11-dehydro-TXB2 levels was found in carriers of both mutations (Rho=0.402, p=0.0034) but not in controls (Rho=0.136, p>0.1). A positive correlation between plasma F1+2 and urinary 11-dehydro-TXB2 levels was found in carriers of either mutation: FV Leiden: Rho=0.475, p=0.019; FII A20210: Rho=0.441, p=0.021. Results of the present study suggest a possible relevance of platelet activation in deep venous thrombosis and the positive role of aspirin in preventing venous thromboembolism.

Posters
Vascular Risk Factors

P008

HIGH PREVALENCE OF METABOLIC AND IMMUNOLOGIC RISK FACTORS IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

Pratesi G, Fedi S, Sofi F, Pratesi C, Pulli R, Rogolino A, Marcucci R, Cellai AP, Alessandrello Liotta A, Prisco D, Abbate R, Gensini GF

Dip. di Area Critica Medico-Chirurgica, Università degli Studi di Firenze, Centro Trombosi, A.O. Careggi, Florence, Italy

In recent years new risk factors for atherosclerotic disease have been identified. The aim of this study was to evaluate the prevalence of hyperhomocysteinemia, high levels of Lp(a) and PAI-1 activity and aCL positivity in a single cohort of patients with peripheral arterial disease (PAD). One-hundred and twenty-six PAD patients (97 males and 29 females, mean age 68.9 years) and 198 age and sex-matched controls (153 males and 45 females, mean age 66.8 years) were enrolled. Hcy concentrations were evaluated by FPIA method, Lp(a) and aCL by ELISA and PAI-1 activity by a chromogenic method. In PAD patients Hcy plasma levels were significantly higher than in controls both in males (median:15.3 μmol/L vs 9.5 μmol/L) and in females (median:12.1μmol/L vs 8.4 μmol/L). Hyperhomocysteinemia defined as Hcy plasma levels above the 95th percentile of the control subjects was detected in 55/126 patients (43.7%) and in 8/198 (4%) controls. Lp(a) levels were significantly higher in PAD patients (median: 208.5 mg/L,) than in control subjects (median: 92 mg/L) and Lp(a) levels >300 mg/L were found in 50/126 (39.7%) patients with PAD and in 20/198 (10.1%) controls. Elevated PAI-1 plasma levels (>15 IU/mL) were detected in 33/126 (26.2%) patients and in 16/198 (8.1%) controls. aCL (IgG> 16.5 GPL and/or IgM> 6.5 MPL) were found in 44/126 (34.9%) PAD patients and in 6/198 (3.1%) controls. At multivariate analysis, adjusted for sex, age and the traditional cardiovascular risk factors, Hcy and Lp(a) levels and the presence of aCL remained independent risk factors for PAD (OR = 16.2, 95% CI = 5.2–50.6; OR = 6.9, 95% CI =2.7–17.8; OR = 22.9, 95% CI = 5.9-88.9, respectively, $p< 0.0001$). In conclusion, our results indicate an association between PAD and elevated Hcy and Lp(a) levels and suggest a role for the aCL as a risk factor for the PAD. The identification of these risk factors may offer a tailored strategy for secondary prevention.

P009

WEIGHT LOSS REDUCES PERSISTENT PLATELET ACTIVATION IN WOMEN WITH ANDROID OBESITY

Falco A,* Guagnano MT,° Ciabattoni G,§ Marinopiccoli M,* Nutini M,* Sensi S,° Patrono C,* Davì G*

**Center of Excellence on Aging and Departments of °Medicine & Aging and §Drug Sciences, "G. D'Annunzio" University, Chieti, Italy*

Background. Obesity, and in particular the degree of abdominal adiposity, is associated with enhanced cardiovascular morbidity and mortality, through mechanisms possibly linking the metabolic disorder to platelet and vascular abnormalities. We investigated the association of obesity with enhanced lipid peroxidation and platelet activation and its potential reversibility. *Methods.* Repeated blood and urine samples were obtained from 69 obese (body mass index >=28 kg/m^2), otherwise healthy women (25 with gynoid obesity and 44 with android obesity as defined by the waist/hip ratio) and 24 age-matched non-obese women. Plasma C-reactive protein, insulin and leptin levels, and urinary excretion of 8-iso-prostaglandin F2 α- and 11-dehydro-thromboxane B2 were measured by previously validated immunoassays. In order to assess the causal relationship between abnormal body weight and indices of lipid peroxidation and platelet activation, we also investigated the short-term effects of diet-induced weight loss in 20 women with android obesity. *Results.* Obese women had abnormally high levels of lipid peroxidation and platelet activation as compared to controls. Moreover, both urinary 8-iso-prostaglandin F2 α_and 11-dehydro-thromboxane B2 were significantly higher in android than in gynoid obesity. Based on multiple regression analyses, C-reactive protein levels and waist/hip ratio >=0.86 predicted the rate of *in vivo* lipid peroxidation, independently of insulin and leptin levels. Successful weight loss was achieved in 11 of 20 women with android obesity and was associated with statistically significant reductions in C-reactive protein (from 1.56±0.93 to 0.98±0.31 mg/L), urinary 8-iso-prostaglandin F2 α_ (from 469±131 to 330±60 pg/mg creatinine) and 11-dehydro-thromboxane B2 (from 1169±525 to 534±181 pg/mg). In contrast, the same indices remained unchanged in the 9 obese women who failed to reduce their body weight. *Conclusions.* Obesity, and in particular an abdominal fat pattern, is associated with enhanced lipid peroxidation and persistent platelet activation in otherwise healthy women. These abnormalities are driven by inflammatory triggers related to the degree of abdominal adiposity and are, at least in part, reversible following successful weight loss.

P010

C-REACTIVE PROTEIN AND PROGNOSIS IN ELDERLY PATIENTS WITH ISCHEMIC STROKE

Ceccarelli E,* Masotti L,° Donati C,* Forconi S,* Cappelli R*

**Istituto di Medicina Interna e Geriatria, Università di Siena; °U.O. di Medicina Interna, Ospedale di Cecina, Livorno, Italy*

Introduction. While in acute coronary syndromes elevated values of C-reactive protein (CRP) have been related to bad prognosis, in ischemic stroke this relation is unclear, especially in the elderly. *Aim of the study.* To evaluate the role of CRP on short and long-term prognosis in over 75-year old, elderly patients with acute ischemic brain attack. *Methods.* We retrospectively evaluated CRP values (turbidimetric method, cut-off < 0.3 mg/dL), performed within 24 hours from hospital admission, in 288 elderly patients, 185 females and 103 males with mean age±SD 82.92±6.78 years, discharged with a diagnosis of transient ischemic attack (TIA) or stroke. We studied the relation between CRP values and short-term prognosis (30-day mortality, length of hospitalization and physical disability measured by the modified Rankin scale score) and long-term prognosis (12-month mortality and re-hospitalization). *Results.* Mean values of CRP were significantly higher in patients with stroke with respect to patients with TIA (5,85 vs 3,24 mg/dL, $p<0.05$), in patients with

acute ischemic area documented by brain computerized tomography (CT) with respect to patients with chronic ischemic damage at CT or negative CT (6.16 vs 4.10 vs 2.74 mg/dL respectively) and in patients with hemodinamics carotid stenosis respect patients with not hemodynamic carotid stenosis or without carotid plaques (6.34 vs 3.95 vs 0.53 mg/dL respectively). Mean values of CRP resulted significantly higher in patients who died in the first 30-days from ischemic attack than in survivors (10.7 vs 4.3 mg/dL, $p<0.05$). Length of hospitalization and physical disability score rose with increasing values of CRP. CRP values also influenced the 12-month re-hospitalization for cerebrovascular events. We did not find a relation between CRP values and 12-month mortality. *Conclusions.* Our study, with the limitations of the retrospective studies, shows that elevated CRP at hospital admission is a negative prognostic marker in elderly patients with ischemic stroke, above all for short-term prognosis.

P011

LONG-TERM SAFETY AND EFFICACY OF HYDROXYUREA IN 25 YOUNG PATIENTS WITH HIGH-RISK ESSENTIAL THROMBOCYTHEMIA

Finazzi G, Ruggeri M, Rodeghiero F, Barbui T

Divisioni Di Ematologia, Ospedali Riuniti, Bergamo e Ospedale S. Bortolo, Vicenza, Italy

The optimal treatment of young patients with essential thrombocythemia (ET) and a high risk of bleeding and thrombosis is uncertain. There is concern that long-term use of hydroxyurea (HU) may be leukemogenic, but non-mutagenic alternatives, such as anagrelide or interferon, are costly, frequently associated with side-effects and of still unproven efficacy on hard clinical end-points. We report here our experience with the long-term use of HU in a consecutive cohort of young patients with high-risk ET. We identified 25 ET patients aged less than 50 (median 42, range 18-49 years) who started on HU therapy before 1.1.1997 and were previously untreated. Hydroxyurea was given for the occurrence of a major vascular event (13 cases, 52%) and/or platelet count persistently above $1,000x10^9$/L. The median platelet count at the start of HU was $933x10^9$/L (range $426-3,200x10^9$/L). Therapy was aimed at maintaining platelet count below $600x10^9$/L or below $400x10^9$/L in those patients who had thrombosis with a platelet count between 400 and $600x10^9$/L. After 8 years' median follow-up (range 5-14 years), no patient had to withdraw the drug for intolerance or adverse effects. One case of transient ischemic attack was registered (4%) but no major thrombosis, bleeding, leukemic or neoplastic transformation or death occurred. These data compare well with those recently reported for the long-term use of anagrelide in a similar population of 35 young ET patients (median age 38, range 17 to 48 years) followed for a median of 10.8 years (range 7-15), showing a 20% rate of thrombosis, 20% of major bleeding and 24% of anemia (*Storen and Tefferi, Blood 2001; 97:863*). Until comparative studies are published, HU remains a first-choice drug also in younger patients with high-risk ET.

P012

HEMOSTASIS IN SUBJECTS WITH A FAMILY HISTORY OF TYPE 2 DIABETES AND HYPERTENSION

Trifiletti A, Lasco A, Scamardi R, Cincotta M, Gaudio A, Barbera N, Frisina N

Department of Internal Medicine, University of Messina, Italy

First-degree relatives of type 2 diabetic and/or hypertensive patients are at increased risk of cardiovascular diseases. The aim of this study was to verify some possible hemostatic alterations in first-degree relatives of type 2 diabetic, normotensive and hypertensive, patients. In 78 non-diabetic, normotensive first-degree relatives of type 2 diabetic (47 normotensive and 31 hypertensive) patients and in 36 normoglycemic, normotensive subjects with no family history and/or hypertension, we evaluated plasma levels of fasting glucose and insulin, tissue-type plasminogen activator (t-PA), plasminogen activator-inhibitor (PAI-1), D-dimer (DD) and prothrombin fragment 1+2 (F1+2). Insulin resistance, calculated by the HOMA model, and plasma levels of t-PA and PAI-1 were significantly higher in diabetics' relatives than in controls. As far as the thrombin activation indices are concerned we detected a significant increase of DD and F1+2 in hypertensive diabetics' relatives compared to other study subjects. In conclusion our data indicate that familial predisposition influences the hemostatic system in first-degree relatives of diabetic and/or hypertensive patients.

P013

INCREASED PROTHROMBOTIC POTENTIAL OF BLOOD MONONUCLEAR CELLS DURING *HELICOBACTER PYLORI* INFECTION

Semeraro N, Binetti BM, Pentimone A, Rossiello MR, Di Leo A,* Berloco P,* Gresele P,° Momi S,° Colucci M

*Dipartimento di Scienze Biomediche, Università di Bari; *IRCCS "S. de Bellis", Castellana; °Dipartimento di Medicina Interna e Medicina Vascolare, Università di Perugia, Italy*

Helicobacter pylori (HP) and some HP virulence factors have been recently shown to activate human mononuclear cells (MNC) for the production of tissue factor (TF) and plasminogen activator inhibitor-2 (PAI-2). To assess whether HP influences the cell procoagulant-fibrinolytic potential *in vivo*, we evaluated the production of TF and PAI-2 by blood MNC in 61 patients with chronic gastritis, who were categorized into HP-positive (31 patients, 13 men, aged 25-75y, mean 45) and HP-negative (30 patients, 12 men, aged 21-77y, mean 42), by a commercial urease test (CLO test) and PCR. In the same subjects we also measured plasma levels of t-PA, PAI-1, TAFI, thrombus precursor protein, D-dimer, lipoprotein(a) and P-selectin, using commercial immunological assays. TF antigen expressed by MNC after incubation for 20 h at 37°C, in the absence of any stimulus, was significantly higher in HP-positive than in HP-negative patients (mean±SEM: 82±16.5 vs 46.4±5.3 pg/10^6 cells $p<0.05$). MNC TF was functionally active (as revealed by clotting assays) and TF activity was closely correlated with antigen levels (r=0.90). Moreover, PAI-2 antigen accumulated in the MNC culture medium after a 20-h incubation period at 37°C was significantly higher in HP-positive than in HP-negative patients (4.9±0.7 vs 2.9±0.5 ng/10^6 cells) and was significantly correlated with cell

TF (r=0.45, $p<0.01$). No differences between the two patients' groups were found in all the studied plasma parameters. Our data suggest that HP infection is associated with functional abnormalities of MNC resulting in the coordinate expression of TF and antifibrinolytic activity which may contribute to the inflammatory reaction of gastric mucosa elicited by HP. In addition, considering that blood-borne (leukocyte-derived) TF has been proposed as an important pathogenetic factor in thrombus development, the reported changes in cell coagulation-fibrinolysis balance may represent a possible link between HP infection and ischemic heart or brain disease.

P014

ADVANCED GLYCATION END PRODUCTS ACTIVATE ENDOTHELIUM THROUGH THEIR SIGNAL-TRANSDUCTION RECEPTOR. A MECHANISM FOR AMPLIFICATION OF INFLAMMATORY RESPONSES

Basta G, Lazzerini G, Massaro M, Simoncini T, Tanganelli P,* Fu CF,^ Kislinger T,^ Stern DM,^ Schmidt AM,^ De Caterina R°

*CNR institute of CIinical Physiology, Pisa, °Chair of Cardiology, G. D'Annunzio University, Chieti, Institute of Pathology, *University of Siena, Italy; ^Columbia University, New York, NY, USA*

Background. The products of non-enzymatic glycation and oxidation of proteins, the advanced glycation end products (AGEs), form under diverse circumstances such as aging, diabetes, and kidney failure. Recent studies suggested that AGEs may form in inflamed foci, driven by oxidation or the myeloperoxidase pathway. A principal means by which AGEs alter cellular properties is through interaction of AGEs with their receptor (RAGE) on endothelial cells, enhancing vascular activation. *Methods and Results.* AGEs, RAGE, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and E-selectin are expressed in an overlapping manner in human inflamed rheumatoid synovia, especially within the endothelium. In primary cultures of human saphenous vein endothelial cells, engagement of RAGE by heterogeneous AGEs or N-epsilon(carboxymethyl)lysine-modified adducts enhanced levels of mRNA and antigen for vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and E-selectin. AGEs increased adhesion of polymorphonuclear leukocytes to stimulated endothelial cells in a manner reduced on blockade of RAGE. *Conclusions.* AGEs, through RAGE, may prime proinflammatory mechanisms in endothelial cells, thereby amplifying proinflammatory mechanisms in atherogenesis and chronic inflammatory disorders.

P015

INHIBITION OF ENDOGENOUS NITRIC OXIDE IS SUFFICIENT TO INDUCE ENDOTHELIAL ACTIVATION AND POTENTIATES CYTOKINE INDUCTION OF ADHESION MOLECULE EXPRESSION

Lazzerini G, Basta G, Del Turco S, De Caterina R°

CNR Institute of CIinical Physiology, Pisa, °Chair of Cardiology, G. D'Annunzio University, Chieti, Italy

We investigated the effects of suppressing endogenous nitric oxide (NO) production by the NO synthase inhibitor L-monomethyl-arginine (L-NMMA) (0.5-10 mmol/L), given alone or in combination with interleukin (IL)-1α (0.05-0.5 ng/mL), on human umbilical vein endothelial cells (EC). The expression of vascular cell adhesion molecule-1 (VCAM-1, by enzyme immunoassay), barely detectable at baseline, was significantly increased by L-NMMA, with a plateau of expression occurring at 5 mmol/L. This was paralleled by a spotty, but clear-cut, increase in U937 monocytoid cell adhesion, as measured by a rotational adhesion assay. Maximum activation induced by L-NMMA alone never however exceeded 20% of the maximum response to IL-1α. When EC incubated with L-NMMA were stimulated with otherwise subthreshold concentrations of IL-1α (0.05-0.5 ng/mL), these determined a higher VCAM-1 expression than in the presence of L-NMMA alone. Supra-threshold IL-1α concentrations had an additive effect. Northen analysis indicated that VCAM-1 mRNA levels, not detected at baseline, were induced even by the sole L-NMMA treatment, and that the effects of L-NMMA and IL-1α were again at least additive. Electtrophoretic mobility shift assay for transcription factors NF-κB, AP-1, IRF-1 and GATA demonstrated NF-κB activation by the NO synthase inhibitor alone, and additive effects for its administration combined with IL-1α, while levels of the others transcription factor did not change with L-NMMA. Since however NF-κB activation alone is not sufficient for VCAM–1 expression, it is likely that inhibition of endogenous NO also activates some others unknown factors required for the VCAM-1 gene transactivation. These results support the view that the normal endothelial lack of reactivity towards circulating monocytes is the result of an active inhibition of endothelial activation by endogenous NO.

Posters
Homocysteine

P016

HOMOCYSTEINE LEVELS IN PATIENTS WITH MIGRAINE WITH AURA

Erba N,* Moschiano F,* De Micheli V,* Schieroni F,* D'Amico D, Ciusani E, Ariano C, Leone M, Grazzi L, Bussone G

*Ist. Neurologico "C.Besta", Milan, *Ospedale "L. Mandic", Merate, Lecco, Italy*

Migraine with aura (MA) has been established as an possible risk for ischemic stroke, in particular in young adults. There is some evidence that a thrombophilic state is present in MA. Mild hyperhomocysteinemia has been shown to be an independent risk factor for ischemic stroke but there is not a strong evidence of hyperhomocysteinemia in patients with MA. In this study we investigated the homocysteine plasma levels in 107 patients (35 M, 72 F, age 30.14±9.4) suffering for MA and in 87 healthy volunteers (46 M, 41 F, age 36±9). The diagnosis of MA was made according to the 1988 International Headache Society Classification of Headaches. Student t-test was used for statistical analysis. The results showed a significant difference in homocysteine levels between patients and controls (see Table below).

	Patients			Controls			p
	n.	x	sd	n	X	sd	
All	107	12.2	11.7	87	9.7	3.9	0.04
Females	72	9.71	7.3	41	8.2	1.9	n.s.
Males	35	17.36	16.55	46	11.29	4.69	0.039

When female and male subjects were considered separately, the statistical significance was confirmed only for males. Our results suggest a possible association between hyperhomocysteinemia and MA. Further studies are needed to confirm our data and to understand whether higher homocysteine levels may be one of the prothrombotic factors which lead to an increased risk for ischemic stroke in young adults with MA.

P017

HIGH FREQUENCY OF METHYLENETETRAHYDROFOLATE REDUCTASE GENE MUTATION IN DOWN SYNDROME

Testa S,* Manna A,° Cavalli P,# Riboli B,# Mazzei G,^ Galli L,^ Denti N,* Morstabilini G*

**Centro Emostasi e Trombosi e #Servizio di Citogenetica, Ospedale di Cremona; °Servizio di Ematologia, Ospedale di La Spezia; ^Laboratorio Analisi, Ospedale di Sospiro, Italy*

Trisomy 21, or Down's syndrome (DS), has received renewed attention as recent research has focused on meiotic non-disjunction and DNA methylation. Considering that the CBS gene is localized on chromosome 21 and is overexpressed in DS patients and that maternal MTHFR C677T polymorphism has been recently associated with an increased risk of fetal DS, we studied a DS population to evaluate MTHFR and CBS gene mutations and their correlation with t-Hcy, folate and vitamin B12 plasma levels. In a Mental Illness Institute (Cremona, Italy), 25 DS patients compared with 25 non-DS patients, cross-matched for age, sex, race, geographic area of birth, were enrolled in the study, after informed consent of the ethical committee. Mean age of the two groups was 45y (range 32-56y). Hcy, folate, vit. B12, MTHFR (677CT) and CBS (ins 68bp, T833C) genotype were evaluated. In DS patients the allelic frequency of MTHFR C677T transition was 52% (homozygosity was 36%), while in the control population the MTHFR C677T transition was 42% and 16% respectively. One case and one control were carriers of both CBS68bp insertion and the C677T transition, but this condition does not result in increased Hcy plasma level. Hcy, vitamin, B12 and folate plasma levels were not different between patient and control group. The main finding of the study is the significantly high frequency of homozygosity for the C677T allele of MTHFR in DS patients compared to in the control group. The common MTHFR C677T allele should not be involved in the high prenatal mortality of DS fetuses. Moreover, MTHFR C677T gene polymorphism was not associated with increased Hcy plasma level in DS patients, as expected. This result may suggest that, in many DS patients, CBS enzyme overexpression can reduce the higher homocysteine levels, due to impaired MTHFR activity.

P018

OXIDATIVE STRESS INDUCED BY HOMOCYSTEINE IN HUMAN PLATELETS: THE ROLE OF CALCIUM ELEVATION AND ARACHIDONIC ACID RELEASE

Signorello MG, Pascale R, Leoncini G

Department Of Experimental Medicine, Biochemistry Section, Genoa, Italy

High levels of homocysteine are associated with an increased risk of atherosclerosis and thrombosis. Moreover mild homocysteinemia is an independent risk factor in the development of arterial disease and venous thrombosis. The pathogenic mechanism of homocysteine has not been clarified. The amino acid could exert its pathogenicity through oxidative mechanisms. Furthermore the homocysteine effect on intracellular calcium elevation, arachidonic acid release and reactive oxygen species (ROS) formation in human platelets was investigated. Data show that platelet treatment with homocysteine increases intracellular calcium levels, the lower concentrations being more active than the higher. Moreover homocysteine induces arachidonic acid mobilisation through the activation of c-phospholipase A2 which is a calcium-dependent enzyme present in membrane phospholipids. The amount of arachidonic acid released from homocysteine is comparable to that produced by the calcium ionophore A23187, but is lower than that generated by collagen or thrombin. No cooperation between these agonists and homocysteine was shown. Moreover resting and activated platelets incubated with homocysteine accumulate ROS, suggesting the occurrence of oxidative stress in homocysteine-treated cells. In order to establish a relationship between arachidonic acid release and ROS formation, some experiments have been carried out in the presence of 5,8,11,14-eicosatetraynoic acid (ETYA), a known inhibitor of arachidonic acid metabolism or diphenyleneiodonium (DPI), an

inhibitor of NAD(P)H oxidase and nitric oxide synthase. ETYA reduces ROS formation of about 45%, while DPI is less potent, decreasing ROS formation by about 25%. In addition, the homocysteine autoxidation could also generate ROS but to a smaller extent. Data seem to indicate that arachidonic acid released and the subsequent NADPH oxidase activation induced by the free acid are the main sources of ROS accumulated in platelets treated with homocysteine and could be involved in the pathogenesis of arterial disease and venous thrombosis.

P019

VARIABILITY OF HOMOCYSTEINEMIA IN DIFFERENT POPULATIONS

Rossi L, Lucchetti A, Palla P,* De Marco S,** Carrai M,** Paci A,° Matteucci E,^ Giampietro O,^ Innocenti B

*^Dipartimento di Medicina Interna, Università di Pisa, Laboratorio Analisi Chimico Cliniche e Microbiologiche 1, *Centro Trasfusionale, **U.O. Medicina d'Urgenza, °U.O. Medicina Cardiovascolare, Azienda Ospedaliera Pisana, Italy*

Aim of the Study. Elevated fasting homocysteine (Hcy) levels are associated with an increased risk of thrombosis and with cardiovascular diseases. The range and distribution of Hcy plasma values are strongly influenced by demographic and behavioral variables, such as sex, age, folic acid supplementation and smoking habits. The aim of our study was to verify the presence of elevated (> 15 µmol/L) and high (>30 µmol/L) Hcy levels in patients with cardiovascular and metabolic disease in comparison with two groups of apparently healthy individuals. *Populations and methods.* We enrolled in this study 255 consecutive patients from two disease and two control groups: a) 26 inpatients with cardiovascular diseases; b) 86 patients with type I diabetes; c) 86 volunteer blood donors; d) 57 out-patients. Fasting plasma total Hcy levels from each subject were assayed by an automated FPIA test (Abbott IMx). *Results.* See Table below.

Group	Cardiovascular	Diabetes	Blood donors	Outpatients
N. patients	26	86	86	57
Mean age	64.6	41.9	36.6	45.3
% smokers	69.2	44.2	23.3	49.1
Male/female ratio	2.25	0.75	2.44	1.04
Hcy smokers (mean & s.d.)	14.62±10.85	13.96±15.26	17.31±13.14	13.94±5.14
Hcy non smokers (mean & s.d.)	14.18±4.28	10.96±8.91	13.17±8.27	11.46±4.58
Hcy males (mean & s.d.)	15.15±10.34	14.77±15.41	15.23±10.3	14.31±4.95
Hcy females (mean & s.d.)	12.98±4.89	10.41±8.65	11.43±7.57	11.15±4.54
Hcy total (mean & s.d.)	14.4±8.97	12.29±12.14	14.13±9.7	12.72±4.39
Hcy > 15 umol/L (# and %)	6 (23.1)	11 (12.8)	17 (19.8)	12 (21.1)
Hcy > 30 umol/L (# and %)	1 (3.8)	2 (2.3)	9 (10.5)	2 (3.5)

Conclusions. Hcy values were higher in males but not significantly higher in patients. Higher levels were found in smokers only in the two main groups. The highest rate of elevated Hcy was found among blood donors (10.5% with levels >30 µmol/L). This suggests the appropriateness of periodic monitoring of Hcy in apparently healthy populations.

P020

PLASMA HOMOCYSTEINE IN FAMILIES OF TYPE 1 DIABETIC PATIENTS

Rossi L,* Lucchetti A,* Grandi G, Mariani S, Fagnani F, Calvi D, Forotti G, Boldrini E, Giampietro O, Matteucci E, Innocenti B*

**Laboratorio Analisi Chimico Cliniche e Microbiologiche 1, Azienda Ospedaliera Pisana; Dipartimento di Medicina Interna, Università di Pisa, Italy*

Background and Aims. Elevated plasma homocysteine is an independent risk factor for atherothrombotic disease. After the demostration that moderate hyperhomocysteinemia is associated with thrombosis, many laboratories are becoming interested in total homocysteine (tHcy) measurement. In type 1 diabetes mellitus, elevated total homocysteine (tHcy) seems to be concomitant with the onset of nephropathy. Prior to any renal involvement, plasma tHcy concentrations in type 1 diabetics have been reported either higher or similar or lower than in healthy control subjects. *Materials and Methods.* We measured by fluorescence immuno assay (FPIA, Imx system, Abbott Diagnostics) plasma tHcy levels of 60 healthy controls (age range 20-75 years), 30 type 1 diabetics (age 34±10 yr, no complications, n=10, retinopathy, n=10, nephropathy, n= 10), their non-diabetic relatives (47 parents, 60±10 yr; 36 siblings, 39±13 yr). *Results.* Blood levels of tHcy were higher in healthy control men than in women (geom. mean 13.7 vs 10.5 µM, $p<0.001$), and in smokers and ex-smokers than in non-smokers (13.2, 13.2, 10.9, respectively, $p<0.05$). Plasma tHcy was lower in type 1 diabetics than in control subjects (10.1 vs 11.8, $p<0.01$). Type 1 patients with nephropathy had higher tHcy than patients either with no diabetic complications or with retinopathy (12.4, 9.5, 8.8, $p<0.05$). There was no difference in plasma tHcy between first-degree relatives of type 1 diabetic patients and sex-age-matched controls (11.9 in siblings vs 11.6 in matched controls; 13.5 in parents vs 12.1 in controls). In type 1 diabetes mellitus, plasma tHcy showed a highly significant association (multiple R 0.96, $p<0.001$) with: serum creatinine, SGOT, mean arterial pressure (positive association), lipoprotein (a), bilirubin, copper (negative association). *Conclusions.* 1) in healthy subjects, male gender and smoking are associated with high tHcy levels; 2) tHcy is lower early in the course of type 1 diabetes mellitus (accelerated hepatic transsulfuration?); 3) increased tHcy levels are associated with diabetic nephropathy.

P021

GENETIC DETERMINANTS OF HYPERHOMOCYSTEINEMIA IN PATIENTS WITH RETINAL VEIN OCCLUSION

Marcucci R, Corsini I, Betti I, Giusti B, Brunelli T, Coppo M, Sodi A,* Cappelli S,* Abbate R, Prisco D

*Dipartimento Area Critica Medico-Chirurgica,Università di Firenze; *Clinica Oculistica, Università di Firenze; Centro Trombosi, AO Careggi, Florence, Italy*

A prevalence of about 30% of high levels of fasting homocysteine (Hcy) in patients with retinal vein occlusion (RVO) has been consistently reported. No data are present in the literature on the prevalence of 844ins68 polymorphisms of cystathionine β-synthase (CBS) gene in patients with RVO, and conflicting results exist on the role of C677T polymorphism of methylenetetrahydrofolate reductase (MTHFR) gene. We studied the

prevalence of HyperHcy and its genetic determinants in 55 consecutive patients (24 M/31 F; age: 57 (18-82)) with RVO and in 61 controls (31 M/30 F; age: 55 (16-80)), age and sex-matched. Hcy plasma levels were significantly higher in patients than in controls (12.8 (4.6-9.0) vs 8.7 (5.1-24); p<.001). Hyperhomocysteinemia, defined as a concentration of Hcy above the 95th percentile of controls, was diagnosed in18/55 patients (32.7%) and in 3/61 controls (4.9%). At univariate analysis the OR for RVO associated with hyperHcy was: 36.5 (11-110); p<.0001). The distributions of CBS and MTHFR polymorphisms were in Hardy-Weinberg equilibrium, both in patients and in controls. The distribution of 84ins68 variant of CBS did not significantly differ between patients and controls (patients: +/- 8/55 (14.6%), -/- 47/55 (85.4%)/ controls: +/- 9/61 (14.7%), -/- 52/61 (85.3%); p=ns) and did not affect Hcy plasma levels both in patients and in controls (patients: +/- =12.4+-4.9 μmol/L; -/- =14.9+-8.2 μmol/L; p=ns/ controls: +/- =8.3+-5.9 μmol/L; -/- =8.5+-3.7 μmol/L; p=ns). The distribution of C677T polymorphism of MTHFR significantly differed between patients and controls (patients: +/+ 21/55 (38.1%), ±28/55 (50.9%), -/- 6/55 (11%)/ controls: +/+ 9/61 (14.7%), ±34/61 (55.7%), -/- 18/61 (29.6%); p<.005) and affected Hcy plasma levels in patients or in controls (patients: +/+=19.9+-9.7 +/-=11.5+-3.8 μmol/L; -/- =9.7+-3 μmol/L; p<.005/ controls: +/+= 10.2+-5.3; +/- =8.2+-4.3 μmol/L; -/- =8.1+-2.5 μmol/L; p<.005). These preliminary results confirm the high prevalence of hyperhomocysteinemia in RVO patients but exclude a role of a genetic determinant of RVO.

P022

MEDITERRANEAN DIET PHENOLIC ANTIOXIDANTS DECREASE HOMOCYSTEINE-INDUCED ENDOTHELIAL ACTIVATION

Carluccio M, Ancora MA, Massaro M,[#] Carluccio M,* Visioli F, Distante A,[°] Storelli C,[#^] De Caterina R

*CNR Institute of Clinical Physiology, Lecce and [#]Pisa; Universities of *Milan and [°]Lecce, ^"G. D'Annunzio" University, Chieti, Italy*

Part of the beneficial cardiovascular effects of Mediterranean diets is attributed to the high folate content of such diets, thus reducing homocysteine levels. We sought to determine whether homocysteine promotes the expression of endothelial leukocyte adhesion molecules and tested the hypotheses that a redox-sensitive mechanism is involved and that Mediterranean diet antioxidants influence such early events in atherogenesis. We examined the expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin by surface enzyme immunoassays (EIAs) in human umbilical vein endothelial cells treated with homocysteine (from 10 to 500 μmol/L) or cysteine as control, for 4 to 36 h. Known inducers of adhesion molecules such as bacterial lipopolysaccharide (LPS), cytokines or phorbol myristate acetate (PMA) served as positive controls. In the same settings, cells were also pre-incubated with the dietary antioxidants oleuropein and trans-resveratrol (10-50 μmol/L). The activation of the transcription factor nuclear factor-κB (NF- κB) was assessed by electrophoretic mobility shift assay. The exposure of endothelial cells to homocysteine concentration-dependently increased adhesion molecule expression, without exerting any toxicity. VCAM-1 and E-selectin expression were already increased (50±15% of maximal PMA responses, p<0.01 vs negative control) at 50 μmol/L homocysteine, while increases in ICAM-1 (similar to that induced by LPS or PMA, p<0.01 vs negative control) only occurred after treatment with 500 μmol/L. At the same concentrations, cysteine was ineffective. Co-treatment of HUVEC with oleuropein or trans-resveratrol suppressed homocysteine-induced expression of adhesion molecules and, in parallel, reduced NF-κB activation. In conclusion, homocysteine, in the concentration range achievable in hyperhomocysteinemia, induces the expression of endothelial adhesion molecules presumably by a pro-oxidant mechanism as suggested by the neutralizing effect of two structurally unrelated polyphenolic dietary antioxidants. These results indicate a novel mechanism of induction of vascular disease by homocysteine and the possibility that Mediterranean diets counterbalance some homocysteine vascular effects independent of folate levels.

P023

ELEVATED PLASMA HOMOCYSTEINE BUT NOT 677TT METHYLENETETRAHYDROFOLATE GENOTYPE CONTRIBUTES TO JUVENILE ISCHEMIC STROKE

Brancaccio V, Ames PRJ, Mandarini A,* Iannaccone L, Fasanaro AM,* Scenna G, Margaglione M°

*Unità Emostasi-Trombosi; *Divisione Neurologia, Ospedale "A. Cardarelli", Napoli; °Unità Aterosclerosi e Trombosi, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy*

Background. A C677T mutation in the methylene tetrahydrofolate reductase (MTHFR) gene codes for a thermolabile enzyme which has been linked to the occurrence of arterial and venous thrombosis. *Aims*. To evaluate the contribution of MTHFR genotypes and plasma homocysteine (HC) in patients with juvenile ischemic stroke (IS). *Methods.* MTHFR genotypes (PCR) were assessed in 122 patients (40±7 years) who suffered stroke before 50 years of age and in 100 healthy subjects (39±8 years). Plasma HC (EIA, BioRad) was measured in the same stroke patients and in 66 subjects from the control group. *ResuIts*. The prevalence of the homozygous 677TT MTHFR was 27% in the IS group and 16% in the control group (p=0.05). Overall mean (±SEM) plasma HC was higher in the IS than in the control group (12.4±0.9 vs 9.6±0.7 μmol/L) (p=0.04). When analyzed by genotype, mean plasma HC was significantly different across the four groups, IS with 677TT MTHFR (18.7±2.7 μmol/L), IS with C677T MTHFR and non mutated (9.6±0.4 μmol/L), controls with 677TT (15.9±2.0 μmol/L) and controls with C677T and non-mutated (7.2±0.3 μmol/L)(p< 0.0001, Kruskal-Wallis). Dunn's *post hoc* analysis showed that mean plasma HC in IS patients with 677TT MTHFR genotype was significantly higher than in IS patients with C677T MTHFR and non-mutated (p<0.05). A similar pattern was seen for IS patients with C677T MTHFR and non-mutated versus controls with same genotype (p<0.01). *Conclusions*. Elevated plasma HC contribute to IS not only in patients with 677TT MTHFR but also in patients heterozygous and non mutated. Measurement of plasma HC may identify patients at a high risk of IS requiring HC lowering.

P024

ENDOTHELIAL MARKERS IN HYPERHOMOCYSTEINEMIC PATIENTS WITH VASCULAR DISEASES AT BASELINE AND AFTER VITAMIN SUPPLEMENTATION

Paoletti O, Martini G, Del Bono R, Volpi R, Pontoglio S, Adinolfi G, Bani P, Negrini R, Caimi L

2° Laboratorio Analisi Chimico Cliniche, 3° Laboratorio Biotecnologie, Spedali Civili, Brescia, Italy

Background. Hyperhomocysteinemia is a risk factor for atherosclerosis and venous thromboembolism. Vitamin B12, B6 and folic acid supplementation have been shown to decrease plasma homocysteine (tHcy). Soluble thrombomodulin (TM) and E-selectin (sE-sel) are established markers of endothelial damage; both are increased in peripheral arterial and venous diseases and in hyperhomocysteinemic patients. *Aim of the study.* We measured TM and sE-sel in patients with arterial or venuos vascular disease with hyperhomocysteinemia and after 3 months of daily vitamin supplementation in a subgroup of hyperhomocysteinemic patients. *Methods.* Thirty-five hyperhomocysteinemic subjects (tHcy >15 μmol/L) were selected (group A): 14 males (mean age 50.5 years, range 31-65) and 21 females (mean age 41.1 years, range 24-71), with clinical arterial or venuos vascular disease. Thirty-five matched non-hyperhomocysteinemic patients (group B), with clinical arterial and venous diseases, were taken as controls. A subgroup of 15 hyperhomocysteinaemic patients were tested after 3 months of daily vitamin supplementation (5 mg of folic acid, 500 μg of vitamin B12 and 250 μg of vitamin B6). TM and sE-sel were measured by ELISA (Thrombomodulin-Diagnostica Stago, France and sE-selectin Bender MedSystem Diagnostic, Wien, Austria). Mann-Whitney U unpaired test and Wilcoxon matched pairs test were used for statistical analysis. *Results.* Results are shown in Tables 1 and 2. TM and sE-sel inter-assay CVs were 3.90% (range = 13.12-14.46) and 5.40% (range = 82.9-95.9) respectively. Intra-assay CVs were about the same.

Table 1. Group A vs Group B.

	Group A (n. 35) (mean±SD)	Group B (n.35) (mean±SD)	Difference mean (95% CI)
tHcy (μmol/L)	23.29±9.83	6.66±1.82	16.63 (13.3; 20)*
sTM (ng/mL)	13.55±9.57	7.05±4.63	6.5 (2.7; 9.9)+
sE-sel (ng/mL)	50.06±23.89	35.56±16.74	14.5 (4.9; 24.5)§

*p< 0.0001; +p< 0.0001; §p< 0.01

Table 2. Baseline (T0) and after 3 months of vitamin supplementation (T+).

	T0 (n. 15) (mean±SD)	T+ (n. 15) (mean±SD)	Difference mean (95% CI)
tHcy (μmol/L)	32.58±13.83	7.6±3.04	21.32 (17.4; 32.4)*
sTM (ng/mL)	18.27±13.07	10.32±7.78	6.94 (0.14; 15.75)*
sE-sel (ng/mL)	50.8±17.32	40.82±16.14	10.2 (-2.3; 21.3)*

*p< 0.05

Conclusions. TM and sE-sel are sensitive markers of endothelial damage in hyperhomocysteinemic patients with clinical vascular diseases; their levels are correlated with tHcy concentrations as they decrease after vitamin supplementation and tHcy fall. This fact might be correlated to an effective improvement of the endothelial damage after therapy. These markers could be useful in the follow-up of hyperhomocysteinemic patients and to establish more cost-effective therapeutic regimens.

P025

HYPERHOMOCYSTEINEMIA AND VENOUS THROMBOEMBOLIC DISEASE

Iannaccaro P, Santoro R, Luise F,* Piromalli A,* Papaleo G, Sottilotta G,* Trapani Lombardo V,* Muleo G

*Hemophilia Centre, Azienda Ospedaliera "Pugliese-Ciaccio", Catanzaro; *Hemophilia Centre, Azienda Ospedaliera "Bianchi-Melacrino-Morelli", Reggio Calabria, Italy*

Venous thromboembolic disease in young patients is frequently associated with prothrombotic genetic polymorphisms. Factor V Leiden and the prothrombin G20210A mutation are the prevalent defects in the general population. In recent years, many studies indicated mild-moderate hyperhomocysteinemia as a risk factor of thromboembolic diseases. Whether hyperhomocysteinemia is a risk factor for venous thrombosis is still controversial. In addition, studies on prevalence of the homozygous methylene-tetrahydrofolate reductase (MTHFR) thermolabile variant (C677T mutation) in patients with venous thrombosis gave conflicting results. We report 16 patients (9 males, 7 females), referred to our institutions with objectively diagnosed deep vein thrombosis (DVT, 18 episodes) and pulmonary embolism (PE, 5 episodes) and hyperhomocysteinemia (fasting homocysteine above the upper limit of the normal range, 15 μmoles/L, or post-methionine loading homocysteine above the 95th centile of distribution in healthy subjects, 33 μmoles/L). Congenital or acquired thrombophilia (defects of coagulation inhibitors antithrombin III, protein C, protein S, factor V Leiden mutation, prothrombin G20210A mutation, lupus anticoagulant) were ruled out. Factor VIII:C levels were normal. The median age at the diagnosis of DVT was 39 years (range 22-70). Six episodes of DVT were associated with enviromental risk factors such as orthopedic surgery (2), general surgery (1), cesarean section and post-partum (1), plasters (2). The median fasting homocysteine at diagnosis was 30 μmoles/L (range 18-60); in one patient with a history of DVT of leg and post-partum splenic vein thrombosis, hyperhomocysteinemia was shown by methionine loading. Of course, acquired causes of hyperhomocysteinemia were ruled out. Fourteen out of sixteen patients were homozygous for the C677T mutation of MTHFR gene. The retrospective characteristics of our evaluation and the absence of a control population do not allow us to draw firm conclusions. However, our experience suggests that measurement homocysteine levels is useful in young patients with a history of DVT, even if an enviromental risk factor is present.

P026

PLASMA HOMOCYSTEINE LEVELS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Gianotti A

Servizio di Patologia Clinica, Ospedale S. Carlo, ASL 3, Genoa, Italy

Background. Elevated plasma homocysteine (tHcy) is a significant risk factor for vascular disease including heart disease and stroke since it limits the bioavailability of nitric oxide, increases oxidative stress, stimulates smooth-muscle cell proliferation, changes the elastic properties of vessel walls and generates a prothrombotic state through the activation of factor V. Hyperhomocysteinemia has been shown to correlate with genetic store, diet, vitamin (B6, B12, folate) status and directly with increasing age. *Patients and Results.* We studied, by immunoassay (Immulite 2000), the fasting plasma homocysteine in two groups: 17 COPD (chronic obstructive pulmonary disease) patients versus 17 healthy controls (14 men and 3 women, 50 to 62 years of age) bronchitics and 3 were intrinsic asthmatics (15 men and 2 women, 51 to 63 years of age). The former group showed significantly higher tHcy levels (11.1±5.6 μmol/L±SD) than the latter (8.4±4.2 - $p<0.05$). *Conclusions.* The reported data focus on the importance of monitoring plasma homocysteine levels in COPD patients. It is known that smoking habit, theophylline use and coffee abuse (common factors in these patients) reduce B6 levels; as the biochemical conversion of homocysteine to cysteine is dependent upon two consecutive vitamin B6-dependent reactions, we think that in COPD patients B6 supplementation can reduce the possibility of homocysteine-correlated vascular disease.

Posters
Diagnosis and Treatment of Atherothrombosis

P027

INSULIN ENHANCES VASCULAR CELL ADHESION MOLECULE-1 IN HUMAN ENDOTHELIAL CELLS IN CULTURE

Madonna R,° Pandolfi A,* Pellegrini G,* Lazzerini G,§ Consoli A,* De Caterina R°§

*°Chair of Cardiology, *Department of Medicine and Aging Science, University "G. d'Annunzio" Chieti; §CNR Institute of Clinical Physiology, Pisa, Italy*

Hyperinsulinemia has been proposed among the causes of accelerated atherosclerosis in non-insulin-dependent diabetes mellitus (NIDDM). Inflammation and the subsequent modulation in the expression of several endothelial leukocyte adhesion molecules have a critical role in the initiation and progression of atherosclerosis. We hypothesized that insulin may directly affect the expression of endothelial leukocyte adhesion molecules in the endothelium and, particularly, the expression of vascular cell adhesion molecule-1 (VCAM-1), deeply involved in early atherogenesis. Human umbilical vein endothelial cells, at passage 4-5, were exposed to insulin concentrations ranging from 0.01 to 100 nmol/L, thus covering a range from low physiological to high pharmacological levels. After 16 hours of incubation, VCAM-1 expression was assessed by cell surface immunoassay (EIA). At none of the concentrations tested, did insulin show any cytotoxicity. VCAM-1 levels (mean±SD, with n=3 replicates in each condition) at the various insulin concentrations, expressed as percent of control, were as follows:

* = $p<0.05$ vs control

Thus, insulin significantly increased VCAM-1 expression at concentrations of 0.1-10 nmol/L. In conclusions, in cultured human endothelial cells insulin affects the expression of VCAM-1. This might increase vascular inflammation *in vivo* and thereby foster atherosclerosis in hyperinsulinemic subjects with NIDDM.

P028

MEDITERRANEAN DIET PHYTOCHEMICALS INHIBIT ENDOTHELIAL ACTIVATION THROUGH INTERFERENCE WITH REDOX-SENSITIVE TRANSCRIPTIONAL FACTORS

Carluccio MA, Siculella L,° Ancora MA, Massaro M, Scoditti E, Visioli F,* Distante A, Storelli C,° De Caterina R#°

*C.N.R. Institute of Clinical Physiology, Lecce and #Pisa, *University of Milan and °Lecce, ° "G. d'Annunzio" University, Chieti, Italy*

Epidemiologic studies suggest that Mediterranean diets reduce the risk of cardiovascular disease. Monocyte adhesion to the endothelium is crucial in early atherogenesis, and redox-sensitive mechanisms are involved. The aim of our study was to evaluate whether typical Mediterranean diet phytochemicals affect endothelial leukocyte adhesion molecule expression and monocyte adhesion. *Methods and ResuIts.* We studied several phenols in Mediterranean foods, including oleuropein, hydroxytyrosol, tyrosol, and resveratrol, with or without antioxidant activity. Compounds (1-100 μmol/L) were incubated with human umbilical vein endothelial cells (HUVEC) for 30 minutes, followed by co-incubation with bacterial lipopolysaccharide or cytokines to trigger adhesion molecule expression. Only oleuropein, hydroxytyrosol and resveratrol, possessing a marked antioxidant activity, reduced monocytoid cell adhesion to stimulated endothelium. This correlated with the inhibition of vascular cell adhesion molecule-1 (VCAM-1) mRNA and protein expression, assessed by Northern analysis and cell surface enzyme immunoassay, respectively. The inhibition of VCAM-1 was paralleled by a reduction in the activation of the redox-sensitive transcription factors, nuclear factor-κB (NF-κB) and activator protein–1 (AP-1), at electrophoretic mobility-shift assays. Trasfection studies using various VCAM-1 gene promoter constructs confirmed that phenolic antioxidants repressed VCAM-1 gene transcription, in part by inhibiting NF-κB. E-selectin and intercellular adhesion molecule-1 (ICAM-1) expression were similarly inhibited, indicating a generalized effect on endothelial cell activation. *Conclusions.* Mediterranean diet phytochemicals possessing antioxidant activity may inhibit early events in atherogenesis modulating endothelial gene expression, and can be exploited pharmacologically.

P029

CYCLO-OXYGENASE (COX)-DEPENDENT PLATELET ACTIVATION IS SPECIFICALLY INHIBITED BY LICOFELONE (ML3000), AN INHIBITOR OF COX-1, COX-2 AND 5-LIPO-OXYGENASE

Rotondo S, Krauze-Brzósko K, Manarini S, Martelli N, Evangelista V, Cerletti C

"G. Bizzozero" Laboratory of Blood and Vascular Cell Interactions, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy

Licofelone is an inhibitor of COX-1, COX-2 and 5-lipo-oxygenase in bovine and human platelets and granulocytes *in vitro*. It was specifically developed searching for an analgesic and anti-inflammatory compound with higher gastrointestinal safety than the classical NSAIDs and with advantages over selective COX-2 inhibitors. We investigated the effect of licofelone on platelet activation induced by COX-dependent agonists, such as arachidonic acid (AA), and by agonists only partially dependent on COX activity, such as the combination collagen/adrenalin, thrombin, or its mimetic peptide TRAP. Platelet-rich plasma (PRP) or washed platelet suspensions were prepared from citrated human blood. Aggregation was studied in a lumiaggregometer, monitoring the changes in light transmission after stimulation. Expression of P-selectin and activation of glycoprotein IIbIIIa (GpIIb/IIIa) were studied by flow-cytometry using a specific anti-P-selectin monoclonal antibody and fluoresceinated purified human fibrinogen, respectively. Licofelone, at concentrations as low as 1 μM completely prevented platelet aggregation induced by threshold aggregating concentrations (TAC) of AA (0.87±0.14 mM, n=4) in PRP, and reduced by half platelet aggregation induced by TAC of collagen/adrenalin (0.4±0.3 μg/mL and 2.2±1.6 μM, respectively, n=4). In contrast, licofelone (up to 100 μM) did not affect aggregation stimulated by collagen/adrenalin in aspirin-treated PRP. Washed platelet aggregation induced by TAC of TRAP (20 μM) or of thrombin (0.25 U/mL), as well as TRAP-induced GpIIbIIIa activation and thrombin-induced P-selectin expression were only marginally affected by licofelone concentrations one or two orders of magnitude higher than those fully preventing AA-induced aggregation. This study demonstrates that licofelone specifically inhibits COX-dependent platelet activation. In fact, at very low concentrations it completely inhibits platelet aggregation induced by AA and partially prevents activation by the combination collagen/adrenalin. Failure of licofelone to prevent collagen/adrenalin-induced aggregation of aspirin-pretreated platelets strongly supports COX-1 as a specific target of licofelone. In contrast, licofelone at any concentration tested has substantially no effect on platelet activation induced by thrombin or TRAP.

P030

PHARMACOKINETICS AND BIOCHEMICAL SELECTIVITY OF TWO LOW DOSES OF ENTERIC-COATED ACETYLSALICYLIC ACID

Cerletti C, Dell'Elba G, Manarini S, Pecce R, Di Castelnuovo A, de Gaetano G*

*Istituto di Ricerche Farmacologiche Mario Negri, Consorzio Mario Negri Sud, Santa Maria Imbaro; *Center for High Technology Research and Education in Biomedical Sciences, Catholic University, Campobasso, Italy*

The pharmacokinetics and pharmacodynamic properties of two different doses (80 and 160 mg) of an enteric-coated formulation of aspirin (ASAFLOW®, Belgium) were evaluated in 16 normal subjects (34±5 years old, 9f,7m) after both one day and seven days of treatment. A randomized, cross-over study was designed and plasma levels (kinetics) of aspirin (ASA) and salicylate (SA), serum TxB2 production and urinary excretion of 11-dehydro-TxB2 and 2,3-dinor-6keto-PGF1a were measured. The kinetic parameters after both doses of aspirin show that absorption of ASA and SA was remarkably delayed in respect to after plain aspirin. Plasma levels of SA were about 10 times higher than those of ASA and showed a tendency to accumulate at day 7 after the higher dose. As SA blunts the pharmacological effect of ASA (*Circulation 1985; 72:1185*), the latter finding may be relevant in the long-term use of aspirin. At both doses serum TxB2 was markedly depressed: at 24 hours on day 1, 80 mg induced 68% average inhibition, while 160 mg about 80%, a small but significant difference ($p<0.05$). On day 7, serum TxB2 inhibition

averaged 95% and 99% after 80 and 160 mg, respectively, a small, but highly significant difference ($p<0.0003$). After 7 days of either dose, urine 11-dehydro-TxB2 was inhibited (by 61% and 77%, respectively; $p<0.0001$ in respect to basal values and between doses), but 2,3-dinor-6keto-PGF1a was not. Thus increasing ASA from 80 to 160 mg, that only results in a modest increase of inhibition of serum TxB2, greatly increases the inhibition of thromboxane production *in vivo*. However, even the higher dose of ASA was unable to completely suppress urinary excretion of the endogenous thromboxane metabolite. This might contribute to explaining the partial reduction in ischemic event rate associated with aspirin. In conclusion, low-dose ASA administered for 7 days to healthy volunteers appears to be an incomplete but selective inhibitor of endogenous thromboxane excretion, the dose of 160 mg daily being pharmacologically more effective than the dose of 80 mg daily.

P031

A PARADOXICAL INCREASE IN THE SURFACE EXPRESSION OF VARIOUS CELL ADHESION MOLECULES IN HUMAN ENDOTHELIAL CELLS AFTER TREATMENT WITH STATINS

Massaro M,* Madonna R,° Pandolfi A,° Consoli A,* Zampolli A,** Carluccio MA,* Basta G,** Storelli C,# De Caterina R°**

CNR Institute of Clinical Physiology, Lecce and **Pisa, #the University of Lecce, and °"G. d'Annunzio" University, Chieti, Italy*

By decreasing LDL levels, statins decrease the substrate for LDL oxidation and therefore one of the main triggers of atherosclerosis. Thus, *in vivo* treatment of patients with therapeutic doses of simvastatin decreases urinary 8-iso-PGF2α excretion, an *in vivo* index of lipid peroxidation (*De Caterina R et al., submitted*). However, despite the removal of a main trigger for adhesion molecule expression, therapeutic doses of statins do not decrease circulating levels of soluble adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), E-selectin and intercellular adhesion molecule-1 (ICAM-1) (*De Caterina R et al., data on file*). In order to clarify this apparent paradox we assessed the *in vitro* effects of one commonly used lipophilic statin, simvastatin, on the induced surface expression of three proinflammatory adhesion molecules, VCAM-1, ICAM-1 and E-selectin, and on endothelial nitric oxide synthase (eNOS) in human umbilical vein endothelial cells (HUVEC). *Methods*: Simvastatin (10-1000 ng/mL), activated *in vitro* by alkaline hydrolysis, was incubated with HUVEC for 0-24 h, followed by co-incubation with tumor necrosis factor-α (TNF), lipopolysaccharide (LPS), or advanced glycation endproducts (AGEs) for a further 12 h. After this time, VCAM-1, E-selectin and ICAM-1 expressions were assessed by cell surface immunoassays (EIA), and the eNOS protein levels determined by Western analysis.

	Simvastatin, 600 ng/mL		
Stimulus	VCAM-1 expression % of SE	E-Selectin expression % of SE	ICAM-1 expression % of SE
AGEs 200 mg/mL	153±23	217±20	128±10
LPS 1 µg/mL	150±10	130±13	133±14
TNFα 10 ng/mL	135±23	150±23	130±10

SE: stimulated expression.

Results and Conclusions. Pretreatment with simvastatin increased both the basal and the cytokine-downregulated expression of eNOS. Simvastatin also, slightly but significantly ($p<0.05$), boosted the expression of adhesion molecules induced by TNF, LPS and AGEs, as show in Table 1. These observations suggest the existence of an adverse pleiotropic effect of simvastatin on endothelial leukocyte adhesion molecule expression, likely counteracted *in vivo* by the decrease of LDL.

P032

WHICH STRATEGY TO CHOOSE FOR ANTI-THROMBOTIC MANAGEMENT OF CORONARY ARTERY DISEASE IN HEMOPHILIACS UNDERGOING A PERCUTANEOUS INTERVENTION?

Schiavoni M, Bovenzi F,* Ettorre CP, de Luca L,* De Luca I,* Ciavarella N

*Centro Emofilia, *UO di Cardiologia Ospedaliera, Policlinico-Università, Bari, Italy*

Coronary artery disease (CAD) represents the major cause of morbidity and mortality in the general population. Because of the improved expectancy of life in hemophiliacs, CAD is being observed more frequently also in these people and their need for cardiac interventions in increasing. There is currently little information about cardiovascular approaches to hemophilic people concerning the safest anti-thrombotic management before, during and after percutaneous coronary interventions (PCI) with stent implantation because of a higher risk of bleeding complications. Our personal experience regards a 74-year old severe hemophilic B patient who suffered from CAD which was susceptible to PCI. He received a bolus of F.IX concentrate (30 IU/kg b.w.) i.v. associated with 5,000 IU of unfractionated heparin immediately before cardiac catheterization. During stenting of the I.V.A. he experienced the onset of angina, the hemodynamic picture showed a fresh thrombus formation in the site of the stent with ECG modifications. GP IIb/IIIa blockers were given rapidly i.v. at standard dosage associated with a bolus of 2,000 IU of unfractionated heparin. The physical conditions of the patient progressively improved in about 1 hour, while the ECG returned to normal in a few minutes. The clinical outcome was optimal and he was discharged assuming clopidogrel for 1 month followed by aspirin (100 mg a day)as long term anti-thrombotic treatment. Neither more hemorrhagic events than usual nor thrombotic complications have observed after a 6-month follow up. Our single experience needs larger clinical information in order to choose a well standardized anti-thrombotic strategy for congenital coagulopathic patients undergoing PCI.

P033

EFFECT OF FIBRINOGEN CONCENTRATION AND PLATELET COUNT ON THE INHIBITION OF PLATELET AGGREGATION BY GLYCOPROTEIN IIB-IIIA INHIBITORS

Rocca B,° De Cristofaro R,° Renda G,# Landolfi R°

°Research Center for Physiopathology of Haemostasis, Catholic University School of Medicine, Rome, #Dept. of Cardiology, University of Chieti, Italy

Drugs inhibiting the binding of fibrinogen to its platelet receptors, the glycoprotein (Gp) IIbIIIa, are powerful antithrombotic

compounds, belonging to different classes based on their mechanism of action: RGDS-like peptides compete with fibrinogen to bind GpIIb-IIIa, while antibody molecules directly block the receptor. We explored whether plasma fibrinogen or von Willebrand factor concentrations and platelet counts affect the degree of *in vitro* platelet aggregation inhibition exerted by two different classes of inhibitors, by an RGDS-like compound (Tirofiban®) and an antibody-like compound (ReoPro®). Platelet aggregation was studied in whole blood by the Platelet Function Analyzer (DADE International) with ADP/collagen cartridges, and in platelet-rich plasma (PRP) by Born's method in response to 10 μM ADP. In addition, platelets washed by gel filtration (GFP) were aggregated in the presence of different concentrations of purified fibrinogen. For whole blood samples, the drug concentration which doubled the closure time compared to control samples (2To) was considered for the analysis. The 2To values of samples treated with Tirofiban were positively correlated with plasma fibrinogen concentrations ranging from 138 to 655 mg/dL (r=0.85, n=11, p<0.05). The IC50 values of Tirofiban-treated PRP samples were also positively correlated with plasma fibrinogen concentrations (range: 165-655 mg/dL) (r=0.89, n=9, p<0.05). A similar behavior was observed in experiments with GFP. No relationship was found between the inhibition exerted by ReoPro and fibrinogen concentration. In addition the 2To and IC50 of both drugs were independent of von Willebrand or ristocetin cofactor levels. We assessed the influence of platelet counts in PRP samples adjusted at various platelet concentrations in the presence of a fixed dose of Tirofiban (40 nM) or ReoPro (1.5 μg/mL). The degree of inhibition of both drugs was inversely correlated to platelet counts (Tirofiban: r=-0.9, n=7, p<0.01; ReoPro=-0.81, n=9, p<0.01). Receptor occupancy experiments further demonstrated a inverse dependency between platelet counts and percentage of occupied receptors in the presence of a fixed dose of Reopro. In summary, the degree of inhibition of platelet aggregation by Tirofiban appears to be influenced by fibrinogen and platelet concentrations, while the response to ReoPro appears to be influenced by platelet counts, at least *in vitro*.

P034

EFFECT OF ATORVASTATIN ON COAGULATION AND INFLAMMATORY VARIABLES IN PATIENTS WITH BILATERAL CAROTID STENOSIS

Cortellaro M,* Mussoni L,^ Cofrancesco E,* Camera M,^
Banfi C,^ Tremoli E^

**Istituto di Scienze Medico-Chirurgiche e ^Dipartimento di Scienze Farmacologiche, Università di Milano, Italy*

It is increasingly recognized that statin therapy affects not only plasma cholesterol levels, but also a range of hemostatic and inflammatory variables. These non-lipid effects are of particular importance in patients with an activated coagulation system and endothelial cell damage, such as patients with cardio-cerebrovascular diseases. The effects of statin on coagulation and inflammatory markers, however, are still controversial. We compared, in a randomized, double-blind study design, the effect of atorvastatin 20 mg/day versus placebo on coagulation and inflammatory parameters in normocholesterolemic patients with bilateral critical carotid stenosis. All patients were on antiplatelet therapy. At baseline and 4 months after the onset of atorvastatin treatment, plasma lipids, functional fibrinogen and F1+2, TNFα and IL-6 antigens were measured. In atorvastatin treated patients, LDL cholesterol and triglycerides were reduced (–34% p<.0.001 and –20% p=0.028, respectively), whereas HDL cholesterol was unaffected. Results of coagulation and inflammatory variables (geometric mean and confidence limits) are reported in Table 1 below. Fibrinogen positively correlated with IL-6 (p=0.0013) and negatively with HDL (p=0.004). IL-6 also correlated with TNFα (p=0.003).

Table 1.

	Fibrinogen mg/dL		F1 + 2 mmol/L		IL/6 pg/mL		TNFα pg/mL	
	P (n=22)	A (n=22)	P (n=16)	A (n=14)	P (n=19)	A (n=22)	P (n=21)	A (n=21)
1° (before)	385 (350-423)	395 (365-428)	1.8 (1.3-2.6)	1.7 (1.1-2.5)	2.6 (1.8-3.8)	3.6 (1.8-3.6)	2.5 (2.6-4.5)	3.0 (2.4-3.8)
2° (after)	345 (293-406)	418 (382-457)	1.4 (1.0-2.0)	1.7 (1.2-2.4)	2.2 (1.4-3.3)	4.9 (3.5-7.0)	2.6 (2.1-3.2)	2.6 (2.1-3.2)
Ratio 2°/1°	0.9	1.1	0.8	1.0	0.8	1.4	1.0	0.9

P = placebo; A = atorvastatin.

Conclusions. After atorvastatin treatment, there were no significant modifications of fibrinogen and inflammatory variables.

P035

THE PLATELET GLYCOPROTEIN IIB/IIIA RECEPTOR ANTAGONIST c/E3Fab INHIBITS COAGULATION ACTIVATION IN PATIENT UNDERGOING PERCUTANEOUS TRANSLUMINAL CORONARY ANGIOPLASTY

Musso R, Cultrera D, Sortino G, Ferlito C, Azzaro MP,
Di Francesco E, Fichera E, Giustolisi R

Centro Regionale di Riferimento per l'Emofilia (e la Trombosi) - Istituto di Ematologia, Università di Catania, Italy

Recently, preliminary studies suggested that platelet glycoprotein IIb/IIIa receptor antagonist c/E3 Fab (ReoPro, Lilly) to be very effective in patients undergoing percutaneous transluminal coronary angioplasty (PTCA). In this regard, the main properties of ReoPro include: (1). the combined effects of heparin and ReoPro on platelets; (2). a slow (hours) dissociation of ReoPro from platelets resulting in tapered (~ 48-hour) recovery; (3). a continous redistribution of ReoPro among all circulating platelets for at least 10 days following treatment and (4). the binding of ReoPro to a receptor, αvβ3 vitronectin, that has been implicated in endothelial and smooth muscle cell migration and proliferation, as well as restenosis. Moreover, the new observation that c/E3 Fab prolongs the activated coagulation time in heparinized patients with respect to in those treated without this antiplatelet agent (EPIC trial) raises the possibility that *in vitro* ReoPro would also function as an anticoagulant. To further assess this possibility we evaluated plasma indices of thrombin formation during and following PTCA in 22 patients (8 females and 14 males, aged 40-71 yrs). Plasma releases of β-thromboglobulin (ELISA β-TG) and platelet factor 4 (ELISA PF4) as markers of *ex vivo* platelet activation were also determined. Fibrinopeptide A (FPA), prothrombin fragment 1+2 (F1+2), thrombin/antithrombin III complex (TAT) and D-dimer were monitored by ELISA assays

(Behring Institute, Scoppito). In all patients before PTCA β-TG (91.3±24.3 IU/mL vs 6±2.7 IU/mL in controls) and PF4 (38.2±12 IU/mL vs 7.9±2.8 IU/mL in controls) plasma releases were significantly (p<0.001) highest. FPA (8.8±2.4 ng/mL vs 2.3±0.93 ng/mL in controls) prothrombin F1+2 (5.5±1.8 nM/L vs 1.8±0.36 nM/L), TAT (7.92±1.6 mg/L vs 2.9±0.65 mg/L) and D-dimer (644±178 ng/mL vs 212±86 ng/mL) were markedly increased. After 1-12-48 hour following the bolus of ReoPro, a remarkable reduction of the plasma coagulation values was dynamically observed: β-TG (68.8±10.9 IU/mL, 56.5±16.3 IU/mL, 38±16 IU/mL), PF4 (29.9±16.8 IU/mL, 26.3±10.4 IU/mL, 18.8±7.7 IU/mL), FPA (6.89±3.3 ng/mL, 5.8±1.7 ng/mL, 4.56±2.20 ng/mL), prothrombin F1+2 (4.9±2.80 nM/L, 3.88±2.52 nM/L, 2.2±0.83 nM/L), TAT (6.87±1.94 mg/L, 4.83±1.84 mg/L, 3.18±2 mg/L) and D-dimer (566±219 ng/mL, 408±228 ng/mL, 328±168 ng/mL). It has been suggested that activated platelets can facilitate fibrin formation initiated either by the extrinsic or intrinsic systems. The thrombocytes thus accelerate thrombin generation by 5-6 orders of magnitude by providing a catalytic surface on which coagulation reactions occur. In this connection it is possible that antiplatelet agents may also function as anticoagulants *in vivo*. The results of our study supports the premise that ReoPro restores intraplatelet contents and interferes with the platelet-activation events involved in facilitating thrombin generation further decreasing clot-bound thrombin as well as platelet thrombus formation.

P036

THE ATROCAP STUDY: ATORVASTATIN AND THROMBOGENICITY OF CAROTID ATHEROSCLEROTIC PLAQUE

Cortellaro M,* Cofrancesco E,* Camera M,^ Mussoni L,^ Arbustini E,# Tremoli E^

**Istituto di Scienze Medico-chirurgiche e^Dipartimento di Scienze Farmacologiche, Università di Milano, #IRCCS Policlinico San Matteo, Pavia, Italy*

Statins reduce the risk of acute coronary events out of proportion to their effects on lumen stenosis. Beneficial effects of statins on fibrous cap stabilization have been shown, whereas the effect of these drugs on plaque thrombogenicity has not been reported yet. To evaluate thrombogenicity of bilateral human carotid plaques before and after atorvastatin treatment, 59 patients with bilateral carotid stenosis eligible for two-step carotid endoarterectomy (CEA) were randomly assigned to atorvastatin, 20 mg/day, or to placebo. Histological and immunohistochemical analyses, tissue factor (TF) and tissue factor pathway inhibitor (TFPI) antigen as well as TF activity determinations were carried out in endoarterectomy specimens obtained at baseline and after treatments. Mean TF and TFPI Ag levels in homogenates of plaques removed at the 1st CEA were 55±56 and 32±26 pg/mg respectively. After placebo treatment, TF and TFPI Ag content was greater in the 2nd than in the 1st one (+47% and +45% respectively, p<0.05 and p=0.01). Plaques removed at the 2nd CEA from atorvastatin-treated patients had lower macrophage content than plaques removed during the 1st CEA. TF and TFPI Ag levels, as well as TF activity in plaques removed after atorvastatin treatment were lower (-29%, -18% and –56%, respectively) than those from placebo-treated patients. These findings suggest a preventive effect of atorvastatin on carotid plaque ulceration/rupture supporting a beneficial effect of statins on cerebrovascular events.

P037

ANTI-ANNEXIN V AUTOANTIBODIES ARE MARKERS OF ARTERIAL THROMBOSIS

Fuduli V, Scarmozzino MG, Dardano A, Berlinghieri MC, Consoli D*

*Cattedra di Patologia Clinica, DMSC "G. Salvatore", Università degli Studi "Magna Graecia", Catanzaro; *UO di Neuroscienze, AO Vibo Valentia, Italy*

Annexin V plays an important role as an anticoagulant factor because of its structural and functional interaction with phospholipids on endothelial cells. These Ca^{++} dependent interactions allow proteoglycans to correctly localize annexin V at cellular surface level. Even minimal alterations of this ordinate structure can expose annexin V which can be recognized as epitope and thus stimulate an immunoresponse. Autoantibodies anti-annexin V can amplify this phenomenon, promote progressive endothelial damage at arterial level, and thus may explain some pathogenetic aspects of recurrent episodes of arterial thrombosis. We present laboratory data which correlate with clinical evidence and may support the role of anti-prothrombin and anti-annexin V autoantibodies in the pathogenesis of arterial thrombosis. Forty-eight patients suffering from venous thrombosis and 56 patients of both sex, 20 to 50 years old, with clinical evidence of arterial thrombosis, selected by alterations of standard coagulative parameters, were tested, by immunoassay, for autoantibodies anti-β-2GPI (Radim, Pomezia), anti-prothrombin (Orgenthec Diagnostica GmbH), anti-annexin V (TechnoGenetics, Milan). Two of 48 patients with venous thrombosis showed high levels of anti-β-2 GPI (cut off = 10 U/mL), none of these has been positive either for anti-prothrombin or anti-annexin V autoantibodies. Of 56 patients suffering from arterial thrombosis, 7 were positive for anti-prothrombin (cut off = 10 U/mL) and only 1 was positive for anti-annexin V (cut off = 12.5 ng/mL). We suggest that patients with elevated levels of autoantibodies anti-β-2GPI only are suffering from venous thrombosis. These results indicate that the evaluation of autoantibodies anti-prothrombin allows identification of a population of patients, of young and adult age, with a high incidence of arterial thrombosis. A high level of anti-annexin V autoantibodies may be specific to a small group of patients with early onset of thrombotic event and could be a marker of an acute episode.

P038

METFORMIN DECREASES PLATELET SUPEROXIDE ANION PRODUCTION IN DIABETIC PATIENTS

Sanguigni V, Gargiulo P,* Caccese D, Pignatelli P,° Brufani C,* De Vito F, Marino R,° Lauro R, Di Mario U,* Violi F°

*Department of Internal Medicine, University of Rome "Tor Vergata", *Department of Endocrinology, University of Rome "La Sapienza"; °Department of Experimental Medicine and Pathology, University of Rome "La Sapienza", Italy*

Background. Patients with type II diabetes mellitus are usually treated with oral antidiabetics but it is still unknown if these drugs have antioxidant effects in humans. *Methods.* We studied sixty patients with type II diabetes mellitus, divided into three groups on the bases of hypoglycemic treatment (group A: metformin, group B: glibenclamide, group C: diet). All patients were

followed for at least 1 year. The three sub-groups had similar clinical characteristics. Twenty healthy subjects, sex and age comparable, were enrolled as controls. In each subject, platelet production of superoxide anion (O2-) elicited by collagen, was determined by lucigenin assay. *Results.* Compared to healthy subjects diabetic patients showed higher platelet production of O2- than controls (2.48±0.55 vs 0.93±0.21 nmoles/3×10^8 plts/min, $p<0.001$). Significant differences were also found between metformin, glibenclamide and diet groups; thus the glibenclamide and diet group had higher platelet O2- production than the metformin group (2.94±0.79 and 3.26±0.51 vs 1.25±0.35 nmoles/3×10^8 plts/min, $p<0.001$). It is of interest that no significant difference in platelet O2- production was observed between the metformin group and controls (1.25±0.35 vs 0.93±0.21, $p=0.06$). *Conclusions.* These findings suggest an antioxidant *in vivo* activity of metformin and warrant prospective *ad hoc* studies to further explore this hypothesis.

P039

ROLE OF AT1 RECEPTORS IN ENHANCING OXIDATIVE STRESS IN PATIENTS WITH HYPERTENSION

Sanguigni V, Germanò G,* Pignatelli P,* Caccese D, Lenti L,* De Vito F, Lauro R, Violi F*

*Department of Internal Medicine University of Rome Tor Vergata; *Department of Experimental Medicine and Pathology University of Rome La Sapienza, Rome, Italy*

Background. Recent studies provided experimental evidence that hypertension induces atherogenesis through enhanced oxidative stress and that angiotensin converting enzyme (ACE) system activation may play a pivotal role. AT1 receptors have been shown *in vitro* to enhance formation of superoxide anion (O2-). It has never been studied if this occurs also in human hypertension. *Methods.* Forty-four hypertensive patients were randomly allocated to a treatment with ibesartan, an inhibitor of AT1 receptors, and life-style modification only. In each patient platelet O2- production by chemiluminescence of lucigenin was studied before and after 4 weeks of treatment. Forty healthy subjects matched for sex and age were also studied as controls. To further analyze platelet O2- production we performed an *in vitro* study using dihydroethidium as probe in a cytofluorimetric assay. *Results*: Compared to healthy subjects, hypertensive patients had higher platelet production of O2- (2.68±0.57 vs 0.85±0.22) ($p<0.001$); there was no correlation between blood pressure and platelet O2- production. After treatment no changes of platelet O2- formation were observed in patients allocated to life-style modification; conversely in patients treated with ibesartan had a significant decrease of platelet O2- production (2.69±0.63 vs 1.73±0,41) ($p<0.002$), that, however, was not correlated with blood pressure lowering. The *in vitro* study showed that Irbesartan (1-3-10 nM) inhibited in a dose dependent manner the angiotensin II-mediated platelet O2- production (22%, 53% and 72% respectively, $p<0.005$). *Conclusions.* Patients with hypertension have enhanced formation of O2-, that is mediated by AT1 receptor upregulation. This finding provides new insight to understanding the proatherogenetic activity of the ACE system in humans.

P040

EFFECTS OF THE PLATELET GLYCOPROTEIN IIB/IIIA ANTAGONISTS ABCIXIMAB, TIROFIBAN AND EPTIFIBATIDE ON PLATELET FUNCTION

Gallo L, Nobili L, Cenzuales S, Del Maschio A, Rossi F, Russo U, Rossi E

Hematology and Blood Transfusion Service, L. Sacco Hospital, Milan, Italy

The GPIIb/IIIa, expressed on platelets, is the most important receptor for fibrinogen. Inhibition of soluble fibrinogen binding to activated platelets is the target of antagonists of GPIIb/IIIa complex. In this study we assessed the effect of inhibition of the GPIIb/IIIa binding with the monoclonal antibody (MoAb) directed against that receptor in whole blood samples incubated *in vitro* with increasing concentrations of drugs. We evaluated three different molecules: i) abciximab (a recombinant chimeric Fab fraction antibody) measuring the binding inhibition with CD41, which recognizes the GPIIb/IIIa complex; ii) eptifibatide (cyclic heptapeptide); and iii) tirofiban (nonpeptide) measuring the binding inhibition with PAC-1, MoAb that recognizes an epitope on the GPIIb/IIIa complex of activated platelets at or near the platelet fibrinogen receptor. We found that abciximab inhibited CD41 binding to GPIIb/IIIa in a concentration-dependent manner. PAC-1 binding to activated GPIIb/IIIa receptor was inhibited after exposure to tirofiban and eptifibatide *in vitro* and *in vivo* in a concentration-dependent manner, too. We measured the MoAb binding, expressed as antibody binding capacity, and we calculated the percentage of GPIIb/IIIa receptors blocked by antagonists not available for fibrinogen binding. This method could be used to monitor the efficacy of this therapy in inhibiting platelet aggregation based on a correct dosage which avoids bleeding complications.

P40a

LOW-MOLECULAR WEIGHT HEPARIN FOR CEREBRAL ARTERIES DISSECTION: A CASE SERIES

Bertesi M, Marietta M, Vallone S,* Cappi C, Castelli I, Pozzi S, Torelli G

*Dept. Medical Sciences, Section of Hematology; *Dept. of Neuro-Radiology, University Of Modena and Reggio Emilia, Modena, Italy*

Cerebral arteries dissection is a rare and dramatic disease. Dissection is often traumatic in nature, although in many cases etiology remains unclear. The optimal treatment for such uncommon disease is still matter of debate, because of lacking of trials directly comparing either anticoagulants with antiplatelet drugs, or any treatment with placebo. We describe here 6 consecutive cases of patients (3 male, 3 female; age ranging from 6 to 53 yrs, mean age 33 yrs) affected by cerebral arteries dissection (4 internal carotid arteries, 1 basilar artery, 1 vertebral artery) treated with low-molecular weight heparin (LMWH) with favourable outcome. All patients were diagnosed dissection and consequent thrombosis (4 apparently spontaneous, 1 traumatic and 1 post-partum) by magnetic resonance imaging and catheter angiography scans (MRI/MRA). Each patient was treated at diagnosis with subcutaneous LMWH at therapeutic doses (Enoxaparin 100 IU/kg bid) for three weeks, and then reduced to

a prophylactic dose of 4000 IU/day (2000 IU/day for pediatric patients) for three more weeks. A magnetic resonance angiogram was then performed, which showed complete recanalization of the involved arteries. To our knowledge, very few literature data are available at the moment on the proper treatment of such severe thrombotic complication and in our experience Enoxaparin has been proved to be a safe and useful therapy of thrombosis accompanying cerebral arteries dissection.

Posters
Platelets and Leukocytes

P041

IMMUNOREACTIVE TISSUE FACTOR IS EXPRESSED ON PLATELET SURFACE UPON AGONIST ACTIVATION

Camera M,^* Frigerio M,* Cottell D,° Brambilla M,^ Maderna P,° Rossi F,^ Toschi V,# Tremoli E^*

*^Dept. Pharmacological Sciences, University of Milan, *Monzino Cardiologic Center, Milan, #San Carlo Hospital, Milan, Italy; °University College of Dublin, Ireland*

Several studies have identified tissue factor (TF) as one of the major determinants of the activation of the coagulation cascade at the site of atherosclerotic plaque rupture. Recent data indicate that human platelets contain appreciable amounts of TF which may derive from leukocytes. In this study we investigated whether platelet stimulation with ADP induces the exposure of TF on platelet membranes. In unstimulated conditions flow cytometry analysis, performed with a specific monoclonal anti-human TF antibody, showed that platelet-associated irTF was not detectable in whole blood (WB), platelet-rich plasma (PRP) or washed platelets (WP) obtained from healthy subjects free of medication known to affect platelet function. In contrast, WB and PRP incubated with 10 μmol/L ADP for 15 min showed consistent amounts of membrane-associated irTF, observed also by transmission electron microscopy. The observed expression of TF might be the result of the binding of plasma TF to platelets or rather it may derive from translocation of intraplatelet TF to the membrane. Indeed, consistent amounts of membrane-associated irTF were also found in WP exposed to ADP. Moreover, RT-PCR experiments showed detectable amounts of TF mRNA in unstimulated platelets, suggesting that the platelet itself has the machinery to potentially synthesize TF. ADP induced irTF increase on platelet surface in a concentration-dependent fashion (0.1-20 μmol/L) peaking at 10 μmol/L. Time course experiments (15-240 min) performed in PRP showed that irTF specific fluorescence was maximal at 60 min. P-selectin expression, evaluated as activation-dependent platelet membrane glycoprotein, paralleled that of irTF, indicating that the increase in membrane-associated irTF parallels that of platelet α granule secretion. Our data suggest that platelets may be a source of circulating irTF and that ADP renders this protein available for the interaction with blood or vessel wall components, an event which may contribute to the growth of arterial thrombus.

P042

SRC-DEPENDENT SIGNALING IS A KEY STEP IN THE PROCESS OF AUTOREGULATION OF MAC-1

Piccardoni P, Bagoly Z, Manarini S, Pecce R, Totani L, Martelli N, Piccoli A, de Gaetano G, Cerletti C, Evangelista V

"G. Bizzozero" Laboratory of Blood and Vascular Cell Interactions, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy and Università Cattolica del Sacro Cuore, Centro di Ricerca e Formazione ad Alta Tecnologia nelle Scienze Biomediche, Campobasso, Italy

In a previous study (*Blood 2001; 98:108*) we suggested that an initial P-selectin-triggered Mac-1 binding to its ligand, *per se*, promotes the activation of SRC kinases and phosphorylation of an unknown 110kD protein (P110), these in turn mediating integrin clustering and F-actin accumulation, thus strengthening PMN adhesion. To test this hypothesis, the moAb KIM127 or manganese, to lock β2 integrin in a high affinity state and homotypic aggregation of PMN sheared at 1000rpm, as a sensitive assay of the Mac-1 adhesive function, were used. KIM127 and manganese were both able to induce PMN homotypic aggregation (61±7% and 38±8% of total PMN, respectively) and P110 tyrosine phosphorylation. Specific inhibitors of SRC activity, PP1 and PP2, completely blocked the moAb KIM127 effect (IC50=5 μM) and partially reduced (50% of inhibition at 10 μM) manganese-induced PMN adhesion. P-110 tyrosine phosphorylation induced by both agonists was completely prevented by SRC blockade. Confocal microscopy showed Mac-1 clusters and F-actin patches co-localized at the adhesion sites of KIM127- or manganese-aggregated PMN. Moreover KIM127 specifically stained *activated* β2-integrins recruited in the clusters. Blockade of SRC kinases, of the integrin or of actin polymerization prevented Mac-1 clustering and F-actin accumulation. FACS analysis showed that moAb KIM127 recognizes a subpopulation of β2 integrins corresponding to 7.6±1.7% of the total, that increased to 30±7% in manganese-treated PMN. To better understand the SRC-mediated signal we investigated the nature of P110. Western blot analysis of the immunoprecipitated protein revealed that PYK2, a focal adhesion kinase expressed in PMN, was a component of P110, and was strongly phosphorylated in a SRC and β2 integrin-dependent manner in PMN challenged by KIM127. Our results suggest, in agreement with the initial hypothesis, that SRC kinases, probably through a 110kD protein (PYK2), play a key role in the autoregulation of Mac-1 and that these molecules bridge the high affinity with the avidity state in the dynamic process of PMN adhesion.

P043

HEMOLYTIC HUREMIC SYNDROME/THROMBOTIC THROMBOCYTOPENIC PURPURA-LIKE SYNDROME AFTER SOLID ORGAN TRANSPLANTATION: A CASE SERIES

Pozzi S, Marietta M, Bertesi M, Cappi C, Castelli I, Pinna AD,* Torelli G

*Dept. Medical Sciences, Section of Haematology; *Multivisceral Transplant Unit, University of Modena and Reggio Emilia, Modena, Italy*

Hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP)-like syndrome have been described after solid organ transplantation as a consequence of immunosuppressive therapy with cyclosporin A (Cys A) and tacrolimus (FK506). The mechanism assumed for Cys A is a direct damage to endothelium by reduction of prostacyclin, while for FK506 the mechanism is unknown. Therapeutic options are withdrawal or switch of immunosuppressive agents and plasma-exchange with or without prostacyclin or steroids. However, treatment strategies are heterogeneous, and clinical responses are extremely variable. We describe three cases of HUS/TTP-like in small bowel and multivisceral transplant during FK506 therapy, with different outcomes. The first case is a 57-year old woman, who nine months after small bowel transplant developed thrombocytopenia. Clinical and laboratory data were consistent with TTP-like syndrome. FK 506 was stopped and daily plasma-exchange started, with resolution of microangiopathy. The second case is a 29-year old woman who underwent multivisceral transplantation for intestinal pseudo-obstruction. Nine months later low grade rejection appeared and the patient suddenly became thrombocytopenic; a blood smear revealed microangiopathic hemolysis. Plasma-exchange was started, and FK506 reduced, but renal function worsened and severe pancytopenia appeared. Blood smears confirmed microangiopathy and a biopsy showed bone marrow failure. The clinical picture was complicated by interstitial pneumonia and the patient died of multiorgan failure. The third case was a 24-year old woman who developed sudden thrombocytopenia eight months after small-bowel transplant. Clinical and laboratory data were consistent with TTP-like syndrome. No improvement was seen in spite of FK506 withdrawal and plasma-exchange procedures, and therefore VCR i.v. (0.02 mg/kg each four days) was added to daily plasma-exchange, according to the Italian TTP protocol. After the second dose the patient improved (Plt: 214,000/m^3) and she was discharged after the third administration, with stable platelet values.

P044

GENOMIC RESTRICTION FRAGMENT LENGTH POLYMORPHISM TYPING OF FOUR HUMAN PLATELET-SPECIFIC ANTIGENS IN BLOOD DONORS FROM SOUTHERN ITALY

De Stefano V, Fratellanza A,° Coppola A, Fratellanza G,° Madonna P, Scarcella A,° Formisano S,* Di Minno G

*Centro di Riferimento Regionale per le Emocoagulopatie, *Dipartimento di Immunoematologia e Trasfusione; °Dipartimento di Pediatria, Università degli Studi di Napoli "Federico II", Italy*

The polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) is a rapid, effective and cheap method for genotyping platelet antigens. The frequency of platelet antigens HPA 1, 3, 4 and 5 was determined by the PCR-RFLP technique in one hundred and ten consecutive blood donors referred to our Center. The frequencies obtained were: 69.5%, 26.2% and 4.3% for HPA-1 (a^+/b^-), (a^+/b^+), (a^-/b^+); 45.1%, 37.3% and 17.6% for HPA-3 (a^+/b^-), (a+/b^+), (a^-/b^+); 99.2%, 0.8% and 0% for HPA-4 (a^+/b^-), (a^+/b^+), (a^-/b^+); 80.3%, 18.8% and 0.9% for HPA-5 (a^+/b^-), (a^+/b^+), (a^-/b^+) respectively. With the exception of HPA 3, whose frequency is higher than expected in our sample size, other results were similar to the data reported in other Caucasian populations of the Mediterranean area. Platelet-specific alloantibodies are implicated in neonatal alloimune thrombocytopenia (NAIT), post-transfusion purpura (PTP) and refractoriness to platelet transfusion therapy. So far we have successfully used our HPA genetic

bank for two compatible platelets transfusions in two NAIT. We conclude that the HPA genetic bank is very useful in the diagnosis and in the transfusion support of all patients with thrombocytopenia related to platelet alloantibodies.

P045

POST-TRAUMATIC BLEEDING AND *EX VIVO* FUNCTIONAL PLATELET ABNORMALITIES IN A YOUNG WOMAN WITH PARTIAL QUANTITATIVE DEFECTS OF GP IA AND GP IC. POTENTIAL ROLE OF ANTI GPIA ANTIBODIES

Coppola A, Fratellanza G,* Amoriello A,° Cimino E, Tufano A, Cerbone AM, Di Minno G

*Centro di Coordinamento Regionale per le Emocoagulopatie, Dipartimento di Medicina Clinica e Sperimentale and *Dipartimento di Immunoematologia e Trasfusione, Università degli Studi di Napoli "Federico II", and °Servizio di Immunoematologia e Trasfusione, A.O.R.N. "A. Cardarelli", Naples, Italy*

A 26-years old woman was referred to our Center because of a massive hemorrhage into lower abdomen muscles following a Cesarean section. Her family history was negative for bleeding tendency and she had experienced only prolonged bleeding after tooth extraction ten years earlier. She was at the 29th week of her second pregnancy and a new Cesarean intervention had been planned. Her coagulation tests were entirely normal, while moderate thrombocytopenia (81,000/m^3) was found, together with an abnormally prolonged bleeding time (> 20 min). Platelet functional tests showed impaired sensitivity to ADP (>10 μM) and collagen (>5 μg/mL) and normal response to arachidonic acid and ristocetin. Antiplatelet antibodies were also detected and their elution from the platelet surface enabled identification of IgG antibodies against glycoprotein (GP) Ia (commercial plates coated with specific platelet GP). Cytofluorimetric analysis by monoclonal antibodies showed about 50% reduction on her platelets of GP Ia (CD49b, VLA-2) and GP Ic (CD49f, VLA-6) as well. Qualitatively and quantitatively similar data were found in her father, while her mother showed only a 50% reduction of GP Ia. However, no parent had exhibited a bleeding tendency, abnormalities of bleeding time or functional platelet tests. Before delivery the patient underwent a short course of steroid (0.8 mg/Kg for 2 weeks) treatment and her platelet count rose to 150,000/m^3. Moreover, after cord section, i.v. desmopressin 0.3 μg/Kg was administered. No bleeding complications occurred and the female newborn did not show thrombocytopenia. We conclude that, in addition to the glycoprotein abnormality, the interference of anti-GP Ia antibodies may be involved in this patient's post-traumatic bleeding tendency and her platelet function abnormalities. However, the possibility of a combined effect of the autoimmune thrombocytopenia and of the inherited defects of adhesion molecules deserves further evaluation.

P046

SYSTEMIC MASTOCYTOSIS AND ESSENTIAL THROMBOCYTHEMIA: A CASE REPORT AND AN UPDATE

Spedini P, D' Adda M, Morandi S

Sezione di Ematologia e Centro Trapianto di Midollo Osseo, Divisione di Medicina II, Istituti Ospitalieri di Cremona, Italy

Mastocytosis is a term used to denote a heterogeneous group of disorders characterized by pathologic accumulation of mast cells. Here, we report on a 39-year old man suffering from urticaria pigmentosa (UP) since he was 29 years old, referred to our hospital because of thrombocythemia. On admission, physical examination revealed UP-like skin lesions with a positive *Darier sign* and palpable hepato-splenomegaly. Blood examination did not reveal anemia; the WBC count was 12×10^9/L (blood smear analysis showed 65% neutrophils, 25% lymphocytes and 10% eosinophils) and platelet count was 936×10^9/L. The whole blood tryptase (pro α+β = total) level amounted to 867 ng/mL (normal range < 21 ng/mL) and the serum triptase level amounted to 1067 ng/mL (normal range < 20 ng/mL). Alkaline phosphatase level was 371 U/L (normal range: 60-260) and γ-glutamyltranspeptidase level was 78 U/L (normal range: 0-40). The X-ray of the skeleton revealed diffuse osteoporosis and focal osteolytic lesions in the dorsal spine and in the ribs. A bone marrow biopsy showed increased cellularity with an elevated number of dysplastic megakaryocytes and decreased fat content. Focal aggregates of toluidine-blue-positive mastocytes were prominent. The karyotype analysis was normal. A diagnosis of associated hematologic clonal disease (essential thrombocythemia, ET) to systemic mastocytosis (SM) was made. He was treated with α-interferon with improvement in the clinical symptoms and a return to a normal platelet count and liver enzymes. The occurrence of malignancies resembling ET in patients suffering from SM is very rare. To our knowledge, there have been only nine reported cases of ET arising in patients with SM. Moreover, skin involvement appears to be less frequent in mastocytosis when a myeloproliferative disorder is present. Prognosis may be difficult to predict when two infrequent disorders occur in the same patient and it may be determined by the hematological disease rather than the mastocytosis.

P047

SPLENECTOMY IN PATIENTS WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA

Iannaccaro P, Santoro R, Muleo G

Haemophilia Centre, Azienda Ospedaliera " Pugliese- Ciaccio", Catanzaro, Italy

Splenectomy is the treatment of choise in patients with chronic idiopathic thrombocytopenic purpura (ITP) (lasting more than 6 months) refractory to corticosteroid therapy. It is a curative treatment for two thirds of patients, with a high success rate in the long-term response. However, factors predicting the response to splenectomy have not identified. We evaluated 38 patients (8 males, 30 females, median age at diagnosis 28 years, range 16-71)with chronic ITP and a poor response to steroids, followed in our institution and who underwent splenectomy. The median platelet count at diagnosis was 17×10^9/L (range 0-52×10^9/L). All patients received an initial treatment with prednisone, 1 mg/kg of body weight (b.w.)/day for 28-35 days, with subsequent tapering until withdrawal or maintenance doses. In addition 18 patients received high-dose intravenous immune globulin (HD Ig) 0.4 g/kg b.w. per day for five consecutive days, 5 received anti RhD immune globulin (800-1000 μg per day for three consecutive days), 3 patients had been given α2b-interferon (3 MU, three times in week, for 4 weeks). Response to initial steroid therapy, to HD Ig and to splenectomy was considered partial or complete for platelet count > 50×10^9/L or >100×10^9/L respectively, poor if <50×10^9/L. The median time from diagno-

sis to splenectomy was 20 months (range 6-228) and the median follow-up after splenectomy 63 months (range 3-468, 37 evaluable patients). A persistent complete or partial response (CR or PR) was obtained in 24 and 4 patients, respectively (73.7% overall). Seven patients (18.4%) relapsed (median time from surgery 3 months), 6 of them achieved CR or PR with a new steroid course, 1 is asymptomatic with a platelet count of 30-50×10^9/L. Two patients failed to respond to splenectomy, one achieved CR with cyclosporin A. Three patients had major complications in the post-operative course (1 subphrenic abscess, 1 deep venous thrombosis, 1 superficial vein thrombosis). CR or PR were achieved with splenectomy in 11/13 patients with CR or PR to HD Ig and in 23/25 patients with CR or PR to the initial steroid treatment. The relation between the response to HD Ig or steroid and the response to splenectomy was not statistically significant (by the χ^2 test). In agreement with the literature data, splenectomy in chronic ITP is, in our experience, a therapeutic approach with a high long-term success rate, even if steroid therapy or HD Ig has failed.

P048

COMPLICATIONS IN ESSENTIAL THROMBOCYTHEMIA IN YOUNG ADULTS: A SINGLE CENTER EXPERIENCE

Candoni A, Damiani D, Russo D, Silvestri F, Filì C, Fanin R

Division of Hematology and Bone Marrow Transplantation, University Hospital, Udine, Italy

We retrospectively analyzed the records of 54 consecutive patients younger than 40 years with essential thrombocythemia (diagnosed according to the Polycythemia Vera Study Group criteria) followed in our department between 1990 and 2000. Median age at disease onset was 27.5 years (range 16-39), median platelet count 855×10^9/L (range 650-2.190×10^9/L) and male/female ratio 2.6/1. The mean duration of follow-up was 86 months (median 97 months, range 6-136). Twenty-six precent (14/54) of patients have one (12/14) or more (2/14) cardiovascular risk factors (smoke, dyslipidemia, hypertension, diabetes mellitus). Major complications occurred in 31% (17/54) of patients (cerebrovascular accident in 2, acute myocardial infarction in 4, abortion in 8, venous thrombose in 1 and hemorrhage in 2); none of which was fatal. Minor events (headache, erythromelalgia, paresthesias, minor bleeding) occured in 33% (18/54) of patients whereas 46% (25/54) remained asymptomatic throughout follow-up. Forty-one of 54 patients (76%) received acetylsalicylic acid and 54% (29/54) cytoreductive therapy: hydroxyurea in 7/29, busulphan in 4/29, anagrelide in 1/29, interferon in 11/29, hydroxyurea and interferon in 6/29; 79% of patients responded well to cytoreductive therapy (13/18 responded to interferon). No deaths were observed and overall survival was similar to that of an age and sex matched control population. Our experience documents that young adults with ET are commonly symptomatic (54%) and the maior complications occurred in 31% of cases. Ischemic complications are more frequent than hemorrhagic ones (6/17 vs 2/17). The factors that were found to be predictive for ischemic events were sex (male) and cardiovascular risk factors. Moreover, in our experience, ET in young women is associated with an increased risk of abortion (8 abortions/15 pregnancies) especially in the first trimester (6/8).

Posters
Laboratory Techniques: Modifications of the Hemostasis

P049

LONG-POLYMERASE CHAIN REACTION IN FETAL TISSUE FROM PREGNANT WOMEN CARRYUNG THE FACTOR VIII GENE INVERSION

Belvini D, Salviato R, Are A, Radossi P, Tagariello G

Laboratory Department, Blood Bank, Centre For Blood Disease and Hemophilia Centre, Castelfranco Veneto Hospital, Castelfranco Veneto, Italy

FVIII gene inversion represents the most important causative mutation responsible for severe cases of hemophilia A. Detection of the FVIII inversion by using long PCR requires high quality, non-fragmented DNA because of the very large size of the PCR products. In our opinion, the quality of DNA from boiled fetal tissue is not sufficient for long distance PCR such as that used for FVIII gene inversion. Although there is no evidence that the DNA of this tissue gives significantly different results from other fetal or adult samples, our experience has shown that, DNA extraction and PCR conditions have a crucial role for the efficacy of this reaction in fetal tissues. Recently we have been able to obtain excellent results from chorionic villus samples (CVS) starting from DNA recovered using an effective commercial DNA extraction kit (Quantum Prep Aquagene Genomic DNA kit, Bio-Rad, California, USA). It is worth outlining that some PCR conditions, such as DNA and $MgCl_2$ final concentrations, should be modified compared with those normally used for DNA recovered from peripheral blood leukocytes which are 10 ng/mL of DNA and 2.25 mM $MgCl_2$. In conclusion we want to underline that the results can vary according to DNA and $MgCl_2$ concentrations, but with different results in different samples. Thus we suggest performing a wide range panel of conditions, for each determination, to increase the possibility of obtaining a result rapidly.

P050

VWF:RCO ASSAY: IMPLEMENTATION ON AN AUTOMATED COAGULOMETER

Redaelli R, Borroni L, Mostarda G, Morra E, Baudo F

Department of Oncology/Hematology, Thrombosis Hemostasis Unit, Ospedale Niguarda Cà Granda, Milan, Italy

The VWF:RCo assay is a routine test for laboratory diagnosis of von Willebrand disease (VWD). The ability of patient's plasma to induce agglutination of formaldehyde-fixed normal platelets in the presence of a fixed amount of ristocetin is measured either employing a platelet aggregometer or a slide technique. The disadvantages are the high variability and the low reproducibility, related to platelet preparation, method of agglutination detection and interpretation of results. Moreover, it is time consuming. In order to improve the test performance, we implemented the VWF:RCo assay on the ACL 7000 (IL). *Study population*. Twenty-two normal individuals (N), 18 VWD1, 2 VWD2 and 3 VWD3 patients. Plasmas with known levels of VWF (L1 = 1.33

U/mL; L2= 0.47 U/mL) were used to assess the test variability. *Methods*. VWF:RCo was assayed by the aggregometric method (AGM) and by a specific research cycle on the automated coagulometer (ACL). *Reagents*. Von Willebrand Reagent (Behring); ristocetin (Mascia Brunelli). Platelet suspension containing ristocetin (final concentration 1 mg/mL) and plasma diluitions were similarly prepared for both methods. *Results.*

	VWF:RCo concentration U/ mL (means and intervals)	
	AGM	ACL
N	0.96 (0.42-1.57)	0.91 (0.39-1.93)
VWD1	0.30 (0.07-0.60)	0.22 (0.06-0.49)

The VWF:RCo values determined by AGM and ACL are very well correlated (r: 0.952, Passing-Bablok regression y=1.024 x– 0.09). A linear dose-response curve for the ACL method is observed between 0.05 and 1 U/mL. The coefficients of variation (CV) with ACL are lower than with AGM.

	CV%	
	AGM	ACL
Within run		
L1	18	6.8
L2	19	5.8
Between runs		
L1	17	8.5
L2	19	7.8

Comments. The values of VWF:RCo obtained with AGM and ACL methods are highly correlated. The reproducibility (CVs consistently lower), the easy and rapid performance with time and cost saving of the VWF:RCo assay perfomed by ACL, suggest its routine application for VWD diagnosis.

P051

COMPARISON BETWEEN THREE DIFFERENT SYSTEMS TO MEASURE ACTIVATED CLOTTING TIME DURING CARDIOPULMONARY BYPASS

Paniccia R, Cappuccini G,* Stefano PL,^ Bandinelli B, Evangelisti L, Gazzini A, Lapini I, Sticchi E, Abbate R, Gensini GF, Prisco D

*Dipartimento di Area Critica Medico-Chirurgica, Università degli Studi di Firenze. UU.OO. *Centro Trombosi, Anestesiologia e Rianimazione 1, ^Cardiochirurgia, Azienda Ospedaliera Careggi, Florence, Italy*

Heparin therapy during cardiopulmonary bypass (CPB) is traditionally monitored in the operating room by an activated clotting time (ACT) test, performed by a point-of-care instrument. A new device, Hemochron Junior II (HJII - International Technidyne Corp, USA), which measures ACT by the use of cartridges with celite, has recently become available. A 20 μL blood drop is loaded and flowed into capillaries and when blood flow stops the device registers clotting formation. ACTII (Medtronic) is a second system commercially available for ACT determinations, which uses kaolin-activated cuvettes and registers clotting formation with a mechanical detector. A third point-of-care instrument, Sonoclot (SIENCO Inc, USA), measures blood viscoelastic properties, but among other specific parameters, it also provides a celite-ACT (SonACT). The aim of this study was to compare the performances of these 3 devices. One hundred and eleven samples from 16 CPB patients were obtained throughout the surgical procedure. All samples were assayed in duplicate with two different HJII instruments and once with Sonoclot; 60 samples were also tested in duplicate by one ACTII device. A significant correlation was observed between ACT values obtained with the two HJII (r=0.99, p<0.001) and the duplicates obtained with ACTII (r=0.84, p<0.001). Bland and Altman analysis revealed a good performance for both systems (p<0.001), although ACT values obtained by ACTII were more scattered. Significant correlations were found between ACT values obtained with different devices: HJII vs Sonoclot, r=0.92, p<0.001; HJII vs ACTII, r=0.91, p<0.001; ACT II vs Sonoclot, r=0.91, p<0.001. This study indicates that a significant relationship exists between ACT values obtained with the 3 systems. The use of Sonoclot provides an accurate ACT and other, possibly useful, parameters with a single device.

P052

MONITORING HEPARIN TREATMENT IN DIFFERENT CLINICAL SETTINGS: COMPARISON OF TWO POINT-OF-CARE WHOLE BLOOD COAGULATION ANALYZERS

Paniccia R, Carbonetto F,* Bandinelli B, Palmarini MFG,* Conti P,^ Fedi S, Lari B, Lenti M, Rossi L, Abbate R, Prisco D

*Dipartimento di Area Critica Medico-Chirurgica, Università degli Studi di Firenze, UU.OO. di *Centro Trombosi, Anestesiologia e Rianimazione 1,^Centro Emodialisi, Azienda Ospedaliera Careggi, Florence, Italy*

In different clinical settings, such as cardiopulmonary bypass (CPB), interventional cardiology and hemodialysis, heparin therapy is necessary to prevent thrombosis of extracorporeal circuits. This treatment is traditionally monitored by activated clotting time (ACT). The most widespread system to perform celite-ACT is Hemochron 401(International Technidyne Corp, USA), which registers, by a mechanical device, the time of clotting formation. This technique has been reported to have considerable drawbacks such as lack of sensitivity and poor reproducibility. Recently, a new instrument for measuring the ACT has been introduced (ISTAT, Abbott); this measures clotting time based on the conversion of a thrombin substrate other than fibrinogen and an electrochemical sensor is used to detect this conversion. The aim of this study was to compare the performances of these two systems with special reference to different internal analytic precision and correlation between results obtained. One hundred and sixty-five samples were assayed in duplicate by 2 different Hemochron and 2 different ISTAT devices. One hundred and thirty samples from 20 patients undergoing CPB and 35 samples from 9 hemodialyzed patients were analyzed. A significant correlation between the duplicates from the two Hemochron devices was observed (r=0.99, p<0.001), as well as between those from the two ISTAT instruments (r=0.99, p<0.001). Bland and Altman analysis revealed a good performance for both systems (p<0.001). By analyzing all available data together (n=330) a significant correlation was observed (r=0.96, p<0.001) between ACT values measured by the two different systems. Correlations were significant both for samples obtained during CPB (r=0.94, p<0.001) and during hemodialysis (0.91, p<0.001). No difference was found between ACT absolute values obtained with the two devices. This

study demonstrates that: 1) ISTAT provides reproducible measurements of ACT; 2) the results obtained by the two systems are significantly correlated in two different clinical settings.

P053

EVALUATION OF THE ABBOTT AXSYM HOMOCYSTEINE METHOD

Lonati S,* Accinni R,° Novembrino C,* Ippolito S,* Campolo J,° Galli C,'' Lunghi G,^ Bamonti-Catena F*

**Dip. Scienze Mediche, Università di Milano, IRCCS Ospedale Maggiore, Milan; °Ist. di Fisiologia Clinica-C.N.R, sezione di Milano; ''Scientific Affairs, Abbott Divisione Diagnostici, Roma; ^Laboratorio Analisi Chimico Cliniche e Microbiologia, IRCCS-Ospedale Maggiore, Milan, Italy*

The interest in determining plasma homocysteine (Hcy), as a strong and independent risk factor for cardiovascular disease, is growing, but the available assays do not allow a throughput suitable for medium or high routines. A new fully automated Hcy method on the AxSYM system has been recently proposed. *Aims.* To test the analytical performance of AxSYM Homocysteine and to compare the results with the HPLC and IMx methods. *Materials and Methods.* Fasting plasma samples were obtained by venipuncture from 166 subjects according to the consensus preanalytical rules. Hcy concentration was measured by AxSYM and IMx Hcy FPIA methods and by a rapid isocratic HPLC method with fluorescence detection. Analytical performance data are reported in Table 1. The mean dilution recovery of AxSYM assay was 103% and its linearity was good up to 1:8 dilution.

Table 1. Analytical performance data.

	AxSYM	HPLC	IMx
Imprecision (CV%)			
intraassay	3.9	1.2	1.1
interassay	4.5	2.5	2
Recovery (%)	99.2	100	101
Sensitivity (µmol/L)	0.7	0.05	0.2

Comparison data are reported in Table 2.

Table 2. Comparison data.

	Spearman's correlation	Passing & Bablok method comparison	
	rs statistic (p)	Intercept	Slope
AxSYM vs HPLC	0.83 ($p < 0.0001$)	2.944	0.937
AxSYM vs IMx	0.97 ($p < 0.0001$)	-0.367	1.142
IMx vs HPLC	0.83 ($p < 0.0001$)	2.632	0.805

The AxSYM Homocysteine method showed a good analytical performance, a satisfactory correlation with HPLC and an excellent correlation with the other FPIA method on IMx. The median difference between AxSYM or IMx and HLPC were +17% and +13%, respectively, whereas the median difference between AxSYM and IMx was zero. These results suggest that the new assay is suitable for routine use. The ease of use, complete automation and random accessing of the AxSYM system are time-saving features that will allow many laboratories to include Hcy measurement in the screening procedures to assess the thrombotic risk of healthy subjects and patients.

P054

LABORATORY DIAGNOSIS OF LUPUS ANTICOAGULANTS IN PATIENTS ON ORAL ANTICOAGULANTS. PERFORMANCE OF TWO CONFIRMATORY PROCEDURES

Chantarangkul V, Tripodi A, Clerici M, Mannucci PM

Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, University and IRCCS Maggiore Hospital, Milan, Italy

Diagnosis of lupus anticoagulants (LA) in patients receiving oral anticoagulant therapy (OAT) is problematic because of the clotting time prolongation induced by OAT. Mixing the patient's and normal plasma prior to testing for LA is currently recommended to overcome this problem. Indirect evidence (*Chantarangkul V, Thromb Res 1992; 67:355*) suggests that silica clotting time (SCT) performed at low and high phospholipid concentrations without normal plasma is not affected by OAT. We aimed to investigate the performance of SCT in diagnosing LA in patients on OAT in comparison with STACLOT-LA (Stago) performed with and without hexagonal phospholipids and normal plasma. Blood from 62 patients on OAT because of a previous history of thrombosis attributable to the antiphospholipid syndrome was collected and centrifuged at 2000g. Plasma was filtered and stored frozen at –70 °C. STACLOT-LA was considered diagnostic for LA if the clotting time difference before and after hexagonal phospholipid was above the cut-off (6.6 seconds). SCT was considered diagnostic for LA if the percentage correction of the clotting time after phospholipid increase was above the cut-off (17.5%). Forty-three of 62 plasmas were LA-positive according to STACLOT-LA. The median (range) clotting time difference after hexagonal phospholipid for these 43 plasmas was 38 (9-101) seconds. Thirty-nine of the 43 STACLOT-LA -positive plasmas were also positive according to SCT (sensitivity relatively to STACLOT-LA = 91%). The median (range) percentage correction for these 39 plasmas after phospholipid increase was 64% (23%-79%). Two of 19 plasmas that were STACLOT-LA-negative were SCT-positive (specificity relatively to STACLOT-LA = 89%). In conclusion, SCT performed at low and high phospholipid concentrations without normal plasma may be considered as reliable as STACLOT-LA performed with hexagonal phospholipids and normal plasma to diagnose LA in patients on OAT. Advantages of SCT over STACLOT-LA are easy automation on coagulometers, no need for normal plasma and the relatively low cost.

P055

LABORATORY DIAGNOSIS OF LUPUS ANTICOAGULANTS. EFFECT OF FREEZING-THAWING NON-FILTERED PLASMAS ASSESSED BY STACLOT LA AND SILICA CLOTTING TIME

Chantarangkul V, Tripodi A, Clerici M, Bressi C, Mannucci PM

Angelo Bianchi Bonomi, Hemophilia and Thrombosis Center, Department of Internal Medicine, University and IRCCS Maggiore Hospital, Milan, Italy

Residual platelets in plasma are considered detrimental after freezing-thawing, as phospholipids released from ruptured platelets may quench lupus anticoagulants (LA). We aimed at assessing the effect of residual platelets after freezing-thawing

plasmas tested with two procedures for LA. Blood from 52 patients suspected of having LA were centrifuged at 2,500g. Plasmas were subdivided into 2 aliquots. One was filtered to remove residual platelets and both were frozen and stored at –70°C. Silica clotting time (SCT) at low and high phospholipid concentrations and Staclot® LA with and without hexagonal phospholipids were performed on thawed plasmas. Plasmas were considered LA-positive when both SCT and Staclot® LA performed on filtered plasmas were diagnostic for LA. Forty-two of 52 plasmas fulfilled the diagnostic criteria and were retained for subsequent analysis. SCT on non-filtered plasmas was diagnostic for LA in 42 of 42 plasmas. Though the median (range) percentage correction recorded after phospholipids addition for filtered plasmas, i.e, 67% (36%-83%) was reduced to 54% (25%-81%) for non-filtered plasmas (p<0.001), it was still above the cut-off (i.e, 20.9%). Staclot® LA on non-filtered plasmas was diagnostic for LA in 42 of 42 plasmas. Though the median (range) clotting time difference recorded after phospholipid addition for filtered plasmas, i.e, 40.8 (10-103.5) sec. was reduced to 31.7 (2.8-88.8) sec. for non-filtered plasmas (p<0.001), it was still above the cut-off (i.e, 1.7 sec.). In conclusion, residual platelets do not affect the diagnostic efficacy of SCT and Staclot® LA. However, the fact that the percentage correction for SCT and the clotting time difference for Staclot® LA are reduced by residual platelets, suggests that weak LA may be lost upon freezing-thawing non-filtered plasmas.

P056

USEFULNESS OF THE PLATELET FUNCTION ANALYZER PFA-100 IN MONITORING THE EFFICACY OF EPOPROSTENOL INFUSION

Pergolini P, Cau C, Rolla R, Todesco A, Atzeni N, Brustia A, Cernigliaro C, Pagani L, Bellomo G

Laboratorio di Ricerche Chimico-Cliniche, Università del Piemonte Orientale, Ospedale Maggiore della Carità, Novara, Italy

The usefulness of the platelet function analyzer PFA-100 in investigating platelet function and dysfunction has been investigated in a variety of hemorrhage-prone clinical conditions and to monitor anti-platelet therapy. This laboratory approach allows the measurement of the time required for platelets in citrated whole blood to occlude an aperture cut into a membrane coated with collagen plus either epinephrine (CTEPI) or adenosine diphosphate (CTADP). Here we report the use of a similar approach to optimize epoprostenol infusion during coronary bypass surgery in a patient with heparin-induced thrombocytopenia. As a first step we performed an *in vitro* dose-dependency experiment to evaluate the ability of epoprostenol to increase both CTEPI and CTADP. The minimal drug concentrations required to achieve a significant effect were 1 and 2.5 ng/mL of citrated whole blood, respectively. Before surgery, the patient had a prolonged CTEPI (>300 sec) due to aspirin treatment and a normal CTADP (108 sec). The initial epoprostenol infusion protocol (50 minutes at 4 ng/Kg/min) was ineffective in prolonging CTADP(109 sec) and for these reasons the infusion rate was increased to 10 ng/Kg/min. The new regimen caused a significant increase of CTADP to 276 sec indicating a successful inhibition of platelet function which persisted for at least 60 minutes after the cessation of epoprostenol infusion. After this time, however, CTADP progressively decreased to 166 and 145 sec (24 and 48 hours after the end of infusion) and returned to normal values (58 sec) one month after surgery. These findings suggest that the rapidity and simplicity in use and the excellent sensitivity of PFA-100 to optimize platelet-affecting medication make it extremely suitable for employment in this approach.

P057

ALU-REPEAT I/D POLYMORPHISM OF T-PA GENE: INFLUENCE ON T-PA RELEASE AFTER VENOUS OCCLUSION

Sartori MT, Saggiorato G, Spiezia L, Patrassi GM,* Carraro G, Girolami A

*Department of Medical and Surgical Sciences, University of Padua; *Department of Medicine, Cittadella (PD); Italy*

Endothelial synthesized t-PA is released in both a constitutive and regulated fashion. The cytoplasm storage sites as well as the mechanisms of t-PA secretion are not completely known, and a genetic modulation was recently suggested. In healthy subjects an association between net t-PA release rate and the Alu-repeat I/D polymorphism of t-PA gene intron h, which is in linkage disequilibrium with three other t-PA gene polymorphisms, was described. We aimed to evaluate the possible influence of the Alu-repeat I/D polymorphism on t-PA release after venous occlusion test (VO). Fifty-three patients with a previous arterial or venous thrombosis showing an impaired fibrinolytic capacity, and 43 healthy controls were studied. In each subject the following tests were assayed before and 20' after VO: euglobulin lysis time, t-PA antigen (t-PA:Ag) and activity, PAI-1 antigen and activity; moreover the Alu-repeat I/D polymorphism was determined by allele specific PCR. Defective fibrinolytic potential was due to reduced t-PA release in 25 patients (t-PA group) and to PAI-1 excess in 28 patients (PAI-1 group). No significant differences in both genotype distribution and allele frequencies were observed between patients and controls. The increase in t-PA:Ag after VO (20'/0' levels ratio adjusted for hematocrit) was considerably higher both in controls and in PAI-1 group patients carrying the I allele, either at the homozygous or at the heterozygous level, than in DD genotype carriers (II, ID, DD: 3.71±0.06, 3.52±0.05, 1.96±0.04 in controls, and 2.84±0.03, 2.43±0.05, 2.04±0.04 in PAI-1 group, respectively). Furthermore, a significant difference was seen between ID and DD genotypes in controls (p<0.05), and between II and DD genotypes in PAI-1 group (p=0.03). To a lower extent, a similar influence of the Alu-repeat polymorphism on t-PA release was also observed in t-PA group patients. In conclusion, our data suggest a possible genetic modulation of t-PA regulated secretion.

P058

ACTIVATED FACTOR II/ANTITHROMBIN SYSTEM IN PATIENTS ON ORAL ANTICOAGULANT THERAPY

Preda L, Lattuada A, Rossi E

Hematology and Blood Transfusion Service, L. Sacco Hospital, Milan, Italy

Introduction. We have recently published the performances of a new global test (TGAt) for evaluate the function of activated-factor II/antithrombin system. This test measures the resistance to inhibition of activated-factor II by native antithrombin sub-

stances, in the presence of heparin. The resistance was expressed as prolongation time normalized versus a reference normal plasma (ΔN). The normal range in healthy subjects (blood donors) was 0.70-1.40 (ΔN). It is well known that the activity of factor II decreases during OAT enhancing the inhibition of its activated form. *Purpose.* The aim of this study was to evaluate the normal range of TGAt in OAT. *Patients.* We enrolled 504 consecutive patients on OAT; in 112 (22.2%) with INR< 2.00, 344 (68.3%) with INR 2.00÷3.50 and 48 (9.5%) with INR >3.50. *Methods.* TGAt test consists we measured the clotting time of a plasma sample after addition of the reagent containing *E. carinatus* venom with and without heparin. TGAt, PT and fibrinogen were performed on all patients, while factor II and AT tests were performed on 164 patients. *Results*. The table below shows the mean value of each test in three INR groups.

	TGAt ΔN	PT INR	Fib mg/dL	fact II %	AT %
INR<2.00	1.47	1.63	381	54	116
2.00<INR<3.50	2.13	2.61	378	29	119
INR>3.50	2.41	4.20	428	14	123

In detail, the TGAt mean value and the TGAt cut-off value during OAT are shown in the following Table:

INR	1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00
ΔN									
Mean	1.10	1.49	1.82	2.10	2.32	2.48	2.58	2.62	2.64
Cut-off	0.70	0.93	1.14	1.31	1.45	1.55	1.61	1.64	1.65

Conclusions. The TGAt test during OAT is influenced by factor II levels; in some patients, we found an inexplicable resistance to inhibition of activated factor II. Therefore, during OAT the TGAt test is useful in defining the balance between pro- and antithrombotic agents.

P059

EVALUATION OF THE ACTIVATED FACTOR II/ANTITHROMBIN SYSTEM IN NORMAL PREGNANCY

Preda L, Lattuada A, Rossi E

Hematology and Blood Transfusion Service, L. Sacco Hospital, Milan, Italy

Introduction. We have recently published the performances of a new global test (TGAt) for evaluating the function of activated-factor II/antithrombin system. This test measures the resistance to inhibition of activated-factor II by native antithrombin substances, in the presence of heparin. The resistance was expressed as prolongation time normalized versus a reference normal plasma (ΔN). The normal range in healthy subjects (blood donors) was 0.70-1.40 (ΔN). It is well known that pregnancy is a state characterized by an increase of factor II and fibrinogen, whose levels should be monitored during pregnancy. *Purpose.* The aim of this study was to evaluate the normal range of TGAt in physiological pregnancy. *Patients*. We enrolled 122 women in physiological pregnancy; 23, 48 and 51 women were, respectively, in 1st, 2nd and 3rd trimester. *Methods*. The TGAt test consists of measuring the clotting time of a plasma sample after addition of the reagent containing *E. carinatus* venom with and without heparin. Factor II, AT and fibrinogen as main agonists in the system, were also evaluated, in addition to PT, aPTT, TGCS (global test for PC-PS system). *Results.* The following table shows the mean value of each test in the three trimesters.

Table 1.

	TGAt DeltaN	PT INR	aPTT ratio	Fib mg/dL	TGCS EUC%	fact II %	AT %
1st	0.90	0.96	1.00	371	69	101	99
2nd	0.82	0.93	1.00	434	53	110	97
3rd	0.75	0.90	0.97	485	46	115	100

In detail, the TGAt mean value and the TGAt cut-off value during pregnancy are shown in the following table:

Table 2.

Week	0	5	10	15	20	25	30	35	40
DeltaN									
mean	1.05	0.98	0.91	0.86	0.82	0.79	0.77	0.76	0.75
cut-off	0.70	0.66	0.61	0.58	0.55	0.53	0.52	0.51	0.51

Conclusions. The TGAt test is useful in recognizing clotting activation during phisyological pregnancy.

P060

IN VITRO HEPARIN ANTICOAGULANT INHIBITION INDUCED BY PLASMA EOSINOPHIL CATIONIC PROTEIN

Testa S, Pigoli G, Dolci D, Fantini M, Denti N, Morstabilini G, Alatri A, Ferrari L

Centro Emostasi e Trombosi, Istituto Di Patologia Clinica, Azienda Ospedaliera "Istituti Ospitalieri", Cremona, Italy

Eosinophil cationic protein (ECP) is a highly cationic protein and a major compound of the human eosilinophil cytoplasm granules. The biological function of ECP and the role of eosinophil granulocytes in the coagulation/inflammation pathway are not clarified but it has been shown that ECP has cytotoxic properties, inhibits FXII, and plays a role in platelet activation. Several syndromes characterized by eosinophilia may be complicated by thrombosis and hypereosinophilia was suggested as a cause for thromboembolic events. ECP plasma levels are increased in neoplastic patients showing a partial response to antitumoral therapy. We observed increased sodium heparin infusion needs in patients with hypereosinophilia so, in according with previous reports that showed *in vitro* heparin inhibition by purified ECP, we evaluated *in vitro* interactions between high ECP plasma levels and sodium heparin. High ECP-rich plasma was obtained from a patient with hypereosinophilic syndrome of unknown origin. ECP plasma level was = 75,1 ng/mL (ECP nor-

mal range =1-5 ng/mL), determined by immunometric assay (Immulite, Medical System). Sodium heparin (Heparin Vister) was used at the concentration of 5000 UI/mL. A normal plasma pool was obtained from 40 healthy donors and used to calculate normal ranges and a standard curve of heparin inhibition expressed in seconds through activated partial thromboplastin time (aPTT). Different heparin concentrations were used to obtain a standard curve from 0.05 UI/mL to 0.5 UI/mL, useful to prolong aPTT in the normal plasma pool from 36.9 sec to 116.5 sec. The ECP-rich plasma significantly inhibited aPTT prolongation compared to the normal plasma pool (p=0.027). The present observation confirms, *in vitro*, the inhibition of heparin anticoagulant activity by high ECP plasma levels and could explain increased amount of heparin needed in some patients. It suggest a possible prothrombotic role of ECP in patients with hypereosinophilic activity (neoplasia, parasitic infections, autoimmune disease, allergy) by the inhibition of endothelial heparin-like substances.

P061

ACTIVATED PROTEIN C RESISTANCE AND FACTOR V LEIDEN: COMPETITION OR MUTUAL HELP?

Micelli M, Ranieri P, Coppola B,° Masellis V°

Coagulation Laboratory, Policlinico, Bari; °Department of Biomedicine of Developmental Age, University of Bari, Italy

Introduction. APCR, acquired or congenital, causes an increased risk of venous thrombosis. Congenital APCR can be due to different mutations, the most frequent being the Leiden mutation. The aim of the study was to evaluate physicians' behavior towards the APCR/V Leiden phenomenon and the consequence on health costs. *Methods*. We evaluated whether FV Leiden molecular analysis was or was not matched with APCR analysis in 195 consecutive patients during 2001. We also verified APCR results as regards the molecular analysis. The Leiden mutation was analyzed with the classical Bertina method and the APCR test, both with the conventional and the FV modified method, using the APTT-based assay from Chromogenics-Sweden. *Results*. In 82/195 samples (42%) both DNA and APCR analysis was requested; in 162/195 DNA analysis (83%) resulted wild type and 33/195 (17%) resulted FV Leiden. In 70% of the wild type samples and in 48.5% of the Leiden mutation just molecular analysis was requested. The concordance between DNA analysis and both APCR methods was 100%. We did not find acquired APCR. *Discussion*. In the majority of the requests (58%) only DNA analysis was asked with the loss of clinically useful information: the acquired APCR and mutations different from the Leiden one. DNA analysis performed in 83% of wild type samples elevated the health cost because the DNA analysis costs €110.01. The APCR method costs €9,04 and its sensitivity and specificity is 100%. The preference for the molecular analysis could be due to the diffusion of the *Leiden* term in the scientific literature without knowledge of biological APCR phenomenon and to the false opinion that molecular analysis is definitive and infallible towards the presumed variability of biochemical methods. To assume that normal results with the FV modified method exclude FV mutation and to confirm positive results with DNA analysis to distinguish heterozygotes and homozygotes could be a reasonable strategy. DNA analysis could be the only approach in the lupus anticoagulant. Scientific society guidelines could improve the clinical approach and reduce health costs.

P062

FEASIBILITY OF THE USE OF FILTERED PLASMA IN THE AUTOMATED SCREENING TEST FOR ACTIVATED PROTEIN C RESISTANCE

Iorio A, Accardo P,* Bellesia E,* Tascedda E,* Tonelli L,* Brini M*

*Sezione di Medicina Interna e Cardiovascolare, Dipartimento di Medicina Interna, Università di Perugia; *Dipartimento di Patologia Clinica, Azienda Ospedaliera Santa Maria Nuova di Reggio Emilia, Italy*

Activated protein C resistance (aPCR) and the lupus-like anticoagulant (LA) are the two thrombophilic conditions most frequently found in candidate patients and several fully automated methods are availble for both assays. LA testing is better performed using filtered plasma, to lower the incidence of false negative results. Performing aPCR screening on the same filtered plasma could provide a simple way to optimize the daily practice of the screening laboratory. The aim of this study was to assess the feasibility of aPCR testing on filtered plasma. A hundred plasma samples were selected in 20 consecutive worksessions in which at least on sample was routinely found positive at aPCR testing. For each positive sample, four negative samples were selected. aPCR assay was performed with the IL Test™ APC™ Resistance V (IL, Milan) on a Stago coagulometer (Roche, Milan). Filtered trisodium citrate plasma was prepared with a syringe-driven 0.22 nm PTFE membrane filter (Millipore). aPCR assay was performed both on filtered (F-) and non-filtered (NF-) plasma, within two hours from venipuncture. F- and NF-pooled plasmas were tested in each session to standardize the ratio for the aPCR assay. All the samples were assessed for the factor V Leiden mutation with a standard PCR method. All the twenty patients found positive at NF-aPCR were confirmed heterozygous for the Leiden mutation; all the other samples were found genetically normal. Baseline aPTT values (mean±SD) were 41.9±3.5 and 42.2±3.5 for NF- and F-aPCR, respectively, while aPTT+APC values were 92.5±14.3 and 94.7±15.8. F-aPCR was able to correctly classify all the 100 samples, both using the simple and the standardized ratio (the latter with the same cut-off value as the NF-test). aPCR screening can be performed on filtered plasma with the same performance characteristics as in standard citrated plasma.

P063

MARKED CLOTTING ACTIVATION AND MONITORING OF HEPARIN TREATMENT DURING CARDIOPULMONARY BYPASS

Paniccia R, Fedi S, Noferi D,* Bandinelli B, Pretelli P,* Giusti B, Ilari I, Lucarini L, Sestini I, Abbate R, Prisco D

*Dipartimento di Area Critica Medico-Chirurgica, Università degli Studi di Firenze; UU.OO. *Centro Trombosi, Anestesiologia e Rianimazione 1, Azienda Ospedaliera Careggi, Florence, Italy*

During cardiopulmonary bypass (CPB) a marked clotting activation occurs due to surgery itself and to extracorporeal circulation (ECC), which needs the use of heparin. High doses of heparin are administered during CPB to achieve target anticoagulation as defined by the classical activated clotting time (ACT) test. However, a number of factors such as hemodilution, hypothermia, etc. may impair the efficacy of heparin monitoring by ACT and problems of precision have been reported. The aim of this study was to evaluate the extent of clotting activation,

despite the use of high doses of heparin, during CPB. Blood samples (n=120) from 20 CPB patients were obtained before CPB, after heparin administration, during ECC and after protamine. Two markers of clotting activation, D-dimer (D-D, AGEN) and thrombin anti-thrombin complex (TAT, Behringwerke) were measured; moreover, plasma levels of fibrinogen (Fbg, DADE) and antithrombin III (AT III, Behringwerke) were determined. Heparin anticoagulation was monitored by a new point-of-care whole blood analyzer, the ISTAT Celite-ACT (Abbott, USA). For precision studies ACT was assayed in duplicate. ACT values were correlated with the plasma levels of heparin measured by an anti-Xa-based assay (DADE). During CPB progressive and parallel increases of D-D and TAT values, which were significantly correlated between them (r=0.64 $p<.001$), were found. In contrast, decreases of plasma levels of Fbg and AT III were observed. A good linear correlation was found between ACT duplicates (r=0.99, $p<0.001$). ACT measurements were strictly correlated with heparin plasma levels (r=0.82, $p<0.001$), showing a dose-response relationship. In contrast, no correlation was found between either D-D or TAT values and ISTAT ACT or heparin levels. As a whole, these data indicate that during CPB a progressive activation of coagulation occurs despite the use of high doses of heparin. The ISTAT ACT is a useful tool to monitoring heparin therapy, but it does not mirror the extent of clotting activation during CPB.

Posters
Antiphospholipid Antibodies

P064

ANTICARDIOLIPIN AUTOANTIBODIES ASSOCIATED WITH THE ACQUIRED THROMBOPHILIA OF CHEMONAIVE COLORECTAL CANCER PATIENTS

Di Micco P,* Niglio A,* Izzo T,* Viggiano G,* de Vita F, Diadema MR,° Morgillo F,° Torella R,* Misto G,^ De Lucia D^

**V Division of Internal Medicine; Division of Oncology, Second University of Naples; ^Institute of General Pathology, II University of Naples, Italy*

Colorectal cancer is often associated with alteration of hemostasis. Thrombotic events, in fact, are more frquent than hemorragic disorders and can often represent the first manifestation of the disease. However, many pathways are involved in the acquired thrombophilia of colorectal cancer patients and also antiphospholipid antibodies, as anticardiolipin autoantibodies (aCL), seem to be involved. To investigate the incidence of aCL in chemonaive colorectal cancer patients we screened two groups of patients: the first group (18 patients) suffered from colorectal cancer in ASTER-COLLER B stage, while the second group (9 patients) had colorectal cancer in ASTER-COLLER C and D stages. In both groups aCL (Ig M and Ig G) were screened by an ELISA method. Six (33%) deep venous thromboses (DVT) were recorded in the history of the first group of patients, while four (43%) DVT were recorded in the second group. aCL were found in three patients of the first group (16.6%) and of the second group (33.3%), $p < 0.05$. F 1+2 were high in both group of patients compared to control subjects (2.5±0.5 nM vs 0.45±0.35 nM), and the highest values were observed in the group of patients with aCL (2.8±0.5 nM, $p<0.001$), showing thrombin generation. Our results show the presence of aCL during the natural history of colorectal cancer and seem to confirm their potential role in the pathogenesis of related-acquired thrombophilia, in particular during advanced stage of the disease. The authors feel that the mechanism of thrombin generation in colorectal cancer patients is unknown, but may reflect activation of plasma kallikrein/kinin system on damaged endothelial cells activated by high titers of aCL. However further studies are needed to confirm the role of aCL in colorectal cancer and the acquired thrombophilic state which is related also to other mechanisms such as tissue factor production, homocysteine metabolism, increased thrombin generation, fibrin deposition and many others.

P065

THROMBOTIC RISK AND LABORATORY PATTERN IN 91 CONSECUTIVE PATIENTS WITH LUPUS ANTICOAGULANT AND/OR ANTICARDIOLIPIN-ANTIBODIES

Ghirarduzzi A, Silingardi M, Cattabiani L, Nicolini A, Tonelli L, Iorio A

Internal Medicine I & Clinical Pathology Departments, Thrombosis Center, Azienda Ospedaliera Internal Medicine I and Clinical Pathology Departments, Thrombosis Center, Azienda Ospedaliera S.Maria Nuova, Reggio Emilia, Italy

Background. Antiphospholipid antibodies (APA) are an heterogeneous family of immunoglobulins diagnosed by clotting or immunological tests. Among the APA family, lupus anticoagulant (LA) and anticardiolipin (ACA) IgG and IgM are the most frequently investigated, but their role as risk factors for thrombotic events (TE) is not clearly established, and they not rarely are found in asymptomatic patients. *Aim of the study.* To investigate the association of laboratory pattern and TE in a cohort study. *Methods.* We examined consecutive patients admitted to the Thrombosis Center between January 1997 and September 2000. LA was diagnosed according to the criteria of the ISTH SSC. Reagents used were: PTT-LA and Staclot-LA from Diagnostica Stago, France; dRVVT Screening/Confirm from American Diagnostica, US; Anticardiolipin IgG/IgM from Orgentech, Deutschland. IgG and IgM results were espressed in GPL and MPL units respectively, with a cut off value of 40 U/mL. A logistic regression analysis was performed to evaluate the effect of the laboratory pattern on the risk of being symptomatic. *Results.* Ninety-one patients were enrolled (20/71 male/female, mean age 50 yrs, range 5-85). Patients were classified by the pattern of LA (dRVVT, APTT, both or none) and/or ACA (IgG, IgM, both or none) and for the presence of clinical events (round brackets). The risk of being symptomatic as estimated in the logistic model is shown in squared brackets. Forty-four events were recorded in 37 patients (31.9%): 22 deep vein thrombosis/pulmonary embolism, 7 recurrent superficial venous thrombosis, 11 cerebral ischemic attack, 3 fetal losses, 1 AMI, 1 right atrial thrombosis. ACA IgG resulted the strongest predictor (p = .0026), followed by dRVVT and IgG - dRVVT association. The odds ratio for IgG and dRVTT positivity (gray cells) vs negativity was 5.0 (95% CI 2.03–12.33). *Conclusions.* In our study the highest risk of TE was found in patients with ACA IgG > 40 U/mL and/or dRVVT positivity.

	dRVVT & aPTT	dRVVT	aPTT	LA neg	
ACA IgG & IgM	1 (0) [-]	3 (2) [.60]	1 (0) [-]	3 (2) [.67]	8 (5)
ACA IgG	17 (10) [.40]	7 (0) [.71]	1 (0) [-]	6 (2) [.33]	31 (10)
ACA IgM	4 (0) [-]	5 (3) [.60]	1 (1) [-]	10 (2) [.20]	20 (6)
ACA neg	20 (7) [.25]	7 (2) [.28]	5 (1) [.20]	-	32 (0)
	42 (17)	22 (12)	8 (2)	19 (4)	91 (37)

P066

DETECTION OF ANTIPHOSPHOLIPID ANTIBODIES IN UNEXPLAINED RECURRENT FETAL LOSSES

Ciampa A, Manzo A, Capone F, Volpe E

"G. Moscati" Hospital Haematology Department, Avellino, Italy

Objectives. The aim of our study was to investigate lupus anticoagulant (LA) and/or anticardiolipin antibody (ACA) activity in women with consecutive abortions. *Study design.* Four hundred apparently healthy women (mean age 30 years) who were not pregnant but had experienced recurrent negative fetal outcomes were studied prospectively. Control studies were performed using plasma and serum from 50 healthy control women (mean age 29 years) recruited among LA and/or ACA – subjects. Accurate data on the fetal losses were collected. None of the patients suffered from any underlying obstetrical and/or gynecological disease that could possibly explain the abortions. LA activity was diagnosed using screening and confirmatory procedures based on criteria according to ISTH-recommendations. IgG and IgM ACA isotypes were assayed with the Autozyme kit from Byk-Gulden. Plasma and serum samples from each patient were tested in tandem 8 weeks after the first diagnosis. *Results.* Of the 400 patients tested, 256 women were Apl negative. As to the remaining 144 women (36%) who were Apl^{+}:111 patients (77.08%) were positive for ACA alone; 9 patients (6.25%) were positive for LA alone and 24 patients (16.67%) were positive for both. We found that: 38 women (28%) had experienced only an abortion; 56 patients (41%) 2 abortions; 33 patients (24%) 3 abortions and 19 patients (14%) four or more abortions. *Conclusions*: Our studies confirm the effectively negative implications of Apl in poor pregnancy outcomes. Furthermore, they emphasize the relevance of LA and ACA activity as an important risk factor to be screened for and monitored constantly in women who experience miscarriages, even in those who have had only one abortion.

P067

EVALUATION OF TRANSIENT ANTICARDIOLIPIN ANTIBODY 1 LUPUS ANTICOAGULANT-POSITIVE TESTS IN PREGNANCY

Ciampa A, Manzo A, Capone F, Volpe E

"G. Moscati" Hospital Haematology Department, Avellino, Italy

Objectives. There is a strongly increased risk of poor pregnancy outcome in women with clear evidence of autoimmune phenomena (in particular antiphospholipid antibodies). Our goal was to elucidate the high incidence of a transient LA and ACA activity in normal pregnancy. *Study design*. Four hundred apparently healthy women (mean age 30 years) who were not pregnant but had experienced recurrent negative fetal outcomes were studied prospectively. Healthy controls were 50 women (mean age 29 years) recruited among Apl subjects. Accurate data on the fetal losses were collected. None of the patients suffered from any underlying disease that could explain the abortions. LA activity was diagnosed using screening and confirmatory procedures based on criteria according to ISTH-recommendations. IgG and IgM ACA isotypes were assayed with the Autozyme kit from Byk-Gulden. Plasma and serum samples from each patient were tested in tandem 8 weeks after the first diagnosis. *Results.* Of the 400 patients tested, 256 women were Apl negative. As to the remaining 144 women (36%) who were Apl+: 111 patients (77.08%) were positive for ACA alone; 9 patients (6.25%) were positive for LA alone and 24 patients (16.67%) were positive for both. Furthemore, among the group of patients previously tested negative, 20 patients, once again pregnant, showed abnormal levels of ACA and LA (18 ACA IgG, 1 LA, 1 ACA and LA) with high peaks at the end of the first trimester of pregnancy. Fifteen pregnancies resulted in miscarriages. The remaining 5 patients who were constantly monitored, reverted to having normal levels in the second and third trimester and had successful deliveries. *Conclusions.* Our results show that LA and ACA activity is an important risk factor to consider and monitor constantly. They also indicate the effectively negative incidence of unexpected high transient Apl levels in pregnancy.

P068

THE β(2)-GLYCOPROTEIN 1-DEPENDENT ANTICARDIOLIPIN ANTIBODIES: THEIR IMPORTANCE AS A RISK FACTOR FOR ISCHEMIC STROKE AND MYOCARDIAL INFARCTION

Gristina T,[#] Brusca I,[#] Fazio M,* Sarullo FM,[§] Fiorello F,* Rizzo M,* Sucato R,[#] Cantisano V,[#] Castello A,[§] D'Angelo A,* La Chiusa S[#]

*[#]Servizio di Patologia Clinica, *Divisione di Medicina, [§]Divisione di Cardiologia, Ospedale "Buccheri La Ferla" Fatebenefratelli, Palermo, Italy*

Background. Recent reports have shown the importance of new risk factors for ischemic stroke and myocardial infarction. We investigated the relationship between anticardiolipin antibodies (aCL), and β(2)-glycoprotein 1 (a-β2GP1), their association with increased risk of ischemic stroke (IS) and myocardial infarction (MI) and the occurrence of clinical recurrence of ischemic events such as IS, MI, unstable angina or transient ischemic attack (TIA) *Methods.* One hundred and thirty-nine consecutive patients (mean age 64.8±13.6 years) admitted to the Department of Medicine or to the Intensive Care Unit of our Hospital were involved in the study, and risk factors for ischemic events were recorded. The study group consisted of 86 men and 53 women with MI (n=50), IS (n=60), TIA (n=29). The control group consisted of 50 sex-matched healthy individuals (mean age 55,76±16,93 years). aCL IgG and IgM, a-β2GP1 were measured with ELISA methods. Homocysteine and cholesterol levels as well as other traditional risk factors were also determined (data not shown). *Results.* One hundred and twelve out of 139 patients (80.5%, 95% C.I. 72.82-88.33) had antiphospholipid antibodies, 54/112 had simultaneously aCL and a-β2GP1 (48.21% 95% CI 38.42-58.01), 30 were aCL+ a-β2GP1- (26.79% 95% C.I. 18.11-35.47), 28 were aCL- a-β2GP1+ (25.00% 95% C.I. 16.51-33.49). The risk of trombotic events was significantly increasing in aCL+ a-β2GP1+ patients compared with control group, (odds ratio 5.65, 95% C.I. 2.11-15.13). The odds ratio of aCL+ a-β2GP1– was 0.85 (95% C.I. 0,40-1,82); the odds ratio of aCL- a-β2GP1+ patients was 2.25 (95% C.I. 0.82-6.19). Subsequently 93 patients had ischemic events; 40/93 were aCL+ a-β2GP1+ patients, (43.01%, 95% C.I. 33.31-52.71), 18/93 were aCL+ a-β2GP1- patients, (19.35%, 95% C.I. 11.61-27.10), and 20/93 were aCL- a-β2GP1+ patients, (21.51%, 95% C.I. 13.45-29.56). The risk of thrombotic events in the follow-up also increased in aCL+ a-β2GP1+, (odds ratio 1.92, 95%C.I. 0.89-4.10), but without statistical significance. *Conclusions*. These data suggest that aCL, particularly the β2GP1 dependent variety, is an important predictor of ischemic events.

P069

CATASTROPHIC VASCULAR OCCLUSION SYNDROME IN A PATIENT WITH GAUCHER'S DISEASE TYPE I AND ANTIPHOSPHOLIPID ANTIBODIES

Giuffrida G, Guarnaccia G, Ferlito C, Cipolla A, Di Francesco E, Musso R, Giustolisi R

Cattedra e Divisione di Ematologia con Trapianto, Ospedale Ferrarotto, Catania, Italy

Gaucher's disease (GD) type I (β-glucocerebrosidase deficiency) is characterized by the storage of uncleaved β-glucocerebroside in the cells of the reticuloendothelial system leading to bone marrow infiltration, hepatosplenomegaly and skeletal lesions. Hematologic changes with anemia, thrombocytopenia and thrombocytopathy are common. Recently clotting factor and natural inhibitor deficiencies have also been reported as well as increased levels of antiphospholipid antibodies (APA) but the pathophysiology of such abnormalities is still unclear. We report the case of a 48-year old man who developed massive venous thrombosis 5 years after receiving a diagnosis of GD type I with concurrent APA, lupus anticoagulant (LAC) and anticardiolipin antibodies (ACA). At admission the patient, splenectomized and in enzyme replacement therapy (ERT) at the low dosage-high frequency regimen (imiglucerase-15 U/kg/month), complained rest dyspnea, abdominal pain, inferior limb edema and abdominal distension. A computed tomography of the chest and abdomen showed total thrombosis of the common jugular vein, left brachyocephalic vein, intra-extra hepatic portal vein and of the proximal tract of the mesenteric vein, massive hepatic atrophy, ascites and pleural effusion. Levels of protein S, protein C and antithrombin III were normal, mutations of the allele of metylene thetrahydrofolate reductase (MTHFR-C677T), prothombin 20210 and factor V Leiden were not found. Prothrombin time was slightly prolonged; D-dimer and fibrinogen were increased. Activated partial thromboplastin time (APTT) was prolonged (55"-n.v. =30") and not corrected by mixing procedures (ratio =1.7). Diluted Russell's viper time (dRVVT) was prolonged and ACA were significantly increased (4.7 U/mL-n.v. <1.5). APA are a heterogeneous group of antibodies that are detected in the serum of patients with a variety of conditions, including autoimmune (SLE), infectious (AIDS), and lymphoproliferative disorders and recently have also been reported in GD. Thromboembolic events, thrombocytopenia and recurrent fetal loss are the most frequent clinical manifestations. GD is associated with a significant increase in specific autoantibodies, which may be the result of polyclonal stimulation secondary to the distorted lipid metabolism. In summary we think that global immune dysregulation, which is found in GD can promote APA formation and that in these patients ERT at high dosage may correct these immune dysregulation and may prevent this development of autoantibodies.

P070

APPROACH TO IDENTIFICATION OF LUPUS ANTICOAGULANT INHIBITOR DURING ORAL ANTICOAGULANT THERAPY

Russo U, Sacchi E, Birolini A, Lattuada A, Preda L, Rossi E

Hematology and Blood Transfusion Service, L. Sacco Hospital, Milan, Italy

Introduction. Oral anticoagulant therapy (OAT) is an important tool for the prevention and treatment of venous and arterial thrombosis. One of the most frequent causes of thrombosis is the antiphospholipid antibody syndrome (APS). The laboratory diagnosis of APS presents some difficulties, expecially during OAT. *Purpose.* The aim of our study was to evaluate whether the silica clotting time test (SCT), performed on a mixture (50/50) of the plasma sample and normal pool (NP) plasma, is an useful method to identify LA inhibitor in patients during OAT. *Patients.* We enrolled 360 patients in OAT with different pathologies; 29 of 360 patients were already known to have APS. *Methods.* PT, SCT50/50, SCT50/5 and LAC screen/confirm tests. To improve

the sensitivity of the SCT test for the LA inhibitor we used a small volume (5 μL) of a concentrate of prothrombin complex, instead of 50 μL of NP. *Results*. The range of INR in patients was 1.18±9.21. The cut-off ratio for SCT50/50 and SCT 50/5 was, by our data, respectively 1.30 and 1.35.

Table 1.

SCT ratio	SCT50/50 N pts	SCT50/ 5 N pts
> 2.00	18 (14)*	23 (15)*
cut-off ÷ 2.00	30 (6)*	38 (6)*
TOTAL	48 (20)*	61 (21)*

*Number of known carriers of LA inhibitor.

When the SCT ratio was > 2.00 we considered the presence of LA inhibitor certain and it was not necessary discontinue the therapy for confirmation; when the SCT ratio was near the cut-off or 2.00 it is opportune to discontinue therapy to confirm or exclude the presence of LA inhibitor. *Conclusions*. Our results confirm the usefulness of the SCT50/50 test to detect LA inhibitor in patients during OAT, even those with a high INR. Moreover, the test is well known and simple to perform on automated devices.

P070a

RESISTANCE TO ACTIVATED PROTEIN C CORRELATES WITH ACA IGG AND ANT β2GPI IGG

De Lucia D,* Maisto G,* Del Giudice V,* Marotta R,* Mosavat M,* De Francesco F,* D'Auria G,# Rizzelli L,° Vitolo M,# Esposito C,# Avolio G,# Formicola O,# Russo G#

**Institute of General Pathology, Laboratory of Haemostasis and Thrombosis; II University of Naples; °Laboratory of Clinical Pathology, S.S. Annunziata Hospital; #Laboratory of Clinical Pathology; Loreto Mare Hospital, Naples, Italy*

Recent evidences have showed that resistance to activated protein C (APCR) may be due not only to FV R506Q mutation but also to immunologic disorders (APC Resistance phenotype). In order to evaluate whether the presence of LAC syndrome, ACA (IgG and M) and anti β2GPI (IgG) antibodies could be associated with the APC Resistance phenotype, we studied 50 LAC patients diagnosed according to SSC ISTH criteria. Forty-five out of fifty had a previous history of venous (28) or arterial brain (13) thrombosis or fetal losses (4). We carried out the original and the modified (plasma diluted 1:5 in FV depleted plasma) APCR APTT based assays (Instrumentation Laboratory). We found that the prevalence of APCR original phenotype was significantly higher in patients with ACA (+) IgG and anti β2GPI (+) (24/25) than in those patients ACA (-) IgG and anti β2GPI (-) (1/25). The ACA (+) IgG and anti β2GPI (+) group showed a mean APCR ratio significantly lower than the ACA (-) IgG and anti β2GPI (-) group (p< .001 and p= .002 with the original and modified technique, respectively). The APCR phenotype was lost in 17/25 and showed borderline values in the other 8 patients using the modified technique. A linear correlation between ACA IgG vs original APCR ratio (r= -0.75) was found. However, when the analysis was limited to those patients with an abnormal APCR ratio, the correlation was increased (r= -0.86). We also found a linear correlation when plotting log anti βA2GPI vs log APCR ratio. The authors feel that the APCR ratio phenotype seems to be strongly associated to ACA IgG and anti β2GPI and no merely due to the presence of LAC. Finally, the inverse correlation between APCR ratio and the titre of ACA IgG and anti β2GPI, suggests an effect of these antibodies on the APC phospholipid-dependent inactivation of FVa.

P070b

ANTIPROTHROMBIN ANTIBODIES IN ACUTE CORONARY SYNDROMES

Fedi S, Marcucci R, Alessandrello Liotta A, Cellai AP, Rogolino A, Lenti M, Lucarini L, Lari B, Rostagno C, Comeglio M, Gensini GF, Abbate R

Dipartimento Area Critica Medico Chirurgica, Università di Firenze; Centro Trombosi, Azienda Ospedaliera Careggi, Florence, Italy

Antiphospholipid (aPL) antibodies are a heterogeneous group of antibodies directed in part against phospholipids, such as anticardiolipin antibodies (ACA) and lupus anticoagulant (LA), in part against phospholipid-binding proteins, such as β2 glycoprotein or prothrombin. aPL antibodies may amper the regulation of blood coagulation and they have been associated with venous thromboembolism and arterial recurrent thrombotic events. In the present study we have investigated the role of antiprothrombin (aPT) antibodies in patients with acute coronary syndromes. aPT IgG and IgM determination was performed with ELISA system (Bouty Italiana Laboratori, Milan, Italy) in 59 patients with acute coronary syndrome (45 M, 14 F, age range 38-79 years); 37 UA and 22 AMI patients. 40 healthy subjects (24 M, 16 F, age range 32-65) were also investigated and used for aPT IgM and IgG cut-off determinations. Fifity of these patients underwent percutaneous transluminal coronary angioplasty (PTCA). All the patients were previously investigated for the presence of the hemostasis-related risk factors such as Lipoprotein(a), plasminogen activator inhibitor activity, homocysteine levels, factor V Leiden mutation (present in x/y), G20210A Polymorphism of the prothrombin gene (present in x/y), ACA antibodies IgG and IgM, anti-β2 glycoprotein (β2 GPI) antibodies IgG and IgM determinations. The mean levels of aPT IgG and IgM were significantly higher in patients with respect to control subjects (p < 0.05). The aPT cut-off, defined as the 95° percentile of the distribution of values in controls, was 9.1 U/mL for IgG and 7.0 U/mL for IgM. aPT IgG levels were above this cut-off in 11/59 (18.6%) patients while aPT IgM levels were above this limit in 2/59 (3.4%). At the univariate analysis we have found a significant association between the presence of IgG aPT antibodies and acute coronary syndrome (OR 9.0, IC 1.3-64.0, p=0.04). In conclusion these preliminary results suggest the possible role of aPT antibodies as an additional risk factor for acute coronary syndrome and the need of further studies with a greater sample size to confirm this association.

Posters
Coagulation Disorders

P071

CHANGES OF VON WILLEBRAND FACTOR PATTERN AFTER A SUCCESSFUL ORTHOTOPIC LIVER TRANSPLANTATION IN TYPE 2M "VICENZA" (R1205H) VON WILLEBRAND'S DISEASE

Federici AB, Canciani MT, Baronciani L, Castaldo M, Cozzi G, Burgo I, Mazzaferro V, Mannucci PM

Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Maggiore Hospital and University of Milan; Liver Transplantation Unit, National Cancer Institute of Milan, Italy

Differently from hemophiliacs A and B in whom orthotopic liver transplantation (OLT) can improve factor VIII and IX levels, patients with von Willebrand's disease (VWD) are not expected to have any increase of von Willebrand factor (VWD) after OLT, since VWF is synthesized in endothelial cells and megakaryocytes but not in liver cells. Patients with severe liver failure requiring OLT are also known to have increased levels of proteolyzed VWF. Type 2M *Vicenza* (R1205H) VWD is characterized by reduced levels of VWF and the presence of *supranormal* multimers in plasma. We evaluated the changes of VWF before and after OLT in a 58-year old male with type 2M *Vicenza* (R1205H). Since the correct diagnosis of VWD in 1989, he has been advised to use desmopressin but he had already developed hepatitis B and C infections related to previous transfusions. Gastrointestinal bleeding episodes had become more frequent since 1995 when liver cirrhosis was found. He was later diagnosed to have localized hepatocarcinoma and he underwent OLT in February 2001. During OLT, the patient was given a FVIII/VWF concentrate (EMOCLOT, Kedrion, Italy) with dosage (50-100 U/Kg) adjusted on the daily factor VIII levels. No bleeding complications occurred during or after OLT and FVIII/VWF concentrate was used until postoperative day 25th only. VWF proteolysis was also evaluated by the VWF:RCo/Ag and CB/Ag ratios, the relative percentage of VWF high molecular weight (HMW) multimers and of the VWF native subunit probed by monoclonal antibodies. The patient's data (median values) are shown in comparison with those of his affected son (see table below).

Tests and assay	Patient's son	Patient before OLT	45 d	90 d	180 d after OLT
Platelet count (× 1,000)	241	57	282	161	156
Bleeding time (min)	5	30	5	6	5
VWF:RCo (U/dL)	8	10	16	14	10
VWF:CB (U/dL)	12	20	33	27	17
VWF: Ag (U/dL)	10	31	29	22	14
FVIII:C (U/dL)	12	19	38	31	22
VWF:RCo/Ag	0.80	0.32	0.55	0.63	0.71
VWF:CB/Ag	1.20	0.64	1.13	1.22	1.21
HMW Multimers (%)	23	10	16	18	20
VWF subunit of 225 kDa (% of total VWF)	84	72	77	78	80

Our data demonstrate that the increased bleeding tendency of a patient with type 2M *Vicenza* VWD is mainly due to the VWF proteolysis associated with liver failure, as shown by prolonged BT, loss of HMW multimers and reduced percentage of the VWF native subunit. The OLT cannot significantly modify, as expected, the VWF defect but is effective in reducing the prolonged BT and the number of bleeding episodes related to this acquired VWF degradation.

P072

HEPATIC CHARACTERISTICS OF A COHORT OF ANTI-HEPATITIS C VIRUS POSITIVE PATIENTS WITH CONGENITAL HEMORRHAGIC DISEASES

Coppola A, Sorrentino P, Conca P, Ragucci P, Cimino E, Coppola D, Madonna P, Tarantino G, Di Minno G

Centro di Coordinamento Regionale per le Emocoagulopatie, Dipartimento di Medicina Clinica e Sperimentale, Università "Federico II", Naples, Italy

We evaluated clinical, laboratory and ultrasound features of 99 multitransfused patients with congenital bleeding disorders (89 hemophilia A, 4 hemophilia B, 6 von Willebrand's disease, median age 29.5 years, range 14-72) and a history of hepatitis C virus (HCV) infection. Patients were first referred to our Center in 1999; biochemical screening and clinical evaluation were carried out every three months; ultrasonography was performed every six months. All patients were anti-HCV-positive, infected before 1985 because of non-virally inactivated plasma-derivatives; 16 (16.2%) were also coinfected with HIV and 8 (8.1%) with HBV. After excluding the latter and 7 patients (7.1%) previously treated with interferon, 52/75 patients (69.3%) were HCV-RNA positive (21.3% >850,000 IU/mL, 48% range 1280-752.000 IU/mL). HCV genotype 1b was the most common in these patients (40.3%), but also 1a (23.5%) and 3a (30.5%) were frequently detected, with higher prevalence than in cohorts of anti-HCV positive patients without bleeding disorders in the same geographic area. Among HCV-RNA positive patients, 10 (19.2%) had persistently normal aminotransferase levels and hepato-splenic ultrasonography, whereas the remaining 42 (81.8%) had biochemical signs of chronic hepatitis; however only 8 (15.3%) had a >2-fold increase of aminotransferase levels and 12 (23%) showed ultrasound evidence of more important liver damage (initial lobe caudate and/or left lobe hypertrophy, mild irregular margins) and 6 (11.5%) signs of developed cirrhosis, the latter all with genotype 1b (in 3 cases episodes of hepatic decompensation were reported). Forty patients (52%) had detectable serum cryoglobulins (2 with purpura) and in 2 (3.8%) a low concentration monoclonal γ–globulin was found. Despite the confirmation of low progression rate and non-aggressive clinical course of HCV infection in this cohort of patients, our analysis highlights the need for accurate hepatologic evaluation of patients with congenital bleeding disorders and chronic hepatitis, with potential clinical relevance for treatment approaches.

P073

LABORATORY DATA AND SONOCLOT ANALYSIS FOR COAGULATION ANALYSIS IN ORTHOTOPIC LIVER TRANSPLANT RECIPIENTS

Ruocco L, Bindi ML,* Romanelli A,° Biancofiore GD,* Pavia T, Pellegrini G, Lofaro A

*Laboratorio Analisi Cliniche, Ospedale Cisanello, *I UO Anestesia e Rianimazione-UTI Postchirurgica e Trapianti, Azienda Ospedaliera Pisana e °Istituto Di Fisiologia Clinica, CNR, Pisa, Italy*

Introduction. The aim of the study was to compare blood coagulation analysis by means of the Sonoclot thromboelastographic analyzer (Sienco Inc, Morrison, USA) and standard laboratory tests in orthotopic liver transplant (OLT) patients. *Methods.* Fifty-one patients submitted to OLT for terminal hepatic diseases from January to July 1999 were enrolled in the study. Blood samples were collected and analyzed during the pre-anhepatic, anhepatic and post-reperfusion phases of OLT. Sonoclot's data and standard laboratory tests were compared as follows: time-to-peak vs platelet count (Advia Bayer), rate vs prothrombin time (PT-INR, Sta System Roche), rate and time-to-peak vs serum fibrinogen (F, mg/dL, Clauss method, Sta System Roche), SonACT Vs activated partial thromboplastin time (APTT-sec, Sta System Roche), downward deflection Vs D-dimer (Immuno). Statistical analysis consisted in Pearson's, Fisher's and χ^2 tests as required (confidential limit 95%). *Results.* Patients mean age was 48±8.23 years; 40 (78.4%) were males and 11 (21.6%) females.

1. SonAct Vs APTT: basal phase, $p<0.01$; anhepatic phase, $p<0.01$; post-reperfusion phase, $p<0.001$.
2. Rate Vs PT-INR: basal phase, $p<0.005$; anhepatic phase p= n.s. ; post-reperfusion phase, $p<0.005$
3. Rate was not statistically related to F, but if a cut-off of F= 150 mg/dL was taken, a statistically significant relation was shown in those patients with F less than the cut-off level.
4. F vs time-to-peak: no correlation was found.
5. time-to-peak Vs platelet: the following correlation was shown with a PLT count greater than 50.000: basal phase, $p<0.01$; anhepatic phase, $p<0.001$; post-reperfusion phase, $p<0.05$.
6. Downward deflection Vs D-dimer (cut-off 0.5 mg/L): no significant association was found, even when Sonoclot showed hyperfibrinolysis. *Discussion*. The Sonoclot analysis was shown to be accurate and reliable in monitoring the coagulation pathway in OLT recipients.

P074

DESMOPRESSIN SUBCUTANEOUS ADMINISTRATION ENHANCES PLATELET RESPONSE *IN VIVO* TO PLATELET-ACTIVATING FACTOR IN HEMOPHILIA AND VON WILLEBRAND'S DISEASE TYPE I

Musso R, Cultrera D, Sortino G, Ferlito C, Azzaro MP, Di Francesco E, Fichera E, Giustolisi R

Centro Regionale di Riferimento per l'Emofilia (e Trombosi), Istituto di Ematologia, Università di Catania, Italy

Recently, it has been reported that elevated plasma platelet-activating factor (PAF) concentrations are significantly higher in children with hemophilia A (HA) and von Willebrand's disease (vWD) than in healthy children (*Kavakli K. Thromb Haemost 1999; 81*). It has been also postulated that increased cellular PAF activity may be an adaptative response by the organism in order to prevent hemorrhages and assist primary hemostasis (*Kavakli K Haemophilia 2001;7*). We here report that *in vitro* PAF at different concentrations induced a marked increase of platelet aggregation (PA) in mild HA patients (n=14) and in vWD type I subjects known to be responders to desmopressin (n=12, 6 males and 8 females). PA was performed in platelet-rich plasma (PRP) at 250,000/mL cell count by using a computerized four channel aggregometer (Aggrecoder PA 3210, Menarini, Florence) in the presence of subcritical (0.2 mM) and optimal (1 mM) concentration of PAF (c18:0). PA was performed before and 1 h after subcutaneous desmopressin (Emosint, Sclavo, Italy) injection (0.4mg/Kg). APTT, FVIII:C, vW:Ag and RiCof were also measured. As expected all FVIII complex plasma activities were significantly ($p<0.001$) elevated 1 h after Emosint administration. We also observed that PA was increased at subcritical PAF amount (51.8±13.9%) and higher after desmopressin stimulation (78.9±19.3%) with respect to normal PRPs (13.2±2.8%) ($p<0.001$). By using the inducer at optimal concentration (1mM) the PA was highest either before (70.8±25.3%) or 1 h after desmopressin (86.2±21.4). Our results show that HA and vWD platelets are more sensitive to PAF *in vitro*. *In vivo* desmopressin administration may elicitate a hemostatic action via V2-receptor agonist activity. In our opinion, the platelet mediated events and additional factors which are present in the vascular endothelium and released in the circulation are important elements in the control and/or cessation of bleeding in HA and vWD patients.

P075

GASTROESOPHAGEAL VARICES IN HEMOPHILIC PATIENTS WITH HEPATITIS B OR C VIRUS INFECTION AND LIVER DISEASE PROGRESSION: THE NEXT LIFE-THREATENING CONCERN IN HEMOPHILIA?

Musso R, Cultrera D, Sortino G, Ferlito C, Azzaro MP, Fichera E, Di Francesco E, Giustolisi R

Centro Regionale di Riferimento per l'Emofilia (e la Trombosi) - Istituto di Ematologia, Università di Catania, Italy

With regard to liver disease progression in the hemophiliac population with HBV and/or HCV, esophageal and gastric varices may constitute a new life-threatening concern. In fact, esophageal-gastric-duodenal fiber optical endoscopy cannot be routinely performed owing to obvious hemorrhagic implications especially in patients with high response inhibitor. Some patients are also reluctant to go through this procedure for fear that a possible hemorrhage could occur during the maneuver. In any case, when acute hematemesis occurs as the first sign of esophageal varicel, a prompt therapeutic strategy must be planned. We report here our observation regarding a serious sudden hematemesis and anemic shock in a 41-year old hemophilia A patient, HBV and HCV positive with normal ALT and long standing inhibitor (1.9 BU/mL against human FVIII and 0.6 BU/mL against porcine FVIII, already known high responder to FVIII concentrates). After administration of 8 units of red blood cells, 6,000 IU of human FVIII (Emoclot D.I.) and 5 g tranexamic acid, we decided to perform an explorative surgical laparatomy. Initially, we noted a diffuse sub-serous membrane hypertension in the stomach and distal esophagus as well as dilatation of portal and splenic veins. Further, cutting open the gastric fundus, evident venous varix of the stomach and distal oesophagus were seen together with abundant blood clots. The meticolous surgi-

cal ligation of each venous varices led to a prompt reduction of submucous membrane vein hypertension and stopped bleeding. In the following hours, the patient recovered without re-bleeding. On day 6 after operation, owing to aPTT prolongation with concomitant higher inhibitor to FVIII (30 BU/mL) we started the infusion of activated prothrombin complex concentrate (FEIBA, Immuno) (125 IU/h for 6 days). Thereafter, we infused FEIBA (2.500 IU/day) until the third week. In spite of the aPTT prolongation and high plasma inhibitor, hemostasis was well achieved. No indices accounting for coagulation activation were observed: thrombin-ATIII complex, prothrombin fragment F1+2, fibrinopeptide A and fibrinogen/fibrin degradation products did change slightly. Only platelets decreased from 128,000/m^3 before FEIBA administration to a minimum of 65,000/m^3 during this treatment. In this regard, β-thromboglobulin and platelet factor 4 remained in the normal range, thus suggesting that no platelet activation occurred. On post-operative day 40, the patient was discharged in a good condition. From the present observation we suggest that frequent abdominal ultrasound examinations to document portal-caval-splenic hypertension are nedeed in hemophiliacs who have had HBV and/or HCV infection. Periodic fiber optical endoscopy must also be performed to see possible esophaseal-gastric varices in those patients who have liver disease progression. Moreover, when a serious sudden hematemesis does occur in haemophiliac, urgent hospitalization and meticulous surgical supervision with the hematologist's co-operation are planned. Surgical ligation should be considered the treatment of choise for massive esophageal variceal bleeding instead of endoscopic injections of sclerosing agents. Finally, FEIBA replacement therapy would be a safe choice in hemophiliacs with long-standing inhibitor and a history of response to FVIII in previous surgical operations.

P076

DUODENAL HEMATOMA AS THE PRESENTING SIGN OF HENOCH-SCHOENLEIN PURPURA

Bettega D,* Baratelli GM,° Fumagalli A,* Mastaglio C,# Molteni EE*

**Divisione di Medicina Generale,°Divisione di Chirurgia Generale, #Servizio di Reumatologia, Ospedale Generale di Zona "Moriggia-Pelascini", Gravedona, Como, Italy*

Henoch-Schoenlein purpura is a disease mainly affecting children, characterized by the association of purpura, polyarthralgia, abdominal pain, gastrointestinal bleeding and renal involvement. We describe the case of a 50-year old man, who was admitted to hospital because of abdominal pain together with symptoms of upper gastrointestinal tract obstruction. An esophago-gastric duodenal (EGD) endoscopy and a CT scan of the abdomen showed a narrowing of the duodenal lumen due to a swelling of the visceral wall, while laboratory tests were normal. Thus a laparotomy was performed and surgical exploration surprisingly found a hematoma localized in the muscular wall of the second part of the duodenum. A few days after surgical evacuation of the hematoma the patient relapsed, again presenting severe abdominal pain in association with constipation: direct X-ray examination of the abdomen was consistent with occlusion of small bowel and a test for occult blood in the stool was positive. Moreover he developed recurrent poussées of palpable purpuric skin lesions on both legs and arms, together with arthralgia, erythema, tenderness and swelling of ankles and wrists. Laboratory investigation showed elevation of ESR and CRP, normal PT, APTT and bleeding time, mild normochromic and normocytic anemia and microscopic hematuria with no impairment of renal function. The EGD endoscopy was normal, while colonoscopy was not performed: the skin biopsy showed a picture of leukocytoclastic vasculitis in agreement with the clinical suspicion of Henoch-Schoenlein purpura. The patient received symptomatic medical treatment for pain and constipation, but full recovery was achieved only by giving prednisone by mouth. In our opinion the interest of this case is represented by the unusual kind of presentation, which needed a surgical approach before the full appearance of the classical clinical syndrome.

P077

EFFICACY OF VERY LOW DOSE RECOMBINANT FACTOR VIIA IN A BLEEDING PATIENT WITH FACTOR VIII INHIBITORS UNDERGOING SURGERY

Tamponi G, Schinco PC, Borchiellini A, Pollio B, Valpreda A, Boccadoro M

Department of Onco-Hematology, University of Turin, Molinette Hospital, Turin, Italy

Severe bleeding manifestations of acquired hemophilia have been treated in the last few years with recombinant activated factor VII (rhVIIa, Novoseven, Novo Nordisk, Bagsvaerd Denmark), at least in those countries where it is commercially available, with excellent results, but many financial limitations due to its exceedingly high cost. Recommended dosages span from boluses of 90-120 mg/kg b.w. every 2-6 hrs. to 16-20 mg/kg/h continuous infusion for several days. We report our experience with a 62-years old patient with heavy skin and mucous diathesis and hematuria due to high titer acquired inhibitors to FVIII secondary to advanced breast cancer, who was treated with very low dose rhVIIa. After an initial bolus of Novoseven 90 mg/kg, manufacturers and literature suggested continuous infusion at 16 mg/kg/h. The bleeding stopped for a few days and Novoseven was swiftly tapered as low as 8 mg/kg/h for three days, but she started to bleed once more. Increasing the dose to 10 mg/kg/h assured adequate control of the diathesis. Radical mastectomy was performed two weeks later, despite the inhibitor titer still being high (notwithstanding cyclophosphamide + prednisone as immunosuppression). Novoseven was previously raised and maintained at 30 mg/kg/h, which provided fairly good surgical hemostasis, even though prolongation of post-surgical bleeding was observed. No correlation was found between clinical outcome and laboratory tests (PT, PTT, VII:C level). In view of these results one can conclude that Novoseven is hemostatically effective even in lower dosages than reported, which can have important financial implications, but this does not seem to be true for open wound bleeds. Conventional laboratory monitoring of therapy does not seem to help in assessing outcome.

P077a

PROTEIN C SYSTEM IN PATIENTS WITH MIGRAINE WITH AURA

Erba N,* De Micheli V,* Moschiano F,* Schieroni F,* D'Amico D, Ciusani E, Ariano C, Leone M, Grazzi L, Bussone G

*Ist. Neurologico "C. Besta" Milan, *Ospedale "L. Mandic", Merate, Lecco, Italy*

Many evidences suggest for a correlation between stroke and migraine with aura (MA); in particular MA represents a risk factor for stroke in young adults. The recovery of congenital or acquired alteration of normal coagulation inhibitory system associated with juvenile stroke is know. In this work we studied the prevalence of the alterations of protein-C system, protein C deficiency, protein S deficiency, aPC-resistance associated with factor V Leiden mutation, among 202 subjects suffering for MA and 124 control matched for age and sex. These results confirm the association between reduction of inhibitory protein C system ability and MA suggesting for an increased risk for stroke or migrainous stroke in subjects with these alterations. MA is more frequent in female patients and many risk factors, known as prothrombotic factors, such as the *pill* use and smoke, are able to increase the incidence of stroke in females with MA.

	EA (N° 202)		Controls (N° 124)		X2
	N°	%	N°	%	
APC resistance	11	6	4	3.2	NS
Leiden mutation	11	6	4	3.2	NS
Protein C deficiency	17	8.4	0	0	0.001
Protein S deficiency	11	5.4	0	0	0.004

We propose to investigate for the congenital alteration of protein C system in young patients, females in particular, suffering for MA and to eliminate the modifiable cerebrovascular risk factors.

P078

ACQUIRED HEMOPHILIA ASSOCIATED WITH MYELODYSPLASTIC SYNDROME SUCCESSFULLY TREATED WITH ORAL IMMUNOSUPPRESSIVE THERAPY

Giuffrida G, Ferlito C, Cipolla A, Guarnaccia G, Di Francesco E, Musso R, Giustolisi R

Cattedra e Divisione di Ematologia con Trapianto, Ospedale Ferrarotto, Catania, Italy

Acquired hemophilia (AH) is a rare bleeding disorder caused by an autoimmune depletion of factor VIII C (F VIII:C), due to specific inhibitor. The inhibitor may occur in association with pregnancy or *post-partum*, autoimmune diseases, medication, solid tumors and hematologic malignancies. Association with lymphoproliferative disorders has been reported, while association with myelodysplastic syndrome (MDS) is extremely rare and only one case, to our knowledge, has been described. Treatment has two objectives: permanent inhibitor suppression and management of the acute bleeding episode, but no general consensus exist on the best therapeutic approach. Recently investigations suggest that oral cyclophosphamide and prednisone, without FVIII therapy, may be useful in patients with high titer inhibitor. We report a case of AH associated with MDS treated only with oral immunosuppressive therapy. A 73-year old woman, with a 10-year history of MDS-refractory anemia, developed spontaneous soft tissue hemorrhages, hematuria and progressive anemia (Hb 7.5 g/dL), although platelets number was normal (Plt 207×10^9). Her family history was negative for hemorrhagic diatheses. Coagulation assay showed a normal prothombin time and fibrinogen levels and a prolonged activated partial thromboplastin time (APTT 115''-n.v. 34''). FVIII C level was < 1% (n.v. 60-150); lupus anticoagulant search was negative. An antibody directed against FVIII C was found at high titer (130 BU/mL). A diagnosis of AH was made and oral immunosuppressive therapy with prednisone 1 mg/kg/die and cyclophosphamide 100 mg/die was started. APTT, level of FVIII and inhibitor was measured every 1-week APTT gradually returned to normal value, inhibitor level decreased whereas FVIII levels increased and returned to normal value after 4 weeks (Table 1). One month later, the hemorrhagic diathesis disappeared and Hb increased (11 gr/dL) without blood transfusions. Cyclophosphamide was stopped after 4 weeks and prednisone was gradually tapered off after 3 months. In patients with MDS it has been hypothesized that a dysregulation of the immune system that may favour the development of an abnormal lymphoid clone and in our case, probably, autoantibodies against FVIII.

Table 1. Coagulation profiles.

	n.v.	At visit	1st wk	2nd wk	3rd wk	4th wk
APTT (sec)	34"	115"	82	63	45	33
F VIII (%)	60-150%	< 1	4	41	53	69
F VIII inhibitor (BU/mL)	< 0,01%	130	45	4	2	< 0,01

In conclusion our observation illustrates high titer inhibitor-AH associated with MDS-refractory anemia successfully treated with only oral prednisone and low-dose cyclophosphamide. A causal relationship between MDS and AH remains speculative. Although the clinical course is not predictable and inhibitor may disappear spontaneously, in some cases with high titer inhibitor associated with malignant disease, combined therapy with prednisone and cyclophosphamide may be sufficient to suppress the inhibitor and to arrest bleeding.

P079

BLOOD COAGULATION ABNORMALITIES IN CONGENITAL DISORDERS OF GLYCOSYLATION: A CASE REPORT

Demicheli M,* Santi R,* Contino L,* Pesce F, Spada M,^ Levis A*

**Dipartimento di Ematologia, °Dipartimento Materno-Infantile Azienda Ospedaliera "SS. Antonio e Biagio e C. Arrigo" di Alessandria; ^Clinica Pediatrica, Università degli Studi di Torino, Italy*

Deficiencies in the pathway of N-glycan biosynthesis lead to severe multisystem diseases, that are known as congenital disorders of glycosylation (CDG). Clinical features of CDG are variable, according to the molecular basis of the metabolic defects. In the limited number of cases reported in the literature, the severity of the clinical phenotype correlates with the pattern of biochemical alteration and the subsequent biosynthesis deficiency. The wide range of clinical features includes disorders of nervous system development, psychomotor retardation, dys-

morphic features, hypotonia, immunodeficiency and multiple coagulation disorders. We describe one case of CDG presenting secondary imbalance of blood coagulation, without clinical manifestation. A male child, four months old, was admitted to the Children's Hospital for growth retardation and hypotonia. Clinical evaluation showed liver and kidney enlargment and nystagmus caused by cerebellar hypoplasia. Laboratory findings exhibited elevated transaminase values (ALT 250 U/L), prolonged activated partial thromboplastin time (aPTT), decreased fibrinogen (103 mg/dL) and antithrombin dosage (27%). To explain prolonged aPTT (2.06 Ratio) we executed single coagulation factor dosage: factor IX 34%, factor XI 10 %, factor XII 56%; the others factors were normal. Moreover, protein C level was 22% and protein S 54 %. The isoelectric focusing of serum transferrin revealed hypoglycosylation. Analysis of lipid-linked-oligosaccharide (LLO) in the patient's fibroblasts suggested a diagnosis of CDG-Ia. According to published data, a thrombotic tendency is frequent in CDG-Ia, while an increased bleeding tendency is more frequent in CDG-IIa. The reason why our patient did not show any clinical thrombotic event was probably the concomitant decrease of both procoagulant and inhibitor hemostatic factors.This functional decrease could be due to either altered molecular synthesis or to active site dysfunction.

P080

RISKS AND COMPLICATIONS OF KNEE PROSTHETIC SURGERY IN HEMOPHILIACS: PERSONAL EXPERIENCE IN BARI, ITALY

Patella V, Schiavoni M,* Moretti B, Pesce V, Mascolo V,** Ettorre CP,* Scaraggi A,° Ciavarella N*

*U.O. Clinica Ortopedica II, *Centro Emofilia, **U.O. Clinica Ortopedica III, °U.O. Clinica Medica II, Policlinico-Università, Bari, Italy*

We report on the evaluation of risks and complications concerning 12 knee prosthetic replacement interventions in hemophiliacs based on a 6-year-experience. The best orthopedic outcome has been observed in younger patients, in whom general physical and psychological conditions were more favorable for operation and rehabilitation. The status of the limb to be operated, including factors such as axial deviation at knee level, muscular hypotrophy, alterations and thinning of the skin and subcutaneous tissue, limited joint function were considered. The choice of the prosthesis depended on the anatomic and pathological conditions of the knee, respecting some surgical requirements, as well as an almost complete removal of synovitis, economical bone resection, filling up the femoral and tibial erosions with autologous bone, highly respecting the bone-stock, extreme accuracy in hemostasis. Some surgical details were not understimated: surgical approach in respecting the covering tissues because of the high risk of cutaneous necrosis and infections; correct ligamental balancing; possible detachment of the tibial tuberosity both as a surgical approach to the joint and as an aid to recovery of the post-surgical joint flexibility, optimal pre and post-operative planning requiring polyspecialist cooperation: hematologic evaluation regarding dosage and and duration of replacement with coagulation factors concentrate and management of blood transfusions. Appraisal of infective risk and antinfective therapy mainly in HIV and/or HCV positive patients. The physiotherapist was the main actor in post-operative rehabilitation in order to reach the best articular function and to avoid the loss of range of movement. Excessive post-surgical bleeding, sepsis and the loosening implant were the most dreaded risks and complications. Two prostheses, inserted 4 years previously, became loose because of the scarce quality of the *guest bone*. The outcome in the remaining 10 knee arthroplasties was excellent/good.

P081

AN ACQUIRED INHIBITOR OF FACTOR XIII: CASE REPORT

Valdré L, Legnani C, Pili C, Brusi C, De Fabritiis A, Rodorigo G, Gasperoni M, Lo Manto G, Coccheri S, Palareti G

Dip. Cardiovascolare, U.O. Angiologia, Azienda Ospedaliera di Bologna, Policlinico S.Orsola-Malpighi, Bologna, Italy

Acquired inhibitor of F.XIII, characterized by severe/life-threatening hemorrhages, is rare and few cases have been described. We report a case of a 78-year old man, with active chronic HCV-related hepatitis and moderate autoimmune thrombocytopenia, who in the previous three months was repeatedly hospitalized and transfused for anemia due to macrohematuria and recurrent subcutaneous bleeding. His past history was negative for bleeding. When admitted to our ward he had an extensive muscle hematoma in the right arm and diffuse ecchymoses. Routine clotting tests were normal (except for D-dimer). Plasma F. XIII activity level was 6% and a F. XIII inhibitor was present (5.9 BU/mL). Treatment with prednisone 1 mg/kg/day was started and a small amount of F. XIII concentrate (Fibrogammin, 250 U) was infused on the first day, obtaining no changes in F. XIII activity level, but a sudden clinical improvement and no further signs of blood loss. After 8 days, when F. XIII inhibitor was 2.0 BU/mL, 500 U F. XIII concentrate were infused again, obtaining no elevation of F. XIII activity (8%). On day 18 he was discharged with marked improvement of bleeding lesions without any further concentrate infusion; F. XIII activity was 14% and inhibitor level 1.68 BU/mL. Home treatment was exclusively prednisone (1 mg/Kg/day). Twenty-days after, F.XIII level was stable (10%) and inhibitor level decreased (0.76 BU/mL). Two months later, after a progressive cachetic state, the patient died without any evidence of bleeding. No autopsy was performed. Death was attributed to occult malignancy. In contrast with other reports, the present case of acquired F. XIII inhibitor shows that active bleeding may be treated via infusion of only small amounts of F. XIII concentrate and steroid therapy. Appearance of F. XIII inhibitor is often associated with a serious underlying disease that may lead to death independently of any hemorrhage.

P082

MANAGEMENT OF HEMODIALYSIS IN A HIGH RISK NON-HEMOPHILIC PATIENT AFFECTED BY ACUTE RENAL FAILURE WITH AUTOANTIBODIES AGAINST FACTOR VIII

Ciavarella N, Schiavoni M, Ettorre CP

Centro Emofilia, Policlinico-Università, Bari, Italy

A 77-year old man, suffering from a non-specific dermatitis, was affected by acquired hemophilia (F.VIII: 1.6%; anti-human F.VIII Abs: 50 B.U.) without any documented underlying diseases. The clinical picture, initially characterized by the presence of macroscopic hematuria, was successively complicated by the onset of an acute renal failure with progressive reduction of

diuresis up to anuria in the course of a few days and without any evident explanation. Corticosteroids were started as immunosuppressive treatment. The echographic examinations of kidneys and bladder, as well as prostate were normal. The fast rise of blood urea and creatinine necessitated initiating hemodialysis. In order to allow safe catheterization of the left common femoral vein, recombinant factor VIIa (rFVIIa) was given by intravenous injection at the dosage of 120 μg/Kg b.w. at 2-hour intervals over a period of 12 hours. Compression of the inguinal region was used during therapy. However 12 hours later the patient needed further hemostatic treatment with rFVIIa (120 μg/kg b.w. at 2-hour intervals) over a period of 10 hours because of blood loss from the site of the venous catheter. Seven sittings of dialysis were needed before diuresis was restored. Blood urea and creatinine returned to normal values within 16 days. When the venous device was removed, an intravenous injection of rFVIIa at the dosage of 120 μg/Kg b.w. was given before and after the intervention. No bleeding complications were observed. The management of hemodialysis in our non-hemophilic patient was a dramatic challenge. Nevertheless the high risk of hemorrhagic complications was carefully minimized allowing improvement of renal function.

P083

VITAMIN K RESCUE IN A PREGNANT WOMAN WITH PRE-TERM LABOR CAUSED BY A SUBCLINICAL CELIAC DISEASE

Schiavoni M, Romano F,* Ettorre CP, Parisi C,* Ciavarella N

*Centro Emofilia, *U.O. di Ostetricia e Ginecologia I, Policlinico Università, Bari, Italy*

Celiac disease may be associated with severe vitamin K deficiency, that leads to a deficit of vitamin K-dependent coagulation factors. This phenomenon results in a hypocoagulability state expressed by prolonged PT-INR, often associated with a severe hemorrhagic diathesis. Malabsorption has also been related to some obstetric alterations such as infertility and miscarriage. We report on a 26-year old female at 33 weeks of pregnancy with pre-term labor. Laboratory controls showed a significant prolongation of PT-INR (9.72) together with a slight increase of PTT (R=1.23) without any evident bleeding manifestation. The assays of vitamin K-dependent factors revealed the following defects: F.II: 5%; F.VII: 3%; F.X: 4%; F.IX: 18%; protein C: 23%; free protein S: 20%. The ultrasound showed a small-for-age fetus confirming the non-physiological course of pregnancy. The past history of the female suggested the presence of a subclinical malabsorption, probable cause of the vitamin K deficiency. The emergency led us to administer 10 mg of vitamin K quickly with the aim of restoring the coagulation parameters and allowing the eventual obstetric intervention. Safety value of PT-INR (1.52) was reached in about 4 hours with progressive normalization of vitamin K-dependent factors. Duodenal mucosal biopsy documented the celiac disease. Conservative therapy associated with a correct diet were able to stop labor and to allow the normal progression of pregnancy up to delivery. No further dose of vitamin K was given. The case report highlights the need in emergency to look at possible vitamin K deficiency in high risk females with obstetric alterations and prolonged coagulation tests likely to be due to a subclinical celiac disease.

P084

FACTOR V SAN GIOVANNI ROTONDO: A NOVEL MISSENSE MUTATION (ARG2074CYS) IN THE C2 DOMAIN OF FACTOR V ASSOCIATED WITH REDUCED COFACTOR ACTIVITY

Bossone A, D'Angelo F, Santacroce R, Vecchione G, De Lucia D,^ Di Minno G, Margaglione M*

*Unità di Aterosclerosi e Trombosi, IRCCS. "Casa Sollievo della Sofferenza", S. Giovanni Rotondo; ^Istituto di Patologia Generale e Oncologia, Seconda Università di Napoli; *Genetica Medica, Università di Foggia, Italy*

The clinical phenotypes of FV deficiency shows a wide range of symptoms (epistaxis, menorrhagia, post-traumatic bleeding, etc.) that correlate poorly with circulating FV plasma levels. The majority of the 17 mutations in the FV gene associated with FV deficiency have been found within exon 13, suggesting that this exon is a common site for FV mutations. An index patient with post-traumatic intracranial bleeding and low plasma levels of Factor V activity (5%) and antigen (14%) was identified. Isolation of DNA and PCR analysis were done according to standard procedures. Amplifications of all coding regions of FV gene and intron/exon boundaries were achieved using sense and antisense oligonucleotides designed on the basis of known sequences of the FV gene locus (Genbank accession number Z99572). Sequence analysis of 5'- and 3' untranslated region, all coding regions and intron/exon boundaries revealed, in exon 23, a C-to-T transition in position 1 of codon 2074 (Arg→Cys). The Arg2074 residue is distant from Cys2113. Among possible tertiary structures of the C2 domain containing the Cys2074 residue, obtained using the 3D-PSSM program, we observed a structure in which the two residues were closer. Although preliminar, this modeling yields the possibility of a novel disulfide bridge in the C2 domain of FV. In conclusion, we have identified a mutation that affects a highly conserved residue, which is required for maintaining the structural integrity of the C2 domain of factor V. In addition, we suggest that the new cysteine may interact, forming a new disulfide bridge, possibly with the unpaired Cys2113, a residue that is crucial for binding to phosphatidylserine-containing membranes.

P085

BLEEDING IN PERIPARTUM AND ACQUIRED HEMOPHILIA: GOOD THERAPEUTIC RESPONSE TO ORAL STEROIDS AND SUBCUTANEOUS DESMOPRESSIN INJECTIONS

Musso R, Cultrera D, Sortino G, Ferlito C, Azzaro MP, Di Francesco E, Fichera E, Giustolisi R

Centro Regionale di Riferimento per l'Emofilia (e la Trombosi), Istituto di Ematologia, Università di Catania, Italy

Acquired inhibitor to the factor VIII (FVIII) in non-hemophilic patients is a rare disorder especially among younger females where the autoantibody often occurs in the late post-partum period (*Hauser I et al. Thromb Haemost 1995; 73:1-5*). We herein report the spontaneous occurrence of acquired inhibitor to FVIII in the early post-partum period in two healthy females (29 and 34 years old respectively) associated with life-threatening bleeding complications after their first spontaneous delivery at term. In both none underlying disease was documented during the pregnancy; the only medications they had received in the preceding

months were oral iron compounds and folic acid. The standard coagulation and biochemical parameters recorded three weeks before the delivery were in normal range. They were admitted to gynaecological emergency unit for spontaneous delivery at term without bleeding complications. During the early post-partum in both females, 48 hrs and 72 hrs respectively, unrestrainable metrorrhagia resulted in severe anemia which required prompt blood transfusions. Coagulation studies revealed prolonged aPTTs (90s and 110s respectively, normal 29.4s) with normal PTs and platelet counts (331×10^9 L-1 and 282×10^9 L-1). APTT was not corrected after 2 hours' addition of normal plasma (1:1 mix of normal and patient). In addition, their FVIII:C levels were 8.3% and 3.9% respectively (normal 50-150%) and FVIII inhibitor titres (Bethesda assay) were 5.4 and 7.7 Bethesda units/mL-1, while the porcine FVIII inhibitors were 2.1 and 3.3 BU/mL-1. No other biological abnormalities including platelet dysfunction, von Willebrand's defects or FI, FXI, FXII, FXIII deficiencies and lupus anticoagulant were detected. As soon as the FVIII was documented, oral treatment was commenced with 1 mg kg-1 day-1 deflazacort. Yet metrorrhagia still lasted and required blood transfusions for 4 days. On the basis of our previous experiences regarding the usefulness of subcutaneous desmopressin in the management of metrorrhagia of normal adolescent and in perimenopausal women without any congenital bleeding disorders as well as in hemophilia carriers and in von Willebrand disease type I, we decided to start therapy with the analogue hormone desmopressin (Emosint, Sclavo, Italy) at doses of 0.4 mg/kg twice a day (4 days). We observed a prompt resolution of the uterine bleeding in both patients. A progressive increase of the hemoglobin level in the post-partum period was seen. Thereafter, in one patient deflazacort maintenance therapy was given for 3 weeks at doses ranging from 0.5 mg/Kg1 to 0.2 mg/kg1/day1 with FVIII:C increase to 35% and inhibitor decrease to 0.91 BU mL-1. At week eleven, the inhibitor completely disapperead and FVIII:C was 88%; thus deflazacort therapy was stopped. The patient is at present (January 2002) without any treatment (31 weeks of follow-up). In the second patient the steroid maintenance therapy ranged from 0.5 mg to 0.25/mg/kg1/day1 for ten weeks with FVIII:C 20% while the inhibitor was 1.21 BU mL-1. No other disease nor bleeds have been so far observed. Until now, she is on therapy with deflazacort 39 weeks from onset of treatment and the inhibitor is persistently detectable (2.1 BU mL-1). From our observation we suggest that in the early post-partum period an acquired inhibitor to FVIII can emerge when life-threatening uterine bleeding occur. Early treatment with steroids must be started as soon as the diagnosis is made to eradicate the inhibitor. Subcutaneous desmopressin can be used to successfully manage bleeding complications.

P086

ACQUIRED HEMOPHILIA IN AN ELDERLY PATIENT WITH CHRONIC MYELOID LEUKEMIA

Musso R, Cultrera D, Sortino G, Giustolisi G, Ferlito C, Azzaro MP, Di Francesco E, Fichera E, Giustolisi R

Centro Regionale di Riferimento per l'Emofilia (e Ia Trombosi), Istituto di Ematologia, Università di Catania, Italy

Inhibitors to coagulation factor VIII (FVIII:C) are classically reported in patients with various autoimmune conditions, with malignancy or pregnancy, skin diseases and immune dysregulations including lymphoproliferative disorders as well as after administration of several drugs, especially in the elderly population. To date, the occurrence of an auto-antibody to FVIII:C has been described in one patient with chronic myelogenous leukemia (CML) while he was receiving interferon-α (IFN-α) (*English KE. Am Pharmacother 2000;34:737-9*). We herein report the first case of an association between CML and acquired hemophilia (AH). The male patient, born in 1923, had prostatic hypertrophy in 1987 treated for years by estrogen-depot injections. In May 2001, hematomas and bruises appeared on the upper and lower limbs, on the back and chest. He was pale with diffuse pain, fever and dyspnea. No splenic enlargement was present; hematologic parameters showed anemia (Hb 8.3 g/dL), enhanced white blood cell (WBC) count (43.7×10^3/mL) with blast myeloid cells and increased platelet count (736×10^3/mL). Investigations revealed aPTT of 61s (normal 28-38) which did not correct on mixing with normal plasma. FVIII:C level was 7.7%, von Willebrand factor antigen was 192%. Assays detected a FVIII inhibitor (8.8 Bethesda units). Lupus anticoagulant, platelet dysfunction, von Willebrand's defect, or FI, IX, XI, XII or XIII deficiencies were excluded. Owing to potential hemorrhagic risk bone marrow biopsy was not performed. So, to ascertain CML diagnosis the peripheral blood samples were used for cytofluorimetric and cytogenetic evaluations and the PCR assay was employed to detect the presence of brc-abl mRNA. This latter molecular hallmark fusion gene of CML was found. The treatment of the acute bleeding, the eradication of the autoantibody and the chemo-therapeutic protocol were the three objectives of therapy. Hemostasis was rapidly achieved using FEIBA 50 U/kg (3,000 units) 12-hourly. His symptoms improved and after 4 days of treatment (total 18,000 units of FEIBA) FEIBA was stopped. Intravenous methylprednisolone (80 mg daily) was commenced simultaneously. Hydroxyurea at dosage of 1,500 mg daily was administered. No red packed cells units were infused. For one week prednisone, 0.8 mg/kg^{-1} was orally prescribed and gradually tapered off. Complete disappereance of autoantibodies to FVIII was achieved 3 months after initiation of corticotherapy. No bleeds were recorded at recent follow-ups (October 2001). Despite hydroxyurea treatment the circulating WBC (46×10^3/mL) lasted increased. In August 2001 we began chemotherapy with busulphan (4 mg/daily) with a good clinical response (WBC 13×10^3/mL and no peripheral blast cells in January 2002). This report confirms that in older populations various factor may alter the immune system and trigger the onset of autoimmunity (*Mishra N et al. Clin Geriar Med 1998; 14:515-42*). The reduction of immune tolerance could lead to the proliferation of pathological clones or the production of autoantibodies. Because bleeding is often severe and polymorphous, a prompt and correct diagnosis is necessary in order to provide adequate therapeutic options. Usually, prednisone is the first-choice treatment to eradicate the inhibitors (*Green D et al. Thromb Haemost 1993; 70:753-7*). Anyhow, to our knowledge this is believed to be the first reported case of spontaneous FVIII inhibitor associated with the onset CM.

P087

MOLECULAR AND GENETIC ANALYSIS OF A COMPOUND HETEROZYGOUS FOR DYSPROTHROMBINEMIA AND HYPOPROTHROMBINEMIA

Akhavan S,* Luciani M,° Lavoretano S,* Mannucci PM*

**Angelo Bianchi Bonomi Hemophilia Center, IRCCS Maggiore Hospital, Milan; °Pediatric Bambino Gesù Hospital, Rome, Italy*

Human prothrombin is encoded by a gene 21 kb in length, located in chromosome 11 p11-q12 and containing 14 exons separated by 13 intervening sequences. Sites cleaved in prothrombin during production of the fully active α-thrombin are at Arg155 and Arg284 for thrombin and at Arg271 and Arg320 for factor Xa. These cleavages create *activation peptide*s and produce intermediate molecular forms, including meizothrombin (single cleavage at Arg320) and meizothrombin des fragment 1 (cleavages at Arg155 and Arg320). The latter intermediate is normally converted to free fragment 2 and α-thrombin by a factor Xa cleavage at Arg271 and a final autocatalytic cleavage at Arg284. Prothrombin deficiency is an autosomal recessive bleeding disorder, and is expressed clinically only in individuals who inherit abnormal alleles from both parents, who are often consanguineous. Two phenotypes can be broadly distinguished when functional and antigenic plasma levels of prothrombin are measured: hypoprothrombinemia, with concomitantly low levels of coagulant activity and antigen (type I), and dysprothrombinemia, with low activity but borderline or normal antigen levels (type II). Genetic and biochemical analyses show that these disorders are the result of substitution, deletion or insertion of a single nucleotide in the prothrombin gene, resulting in the substitution of an amino acid in the protein or a premature stop codon. To date, 32 such defects in prothrombin have been identified. We studied a patient with prothrombin deficiency. The proband is a 2-year old male. He presented with prolonged screening of coagulation tests prior to surgery for a dacryostenosis at the age of 10 months. He had no prior history of bleeding. Laboratory studies indicated an isolated low prothrombin level of 20%. The proband underwent dacryostenosis with post-operative bleeding complications. At the age of 18 months, he also had a post-traumatic epidural hematoma without any fracture. In both of the cases treatment with Prothromplex was necessary. His parents are not consanguineous and had no clinical history of bleeding. Sequencing of PCR-amplified genome DNA revealed two different mutations in heterozygous forms, a G to A transition in exon 8 at position 7312 resulting in the replacement of arginine 271 by a histidine, and a 2bp deletion at 20060-61 resulting in a frameshift leading a premature termination codon in exon 14. Arginine 271 is located at a factor Xa cleavage site. The substitution by a histidine prevents the formation of fully functional α-thrombin and cause a dysprothrombinemia phenotype. The inheritance of hypoprothrombinemia from the father and dysprothrombinemia from the mother was confirmed by nucleotide sequencing of the prothrombin gene of these family members.

P088

VON WILLEBRAND FACTOR IN AUTOLOGOUS STEM CELL TRANSPLANTATION

Lattuada A, Rosti A, Sacchi E, Carraro MC, Libera L, Ferrandi P, Rossi E

Hematology and Blood Transfusion Service, L. Sacco Hospital, Milan, Italy

Hematopoietic cells reside predominantly in the bone marrow and can be mobilized in large numbers in peripheral blood by the administration of CSF (colony-stimulating factor). Apheresis products containing CSF-mobilized peripheral blood cells are now widely used instead of bone marrow for autologous transplantation, because the hematopoietic recovery is faster using peripheral blood cells than using bone marrow stem cells. This procedure require high-dose chemotherapy (HDC) and consequently marrow dysplasia and endothelial damage whoise mechanisms is not yet well known. Thrombotic events are frequent in these patients, venous-occlusive disease (VOD), and thrombotic thrombocytopenic purpura (TTP) being the most important complications. In TTP platelet adhesion the damaged subendothelium could justify the prolonged thrombocytopenia and von Willebrand factor (VWF) is one of the most important proteins involved in platelet adhesion and aggregation. With this background we studied 6 patients during autologous stem cell transplantation to evaluate the change of concentration and activity of VWF, the VWF cleaving protease, platelet count. The results (mean±SD) observed before and after high dose chemotherapy and before and after reinfusion (R) are as follows:

	VWF:Ag	VWF-protease	VWF:CB	Plts $\times 10^9$/L
Before HDC	97±33	107±22	64±22	333±97
After HDC	112±36	92±20	98±10	220± 33
Before R	159±37	76 ±24	186±19	177±85
After R	156±25	75± 22	197±47	46±18

Conclusions. In autologous stem cell transplantation the increased concentration and activity of VWF could explain the occurrence of TTP and VOD, and justify the prolonged thrombocytopenia and platelet transfusion refractoriness.

P089

ACTIVATED RECOMBINANT FACTOR VII CONTINUOUS INFUSION IN PATIENTS WITH HEMOPHILIA AND INHIBITORS

Tagariello G, Radossi P, De Biasi E, Risato R, Davoli P

Blood Bank, Center for Blood Diseases and Hemophilia Center, Castelfranco Veneto Hospital, Castelfranco Veneto (TV), Italy

Activated recombinant FVIIa (rFVIIa) is effective in hemophiliacs with inhibitors. Major problems of this therapy are its high costs and the short half life of the product which needs repeated and short interval administrations. Continuous infusion (CI) treatment avoids peaks and troughs and makes this therapy cheaper. Other questions still open are represented by the absence of a general consensus on the optimal regimen for CI and that this mode of administration is not recommended by the manufacturer. In the recent past major surgery was quite impos-

sible in hemophiliacs with inhibitors and rFVIIa has provided a valid and efficacious therapeutic option. We have previously described two cases of total hip replacement (THR) made possible by using rFVIIa CI (*Haemophilia 2000; 6: 581*) and we have now experienced the possibility of this treatment not only for elective surgery, but also for major hemorrhages or other procedures which have to be treated in a hospital setting. Since the January 1st, 2001 we have treated 6 patients by CI. Two had elective major surgery: one THR of a patient who started replacement therapy with FVIII concentrates because of the low titer of inhibitor. On the 7th day the titre of inhibitor rose and replacement therapy with FVIII became ineffective. rFVIIa was initiated by CI and continued for seven days with a total dose of 126 mg. The second case was femur ostheotomy, treated successfully for 8 days. Two ileo-psoas hematomas, four lithotrixia sessions for renal calculosis and one gastric massive hemorrhage were treated with minimum amounts of rFVIIa (100-120 μg/kg/bw bolus infusion followed by 100-120 μg/kg/BW in six hours CI) successfully. In conclusion we think that the use of rFVIIa by CI represents an effective and flexible therapeutic option in different types of bleeding in patients with hemophilia and inhibitors.

P090

ACTIVATED RECOMBINANT FVII (NOVOSEVEN) CONTINUOUS INFUSION FOR TOTAL HIP REPLACEMENT IN A PATIENT WITH FACTOR VII CONGENITAL DEFICIENCY

Radossi P, Bisson R,* Davoli P, De Biasi E, Risato R, Tagariello G

*Blood Bank, Centre for Blood Disease, Hemophilia Center and *Orthopedics, Castelfranco Veneto Hospital (TV), Castelfranco Veneto, Italy*

Inherited FVII deficiency is a rare autosomal recessive disorder which can cause hemarthrosis and, in the long-term, severe arthropathies comparable to those seen in hemophiliacs. Treatment includes prothrombin complex or plasma-derived concentrates. More recently activated recombinant FVII (rFVIIa), provides a further option for replacement therapy in these patients, without the risk of transmitting blood-borne infectious diseases. A 28-year old female (kg 55) with severe FVII deficiency (FVII:C <2%) was admitted in June 2001 for total hip replacement. Therapy with rFVIIa started with a bolus injection (BI) of 60 KUI (21 μg/kg) that was repeated twice during the operation. CI begun at the end of the operation with a dose of 6.3 μg/kg/h, for days 1-2, 4.5 μg/kg/h for days 3-5, 3.6 μg/kg/h for day 6, 2.7 μg/kg/h for day 7, 1.8 μg/kg/h for days 8-10, 2 BI of 120 KUI in days 11-12. A total of 0.93 μg/kg rFVIIa was used. FVII plasmas levels ranged from 7.8 U/mL following the BI to 1.2 U/mL in the 9th day. Prothrombin time was within the normal range during all the period. ATIII, platelets and D-Dimer were monitored daily always within the normal range. The procedure underwent without any serious adverse effects and the patient started early mobilization. In conclusion we think that rFVIIa represents an effective replacement therapy for major surgical procedures also in patients with severe FVII congenital deficiency. CI maintains stable plasma factor level avoiding peaks observed with repeated BI. Thranexamic acid appears to be useful and saline parallel infusion seems sufficient to protect from local thrombophlebitis.

P091

SUCCESSFUL ADMINISTRATION OF RECOMBINANT ACTIVATED FACTOR VII AND HIGH-DOSE INTRAVENOUS IMMUNOGLOBULIN IN AN OLD PATIENT WITH AN ACQUIRED FACTOR VIII INHIBITOR, SEVERE HEMOTHORAX AND ILIOPSOAS HEMORRHAGE

Loffredo F, Coppola A, Garofano T, Madonna P, Tufano A, Di Minno G

Centro di Coordinamento Regionale per le Emocoagulopatie, Dipartimento di Medicina Clinica e Sperimentale, Università "Federico II", Naples, Italy

An 80-year old male was admitted to our center because of a 2 month history of bleeding tendency (cutaneous and muscle hemorrhage, mainly after trauma), in the presence of a prolonged APTT (87.3 sec) and normal PT. His family and personal history was uneventful as to bleeding tendency and the patient had experienced two myocardial infarctions, with documented multi-vessel coronary artery disease and bypass graft treatment. Moreover frequent arrhythmias (atrial fibrillation, ventricular tachyarrhythmia) occurred. On admission, his laboratory tests confirmed APTT prolongation, and nearly undetectable FVIII:C (0.3%) and a 2.5 BU/mL FVIII inhibitor were found. No signs of autoimmune disease or malignancy were detectable at clinical, laboratory or instrumental examinations. No new drugs had been introduced over the last two years. Three days after admission the patient had severe pain in the lower abdomen and lower left limb, that worsened during extension of the hip joint, and a 2 g/dL hemoglobin loss in 12 h was found. A CT scan showed a diffuse hemorrhage into left iliopsoas muscle, with dislocation of abdominal organs. Furthermore persistent cough and dyspnea lead to the discovery of a massive left hemothorax, without other clear-cut signs of pulmonary disease. Recombinant activated factor VII (rFVIIa) was started and, because of his cardiovascular state, low doses (60 μg/kg every 4 hrs) were employed for 7 days. In parallel a high dose intravenous ummunoglobulin (HDIg) course was administered (0.4 g/kg for 5 days) and then prednisone 1 mg/kg was started. Over the following 2 weeks APTT progressively shortened and the inhibitor titer reduced (55.8 sec and 1.8 BU/mL, respectively, after 8 days; 38.6 sec and 0.5 BU/mL after 15 days). No further bleeding occurred. In this patient at high thrombotic risk, relatively low doses of rFVIIa were useful and safe in the management of his severe bleeding. Moreover, combined with immunosppressive treatment, the first-line use of HDIg enabled a fast control of FVIII inhibitor.

P092

THROMBOPHILIC FAMILIES: A DIFFERENT GENOTYPE/PHENOTYPE CORRELATION

Fuccio A,* Sibillo A,* Salemme F,° Aversano V,# Sibillo R°

**Area di Biologia Molecolare Clinica, °Laboratorio di Emostasi e Coagulazione, Hermes Centro Medico Polispecialistico, Caserta; #Chirurgia Vascolare, Casa di Cura Villa Fiorita, Capua (CE), Italy*

Single-point mutations in the gene coding for prothrombin (G20210A) and factor V (G1691A) are associated with an increased risk of thrombosis. We describe two pedigrees including heterozygous subjects for prothrombin and factor V mutations. A complete set of plasma and DNA determinations relat-

ed to hemostasis was performed on both families. In the first family the proband is a 33-year old male who suffered from deep-vein thrombosis, he was heterozygous for factor V Leiden. We completed the analysis in the remaining family members: the mother (64 years old) and the sister (30) were heterozygous for factor V Leiden; the father (71) and the two brothers (41 and 36) were heterozygous for G20210A mutation. None of them has had thromboembolic disease, even if some of them have been exposed to risk factors such as pregnancy and surgical procedures. In the second family the proband (35 years old) experienced recurrent phlebitis (27) immediately after her second pregnancy and one event of cerebral ischemia (32) after a surgical procedure; she was heterozygous for factor V Leiden. The younger sister (29), heterozygous for both factor V and G20210A mutations, had a spontaneous abortion during the first trimester of pregnancy. The older sister (37), heterozygous for G20210A mutation, had no thrombosis even after two pregnancies and one surgical procedure. The brother (25), heterozygous for factor V Leiden, had no thromboembolic disease. These cases suggest that the genotype/phenotype correlation may not be as strong as described and support the complexity of thromboembolic disease. Individuals heterozygous for the same mutant gene exhibit symptoms or not despite having been exposed to risk factors.

P092a

EFFECTS OF TRANSDERMAL HORMONE REPLACEMENT THERAPY ON COAGULATION AND FIBRINOLYSIS

Papa ML,* Capasso F,* Albolino L,* Pudore L,* Torre S,* Russo V,* Iaccarino M,° Cipolletta M,* D'Ambrosio A,° Pinto A,° Curcio M,° Sica G,# De Lucia D#

**Laboratorio di Emostasi e Trombosi, Ospedale San Giovanni Bosco, Naples; °Unità Operativa Ostetrico-Ginecologica, Clinica Mediterranea, Naples; #Istituto di Patologia Generale ed Oncologia, II Università di Napoli, Italy*

Observational studies have suggested that hormone replacement therapy (HRT) may reduce the risk of arterial disease in healthy post-menopausal women. Hovewer an early increased risk of myocardial infarction was reported in women with coronary hearth *disease (JAMA 1988*). Furthermore, it was observed in primary prevention trials that HRT increases both the rate of venous thromboembolism(VTE) of 2-4 fold and of cardiac ischemic events (*ISTH, Paris; 2001*). Whether the possible cardioprotective effect of oestrogen depend on the route of administration(oral versus transdermal) is still unknown even though the users of oral HRT exhibit changes of hemostatic and inflammatory variables. At present, transdermal HRT is increasingly prescribed because offers some advantages to women but the beneficial effects on thrombotic risk markers must bee tested in prospective studies too. We examined 100 healthy post-menopausal women aged 45-60 years, 50 HRT non-users and 50 transdermal HRT users to observe effect of therapy on coagulation and fibrinolytic systems during the first year of treatment. 100 healthy pre-menopausal women acted as control group. Factor VIII (VIII:C), factor VII(VII:C); the natural inhibitors: antithrombin (ATIII), protein C (PC), protein S (PS); the resistance to activated protein C were carried out on automated coagulometer ACL7000 (IL, Milan, Italy). The free tissue factor pathway inhibithor (TFPI), tissue plasminogen activator (t-PA), plasminogen activator inhibithor type 1 (PAI-1) were measured with Elisa techniques. (Stago' Asniers, France). The HRT users had levels of VIII:C and VII:C similar to non-users even if higher compared to pre-menopausal group (VIII:C 126±58, 120±59, 85±15, p= 0.001. VII:C 113±23, 103±19, 90±16, p=0.001). The ATIII and PC did not change among the three groups, the levels of PS were a little lower compared with those of controls. The values of APC ratio (nAPC_SR) were the same in two populations of women (nAPC_SR 1.02±0.7, 1.02±0.8, 1.1±25, p= 0.02). No variations were observed in TFPI concentrations in the HRT group compared with the other groups (9.21±1.65, 9.19±2.55, 5.9±1.60, p= 0.964). There was an increase in fibrinolytic activity in HRT users evidenced by a little decrease in PAI-1 levels compared to those of non users with no variations in t-PA values among the three groups (PAI-1 22.3±18, 32.40±22.4, 11.20±7.3, p=0.042. t-PA 8.11±5.74, 7.89±4.36, 6.51±3.10, p= 0.854 in HRT users, non usres and controls; respectively). Our findings suggest that the lack of a shift in procoagulant- anticoagulant balance to a prothrombotic state as we have observed during transdermal HRT must be confirmed in larger prospective studies. Furthermore, women carriers of a thrombophilic state either congenital or acquired and since at higher risk of thrombosis will benefit from transdermal therapy during post-menopausal period.

P092b

MARKERS OF HYPERCOAGULABILITY AND PROC GLOBAL AS SCREENING TEST FOR THROMBOPHILIA

De Lucia D,* Maisto G,* Del Giudice V,* Marotta R,* De Francesco F,* Mordente S,* Di Alessio P,° Calvanese R,° Gemito G,° Di Onofrio EG,° Di Amico A,° Formicola O,° Russo G°

**Institute of General Pathology, Laboratory of Haemostasis and Thrombosis; II University of Naples; °Laboratory of Clinical Pathology; Loreto Mare Hospital, Naples, Italy*

Thrombosis is a multicausal disease. In most cases two or more risk factors both genetic and acquired are necessary before thrombotic event occurs. Such a combination of risk factors are hyperhomocysteinemia (> 14.5 μ°mol/L), high FVIII:C levels (> 155 IU/dL) or high levels of FII (117.5 IU/dL). It is mandatory to have a screening assay which is able to recognize this combination of defects. The aim of our study was to determine the sensitivity of ProC Global assay to fulfil such demand on a screening test. ProC Global (Dade Behring) is a coagulometric assay measuring the prolongation of an APTT induced by activation of protein C (PC) in the sample. The results are expressed as normilized ratio (NR) or as modified normalized ratio (mNR) using a calibrated normal plasma pool as reference. While NR uses the ratio between the APTT with and without PC activation, mNR uses the difference between both. The results are correlated with levels of homocysteine (HPLC with fluorimetric detection) and the FVIII:C and FII (coagulometric determination; Instrumentation Laboratory). We have investigated 150 patients with hyperhomocysteinemia (> 14.5 mmol/L), aged 51.5 ≥15.5 years, high FVIII:C > 155 IU/dL in 80 patients, high FII > 117.5 IU/dL in 17 patients; both factors are elevated in 11 patients. Patients with FV R506Q mutation and PC/PS deficiencies are excluded from the study. It is possible to detect patients with above mentioned combination of the three risk factors in the ProC Global assay. The sensitivity as function of mNR and NR varied between

92.3% (mNR with cut-off 0.75)and 78% (NR with cut-off 0.80). The sensitivity for the combination of the two risk factors homocysteine and elevated FVIII:C levels is 81.5% (mNR) and 76% (NR), for homocysteine and elevated FII the sensitivity is 85% and 74%, respectively. Among investigated subjects (1455) with thrombophilia we found a high percentage of patients with combined defects. The ProC Global as general screening tests for the whole PC-pathway is able to recognize other thrombogenic risk factors additionally, especially the combination of high levels of homocysteine, FVIII:C and FII.

P092c

G-CSF AND CYTOAFERESIS EFFECTS ON HEMOSTASIS IN CD 34+ CELLS DONORS

Luise F,° Piromalli A,° Sottilotta G,° Morabito F, Iacopino P,* Lombardo VT°

*°Centro Emofilia Servizio Emostasi e Trombosi; *Centro Trapianti Midollo Osseo Azienda Ospedaliera "Bianchi-Melacrino-Morelli" Reggio Calabria, Italy*

Introduction. Peripheral blood is an alternative to bone marrow as source of stem cells for allogenic transplantation. Mobilization of stem cells ($CD34^+$) in peripheral blood is obtained by granulocyte colony stimulating factor. Transient hypercoagulable status has been recently reported in normal donors, likely induced by G-CSF and by extra corporeal circulation. *Aim of study*. To evaluate changes in a hemostatic parameters after administration of G-CSF and stem cell collection by leukapheresis, possibly induced by thrombin generation and by endothelial damage. *Methods*. Prothrombin time, activated partial thromboplastin time, fibrinogen, D-Dimers, antithrombin III, protein C, protein S, thrombin-antithrombin complex, prothrombin fragment 1+2, coagulant factor VIII, von Willebrand Factor, thrombomodulin, plasminogen and homocystein were performed in 20 healthy donors mobilized with G-CSF. Sample of blood were collected from each donor before and after G-CSF and immediately after leukaferesis and 10 days after the G-CSF stimulation. *Results and Conclusions*. Decrease of AT III values were observed, pathological increase of TAT values were observed too. Analysis of F1+2 showed abnormal increase in 10/20 cases. Pathologic decrease of PLG values were observed. These preliminary data showed an activation status of parameters influencing blood hypercoagulability after stem cell collections.The increase of TAT values associated with the ATIII decrease and F1+2 increase gave indirect information of a possible increase of circulating thrombin. The reduction of PLG values could be due to a direct activation of fibrinolitic system as a consequence of either the extracorporeal circulation or a phisiological response to the above mentioned activation status or both No clinical event was associated with parameters evaluated in this study. However, we suggest that stem cell normal donors deserve to be monitored for factors predicting a risk of thrombosis.

Posters
Venous Thromboembolism

P093

ROLE OF THROMBOPHILIC FACTORS IN UPPER LIMB VENOUS THROMBOSIS. A STUDY OF 61 CASES

Brancaccio V, Iannaccone L, Scenna G, Margaglione M,* Ames PRJ

*Unità Emostasi e Trombosi, Ospedale "A. Cardarelli", Napoli; *Unità Aterosclerosi e Trombosi, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy*

Background. Upper limb venous thrombosis (ULVT) is less frequent than iliac and lower limb venous thrombosis. It is often associated with anatomical abnormalitities or central venous catheter (CVC) positioning. The role of genetic thrombophilic factors in ULVT is not well known. *Patients and Methods*. We studied 61 patients with ULVT, documented by phlebography or ultrasound (M/F 25/36; median age 34 yrs, range 15-79). Three cases (5%). had pulmonary embolism (PE); anatomical abnormalities or previous trauma were present in 7 (11%), intense physical stress in 5 (8%), CVC in 5 (8%), postoperative period in 2 (3%), oral contraception in 5 (14%). All patients were tested for: antithrombin, protein C, protein S deficiency, activated protein C resistance, lupus anticoagulant(LA) and anticardiolipin antibodies (aCL), homocysteine levels (33 patients); factor V Leiden (FVL), prothrombin(FII) A20210, TT677 genotype of MTHFR. *Results*. No deficiencies of natural anticoagulants were found. Five patients (8%) had activated protein C resistance and FVL heterozygous; A20210/FII was present in 5 patients (8%); MTHFRTT677 was present in 13 patients (21%). Five patients (8%) had multiple thrombophilic genotypes: FVL+A20210/FII+ TT677/MTHFR 1, FVL + TT677/MTHFR 2, FVL+A20210/FII 1, A20210/FII+ TT677/MTHFR 1. Two patients had an antiphospholipid syndrome (APS) (LA+/aCL- and LA-/aCL+ pattern). Eleven of 33 patients (33%) had high levels of homocysteine: of these 7 had the TT677/MTHFR genotype. A lower limb thrombosis was associated in a case with FVL+A20210/FII genotype while a relapsing case without genetic risk factors was observed in the presence of anatomical abnormalities. No genetic thrombophilia was present in the three cases associated with PE. *Conclusions*. One or more thrombophilic genotype were present in 38% of our patients and 3% and 33% had respectively a primary APS or hyperhomocysteinemia. A complete screening for genetic and acquired thrombophilia must be done in patients with ULVT.

P094

INHERITED FACTOR V LEIDEN MUTATION AND/OR PROTHROMBIN (G20210A) VARIANT CAN AFFECT THE CLINICAL OUTCOME OF BONE MARROW TRANSPLANT RECIPIENTS

Cultrera D, Musso R, Milone G, Indelicato F, Sortino G, Ferlito C, Azzaro MP, Di Francesco E, Fichera E, Giustolisi R

Centro Regionale di Riferimento per l'Emofilia (e la Trombosi), Istituto di Ematologia, Università di Catania, Italy

Among the various complications of bone marrow transplantation (BMT), it is well known that GVHD, CMV disease, respiratory dysfunction resembling adult respiratory disease syndrome, central nervous dysfunction, thrombotic microangiopathy and venous occlusion disease (VOD) are accompained by vascular endothelium activation and/or damage together with an abnormal hypercoagulability. In this regard, the etiopathological role of several inflammatory cytokines and the acquired decrease of the natural anticoagulants such as protein C, protein S and antithrombin III with abnormal thrombin generation has been reported. We report here that inherited conditions predisposing to thrombosis such as FV Leiden mutation and prothrombin (G20210A) variant must also be considered in BMT programs. Nineteen consecutive patients (10 females and 9 males, age ranging 33-58 yrs) undergoing allogenic BMT for malignant hematopoietic disease according to a standardized protocol were evaluated. All patients were monitored for thrombotic events before (day –8) and following BMT (days +7 and +30). Post-transplantation 7 patients had VOD, 2 acute GVHD of the liver and 6 had other diseases. In our cases routine coagulation parameters were performed together with FV:C and FII:C (one-stage method with deficient plasmas from IL, in ACL 3000 Plus automatized coagulometer). Activated protein C resistance (APCR, Behring Institute, Italy) was also determined. Genomic DNA prepared from leukocytes at day –8, day +7 and +30 was used in PCR aplification using the restriction enzyme Mnl 1 for FV mutation and Hind III for G20210A polymorphism of the prothrombin gene. Amongst all of them, 3 were heterozygous for the FV Leiden mutation with APC ratio ≥1.1 and 2 for prothrombin G20210A variant with APCR 1.3. In these 5 patients acute VOD occurred during the aplastic phase. One patient with the FV Leiden mutation had received bone marrow from his brother with the same genetic mutation and without a history of thrombophilia. Our results are summarized in the Table.

	Day -8	Day +7	Day +30	Controls N=15
FV:C	178±31	75±31*	89±25	95±13
FII:C	140±33**	111±22	127±18**	98±23
APCR ratio	2.8±0.9	2.1±1.2 *	1.7±1.3*	3.1±0.9
FV Leiden mutation		3/19	0/19	
Prothrombin (G20210A) variant		2/19	0/19	

* p<0.001 vs day –8 ** p<0.001 vs controls

These data strongly suggest that in patients undergoing BMT, FV Leiden mutation and prothrombin (G20210A) variant may have a predisposing pathogenetic role in the development of clinical VOD and progressive multiorgan dysfunction via vascular endothelium activation and/or diffuse damage. In our opinion, these preliminary genetic tests must be considered not only in patients undergoing BMT but also in their related and unrelated donors.

P095

VENOUS THROMBOEMBOLISM PROPHYLAXIS FOR MEDICAL PATIENTS: IS ADHERENCE TO CONSENSUS GUIDELINES ADEQUATE?

Ageno W, Squizzato A, Ambrosini F, Dentali F, Marchesi C, Mera V, Steidl L, Venco A

Department of Internal Medicine, University of Insubria, Varese, Italy

Introduction. The risk of venous thromboembolism in medical patients is comparable to the risk in general surgical patients. Thromboprophylaxis is recommended for specific medical patients, but its use in clinical practice is unknown. *Methods.* We conducted a retrospective review of the charts of consecutive patients discharged from 2 departments of Internal Medicine, one in the teaching hospital of Varese and one in the non-teaching hospital of Angera, Italy, from October to December 2000. We selected the charts of patients with clinical conditions at increased risk for venous thromboembolism requiring thromboprophylaxis according to consensus statements. The use of antithrombotic drugs and contraindications to prophylaxis were documented. *Results.* We screened a total of 516 charts, 265 in Varese and 251 in Angera and we identified 165 patients (103 and 62, respectively) at risk for venous thromboembolism because of malignancy (53), heart failure (34), stroke (33), acute infections (23), acute respiratory failure (18), acute rheumatic disorders (3), and inflammatory bowel disease (1). Prophylaxis was prescribed to 52 of the 165 patients (31.5%), 34 of 103 in Varese (34%) and 18 of 62 in Angera (27.4%). Patients with stroke and heart failure were significantly more likely to receive thromboprophylaxis (54.5% and 47.2%) than cancer patients (17%) or patients with acute respiratory failure (18.7%) or acute infectious disease (20.8%). Excluding patients with contraindications to antithrombotic drugs, prophylaxis was prescribed to 46.4% of the total eligible population, 58.3% in Varese and 32.7% in Angera ($p<0.05$). *Conclusions.* Prophylaxis of venous thromboembolism is underused in medical patients and the proportion of patients receiving antithrombotic drugs varies with the medical condition which precipitated hospital admission. The low rate of usage of prophylaxis suggests that preventable cases of thromboembolism are occurring and that better education of physicians is required to increase the usage of thromboprophylaxis.

P096

PROPHYLAXIS WITH ENOXAPARIN DOES NOT REDUCE PLASMA LEVELS OF SOLUBLE FIBRIN POLYMERS AFTER ELECTIVE NEUROSURGERY

Sonaglia F, Agnelli G, Piovella F, Severi P, Viganò D'Angelo S, Quintavalla R, Filippucci E

Sezione di Medicina Interna e Cardiovascolare, Università di Perugia; Istituto di Clinica Medica II, Università di Pavia; Ospedale Galliera, Genoa, Ospedale S. Raffaele, Milan; Ospedale Civile di Parma, Italy

Background. Increased pre-operative levels of soluble fibrin polymers, as determined by an enzyme immunoassay (TpPTM), correlate with the development of deep vein thrombosis after elective neurosurgery (*Sonaglia, 1999*). *Aim of the study*: To eval-

uate whether enoxaparin at prophylactic doses (40 mg once daily) reduces post-operative levels of soluble fibrin polymers after neurosurgery. *Methods*. Blood samples were withdrawn on the 10th day after neurosurgery from 162 patients randomized to enoxaparin, 40 mg once daily (78 patients), or to placebo (84 patients) for 7 days. TpPTM utilizes a monoclonal antibody against a conformational epitope of the soluble fibrin polymeric structure. *Results*. TpP average value was similar in patients treated with enoxaparin 2.69±3.15 μg/mL (mean±SD) and in patients receiving placebo 3.57±3.49 μg/mL (p=0.07). In patients receiving enoxaparin the average TpP value was 3.91±3.08 μg/mL (mean±SD) in patients with DVT and 2.43±3.13 μg/mL in patients without DVT (p=ns). In patients receiving placebo, TpP average value was 4.64±3.90 μg/mL (mean±SD) in patients with DVT and 3.03±3.17 μg/mL in patients without DVT (p=ns). Regardless of treatment, the average TpP value on post-operative day 10, was significantly higher in patients with DVT (4.40±3.62 μg/mL mean±SD) than in patients without DVT (2.71±3.15 μg/mL p<0.005). These data confirms the potential role of an increased TpP plasma level as a marker for the development of post-operative DVT. *Conclusions*: Patients undergoing elective neurosurgery have a sustained activation of blood coagulation that is not controlled by in hospital prophylaxis with enoxaparin. This finding may indicate the need for prolonged thromboprophylaxis in patients undergoing neurosurgery for brain tumor.

P097

THE ANNUAL INCIDENCE OF ACUTE VENOUS THROMBOEMBOLISM AMONG THE RESIDENTS OF PAVIA; AN ITALIAN EPIDEMIOLOGICAL INVESTIGATION

Anastasio R,* Buonanno C, Granzow K, Minardi V,* Falaschi F, Bressan MA, Malato A, Siragusa S*

**Unità Malattie Tromboemboliche ed Emorragiche, Cattedra di Ematologia, Università di Palermo, and Servizio Pronto Soccorso Accettazione Policlinico S. Matteo, Pavia, Italy*

The incidence of acute venous thromboembolism (VTE) has not been well documented. Reported annual incidences vary widely, ranging from 43.7 to 145.0 per 100,000 for deep vein thrombosis (DVT) and 20.8 to 65.8 per 100,000 for pulmonary embolism (PE). Few data are available regarding the Italian population. We conducted a population-based study to identify all cases of acute VTE occurring among all residents of Pavia, during a two-year period of analysis. Using the data resources of *Regione Lombardia*, we identified the inception cohort of Pavia residents; among whom, most of the care is provided by *IRCCS Policlinico S. Matteo*. The *Servizio Accettazione e Pronto Soccorso* medical records contain both outpatient and inpatient data; we retrieved all medical records for evaluating the incidence of objectively documented DVT and/or PE. Annual incidence rates were calculated using incident cases of DVT or PE as the numerator and age- and gender-specific estimates of the population referred to our Hospital as the denominator. During the period January 2000 – December 2001, approximately 60,000 residents were evaluated; among whom, 715 (1.2%) were clinically suspected of having acute VTE (521 DVT and 194 PE). The incidence of confirmed VTE was 24.2% (173/715); the annual population-based incidence was 86 per 100,000 (95% CI, 75-97). These data are in keeping with those reported by Silverstein *et al.* (117 per 100,000), and with those expected in the population of Pavia (76,000 residents -ISTAT 2000-, 100 new cases per year). Our investigation has some limits; in fact, although our Institution provides most of the care for Pavia, data from other, small hospitals has not been evaluated. Nevertheless, inclusion of these findings would increase the annual incidence of VTE only slightly. Despite these limits, this is the first population-based analysis of the incidence of acute VTE in an Italian population.

P098

ACUTE MASSIVE THROMBOSES AFTER *CAMPYLOBACTER SPP* INFECTION SUCCESSFULLY TREATED BY CONTINUOUS INFUSION OF ANTITHROMBIN AND HEPARIN: A CASE REPORT

Gamba G,* Lanzarini P, Capezzera M, Montani N

*Departments of Internal Medicine and *Infectious Diseases, University of Pavia, IRCCS Policlinico San Matteo, Pavia, Italy*

Few patients with septic thrombophlebitis, exclusively referred to *Campylobacter fetus* infection, are reported in Medline literature. A 38-year old man was admitted to the Department of Infectious Diseases, due to abdominal pain, fever, diarrhea containing blood and mucus, and occurrence of massive superficial and deep venous thromboses involving both lower extremities and right arm. A *Campylobacter spp* was isolated from the stool before the hospital admission. Treatment with imipenem plus cilastatine and ciprofloxacin and calcium nardroparine 8000 U sc every 12 th hour was started. Four days after, because of rapid aggravation of the clinical features and thrombosis progression associated with the appearence of edema, cyanosis and patches of skin necrosis of the right foot and leg, the patient was transferred to Intensive Care Unit. He was then treated by fasciotomy on right leg and by continuous sodium heparin infusion. Laboratory tests showed AT 46 U/dL, PC 60 U/dL, PS 75 U/dL, PIt 132,000 m^3, HCY 11.2 μM/L; normal values of APA, pANCA, cANCA, ANF, CEA, αFP, CA19-9, β2M; absence of LA and of FV G1691A and FII G20210A mutations. Batteriological cultures and serological test were negative for *Amoeba*, *Bilharzia*, *Salmonella* and *Shigella*, HIV and hepatitis virus searches were also negative. Heparin infusion did not improve the thrombosis progression: the necrotic skin lesions increased in number and size. In association with sodium heparin (1500 U/h) a continuous infusion of antithrombin concentrate (Ambin-Grifols) at doses of 40U/h after a bolus of 2000 U i.v. was given. The patient was then sent to the Department of Internal Medicine to follow his clinical course. The infusion of heparin and antithrombin was continued for 21 days, achieving AT plasma levels in normal ranges (74-110 U/dl). Within a few days, a progressive improvement of the general and local pictures were observed; after three weeks the patient began warfarin treatment. On oral anticoagulant the AT plasma levels maintained normal values, and the patient was discharged 45 days after from the admission, with advanced resolution of the skin necroses. No recurrences of thromboses nor abdominal symptoms were further observed. Recanalization of veins occurred and the skin lesions completely disappeared. A colonoscopy with histological examination, performed after three months, was negative for chronic inflammatory bowel disease.

P099

DERMATAN SULPHATE IN A PATIENT WITH HEPARIN-INDUCED THROMBOCYTOPENIA AND PULMONARY EMBOLISM

Imberti D, Prati C, Croci E,* Taliani MR,° Cavallotti P, Sverzellati E, Agnelli G°

III Unità Operativa di Medicina Interna e Laboratorio di Analisi, Ospedale di Piacenza, °Sezione di Medicina Interna e Cardiovascolare, Università di Perugia, Italy*

Heparin-induced thrombocytopenia (HIT) type II is a potentially life-threatening immune-mediated side effect of heparin administration; paradoxically, despite a low platelet count, patients with HIT type II are at risk of developing thromboembolic venous and arterial complications. Treatment of such complications remains challenging; it is necessary to discontinue heparin administration immediately and to initiate an alternative antithrombotic agent. Dermatan sulphate (DS, Mediolanum Farmaceutici, Italia) is a selective inhibitor of thrombin, characterized by a low-rate of cross-reactivity with heparin. We describe a case of pulmonary embolism in a patient with HIT type II treated with DS. A 68-year old female was admitted to our Department for sudden-onset dyspnea 10 days after surgery for total hip replacement. She had received prophylaxis with nadroparin (5,700 UI sc OID). Ventilation-perfusion lung scan was diagnostic for acute pulmonary embolism (high probability) and the echocolordoppler revealed a thrombus in the greater right saphenous vein. Hematology count showed severe thrombocytopenia (27,000/m^3). Related to the important reduction of platelet count during LMWH administration (before heparin treatment the platelet count was normal), a diagnosis of HIT was suspected and a platelet aggregation test performed; the test was positive for unfractionated heparin and nadroparin, negative for DS. Nadroparin was immediately discontinued. DS treatment was started as a continuous intravenous infusion at the dose of 0.6 mg/Kg/h and continued for seven days, targeting an aPTT ratio of 1.5. The first aPTT ratio was measured after four hours; anticoagulation obtained with DS was very stable, so that only few dose adjustments during infusion were required. Platelet count began to recover on day 3 and on day 5 was 128,000/m^3, allowing the administration of warfarin to be started. No hemorrhagic complications or adverse events were observed. In conclusion in this patient DS produced an effective and safe anticoagulation, allowing a prompt recovery of the platelet count; DS should be considered for the treatment of thromboembolic complications in patients with HIT type II.

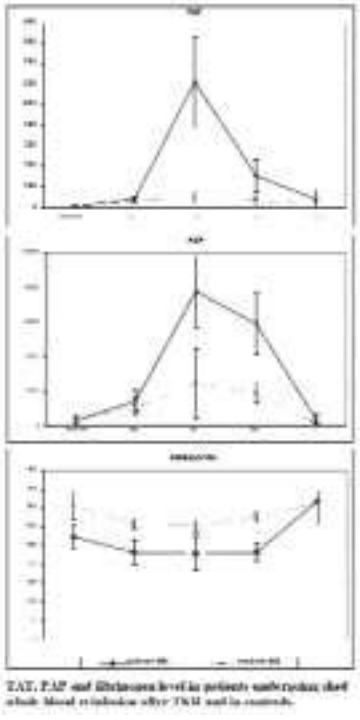
P100

ACTIVATION OF COAGULATION AND FIBRINOLYSIS IN PATIENTS UNDERGOING PERIOPERATIVE SALVAGE AND REINFUSION OF WHOLE BLOOD AFTER TOTAL KNEE REPLACEMENT

Biagini D,* Filippucci E, Agnelli G, Pagliaricci S

*Sezione di Medicina Interna e Cardiovascolare, Dipartimento di Medicina Interna, Università di Perugia; *Centro Ortopedico Umbro, Perugia, Italy*

Background and Objective. Total knee replacement (TKR) is associated with a high incidence of venous thromboembolism. Perioperative salvage and reinfusion of whole blood is commonly used after major orthopedic surgery. The reinfusion of shed whole blood could contribute to the activation of blood coagulation observed in TKR patients. The aim of this study was to assess the activation of blood coagulation and fibrinolysis in patients undergoing perioperative salvage and reinfusion of whole blood after TKR. *Design and Methods.* Consecutive patients undergoing perioperative salvage and reinfusion of whole blood after TKR were included in the study. Patients undergoing TKR without perioperative salvage and reinfusion served as controls. Thrombin-antithrombin complexes (TAT), plasmin-antiplasmin complexes (PAP) and fibrinogen were assayed immediately before surgery (baseline), immediately before (T0) and immediately after (T1) the shed whole blood reinfusion, 2 hours (T2) and 24 hours (T3) after reinfusion. *Results and Discussion.* Plasma level of TAT and PAP increased after surgery in both patients and controls. An increase in TAT and PAP level was observed immediately after reinfusion, with respect to baseline (TAT 610.7 μg/L vs 7.7, $p<0.001$; PAP 7763.0 μg/L vs 317.8, $p <. 0003$) and to controls (47.8 μg/L, $p<0.001$; 2421.3 μg/L, $p<. 02$, respectively). The levels of TAT and PAP remained high 2 hours after the end of reinfusion compared to controls (154.5 μ/L vs 37,6, $p<. 01$; 5889.7 μg/L vs 1862.3, $p<0.001$, respectively) and decreased 24 hours thereafter. No differences were observed at any time in fibrinogen levels between patients and controls.



TAT, PAP and fibrinogen level in patients undergoing shed whole blood reinfusion after TKR and in controls.

Interpretations and Conclusions. TKR is associated with activation of blood coagulation and fibrinolysis. Whole blood reinfusion induces a significant increase of markers of activation of coagulation and fibrinolysis. The activation of blood coagulation associated with shed whole blood reinfusion could be reduced by anticipating antithrombotic prophylaxis before reinfusion.

P101

MANAGEMENT OF ACUTE DEEP VEIN THROMBOSIS IN PERIPHERAL INSTITUTIONS: RESULTS FROM A NATIONAL SURVEY

Mascia B, Falaschi F, Buonanno C, Granzow K, Minardi V,* Anastasio R,* Bressan MA, Siragusa S*

*Servizio Pronto Soccorso Accettazione, Policlinico S. Matteo and *Unità Malattie Tromboemboliche ed Emorragiche, Cattedra di Ematologia, Università di Palermo, Italy*

Acute deep vein thrombosis (DVT) is a very common disease (1-3 cases per 1000). In Italy, the management of DVT is usually demanded of vascular surgeons, angiologists, internists, hematologists and thrombosis units; all of these are usually available at teaching hospitals. The situation is different in small or peripheral Institutions where, in most of the cases, management of acute DVT is demanded of the Emergency Physicians. In order to investigate the magnitude of this problem and the resources available at the peripheral hospitals, we conducted a phone-based survey. One-hundred-thirty-nine hospitals across the country were identified; 119 answered the questionnaire (88.8%). Results are shown in the Table below.

Question	North	Center	South	Total
1. How many of patients do you evaluate per month?				
<1	1	2	5	8
1-5	16	18	25	59
5-10	7	21	7	35
>10	6	6	5	17
2. Do you require specialists to confirm the diagnosis?				
Internal Medicine	14	19	7	40
Surgery (vascular or general)	18	33	29	80
Emergency Department (ED)	1	6	1	8
3. Do you considered clinical judgement appropriate for the diagnosis?	0	2	2	4
4. Do you have specific units for DVT?	2	6	2	10
5. Is UltraSonography (US) available at your hospital?				
Absent	2	4	3	9
Immediate performance	11	21	17	49
Delayed (48 hours)	25	39	36	100
Adequate therapy while waiting for confirmatory US	2	1	1	4

At peripheral Institutions, acute DVT is equally managed across the country. Very few hospitals have specific units (8.4%) or manage DVT in the ED (6.7%); most demand confirmation from internists and/or surgeons or perform delayed testing (84%). While waiting for confirmatory US, adequate therapy is administered in a few cases only (3.4%). More efforts should be made to improve the management of the acute phase of DVT in the EDs.

P102

JUGULAR THROMBOSIS RELATED TO CENTRAL VENOUS CATETHER IN ONCOLOGICAL PATIENTS: OUR EXPERIENCE IN 2001

Di Micco P, Torella R, Niglio A

V° Division of Internal Medicine, II University of Naples, Italy

Placing of a central venous catether is now a common practice during prolonged chemotreatment and catether-related thrombosis seems to be an infrequent complication. However in the last months 7 patients were referred to our Institute for suspected internal jugular thrombosis, and in particular 6 of these for catether-related thrombosis. The 6 patients had neoplasm in chemotreatment (1 Waldenström's disease, 1 multiple myeloma, 1 peritoneal metastasis from ovarian cancer, 1 gastric carcinoid, 1 ganglioneuroblastoma, 1 lung cancer). Additional thrombotic risk factors in the 6 patients included prolonged bed rest, bacteremia, and cancer-related procoagulants. The period between insertion of catether varied from 1 week to 2 months. Jugular vein thrombosis was confirmed by ultrasound examination in all cases. Conservative therapy was tried including anticoagulation therapy with warfarin (INR 2-3), unfractioned heparin or low molecular weight heparin or thrombolysis (only in one case for presence of floating thrombus). Surgical treatment was needed only for one case. Clinical episodes of pulmonary embolism were not recorded. In conclusion we can assert that jugular thrombosis was not rare in patients with an implanted central venous catether and this condition appears be related to artificial surface besides to other thrombotic risk factors such as cancer and its related chemotherapy, bacteremia and prolonged bed rest. However in the our experience jugular thrombosis related to central venous catether had a good prognosis by itself, in fact no clinical case of pulmonary embolism appeared, but the underlying disease often seems to be related to a worse prognosis and determines the real outcome of the disease.

P103

TUMOR CELL CONDITIONED MEDIA-INDUCED TISSUE FACTOR EXPRESSION BY ENDOTHELIAL CELLS IS COUNTERACTED BY LOW MOLECULAR WEIGHT HEPARIN (DALTEPARIN) AND UNFRACTIONATED HEPARIN

Vignoli A, Marchetti M, Suardi S, Barbui T, Falanga A

Hematology Division, Ospedali Riuniti, Bergamo, Italy

Tumors are known to activate the hemostatic system of the host increasing the risk of thrombotic complications: in this setting, a key role is played by the tumor cell/endothelial cell interactions. Heparins are potent antithrombotics, which can potentially interfere with these interactions. In this study, we wanted to: 1. characterize tissue factor (TF) expression by endothelial cells (EC) exposed to tumor cell conditioned media (CM), compared to a standard inflammatory stimulus (bacterial endotoxin, LPS); 2. evaluate whether a low molecular weight heparin (LMWH) (dalteparin) and unfractionated heparin (UFH) are able to interfere with tumor CM- or LPS-induced endothelial TF. Micro- (HMEC-1 line) and macro-vascular (HUVEC) human EC were incubated with CM from two human breast carcinoma cell lines [i.e, MDA-MB-231 (highly metastatic), and MCF-7 (low metastatic)] or LPS (10 μg/mL), in the presence or absence of LMWH or UFH (0.01-10

IU/mL). After 4 h incubation, EC samples were tested for TF activity (TF:Act), by the one-stage clotting assay, or antigen (TF:Ag), by ELISA. *Results*: CM from MDA-MB.231 and LPS alone (but not CM from MCF-7) significantly increased TF expression by both EC types. TF expression was significantly counteracted by heparins. Specifically, in HMEC-1, 10 IU/mL dalteparin reduced TF:Act induced by CM of MDA-MB-231 or LPS by 53±9% and 57±11%, respectively, while UFH (10 IU/mL) by 55±11% and 43±17%, respectively. These results were confirmed by the TF:Ag analysis. In HUVEC, at the same concentration (10 IU/mL), dalteparin reduced both MDA-MB-231 CM- and LPS-induced TF:act by 48±13% and 26±9%, respectively. Instead, UFH did not counteract the MDA-MB-231 CM induction of TF, but reduced the LPS-induced TF:Act (29±10% reduction). This study suggests that LMWH and UFH may act differently in reducing the activation of blood coagulation triggered by tumor cell-derived products or by LPS in distinct EC types.

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ARISTOS ITALIAN REGISTRY ON THROMBOEMBOLISM IN ONCOLOGY SURGERY

Agnelli G, Rossi R, Sonaglia F, Bolis G,° Capussotti L,#
Scarpa RM,§ Tonelli F,§ Valarani B,@ Sardina M, Gussoni G* on behalf of the ARISTOS Group

*Sezione Medicina Interna e Cardiovascolare, Dipartimento di Medicina Interna, Università di Perugia; °Ginecologia Clinica Mangiagalli, Milan; #Chirurgia Generale I, Ospedale Mauriziano, Torino; §Urologia Ospedale S. Luigi, Orbassano (TO); §Cattedra di Chirurgia Generale, Università di Firenze; @Hyperphar Research, Milan; *Italfarmaco S.p.A, Milan, Italy*

Background. Cancer patients have a high risk of venous thromboembolism (VTE). The incidence of VTE after cancer surgery is two-fold higher than that after non-cancer surgery. Venography-detected deep vein thrombosis (DVT) is currently used as the endpoint in clinical trials on the prevention of VTE. However, the clinical relevance of asymptomatic venography-detected DVT is unclear. The study population of clinical trials on VTE prophylaxis in cancer surgery is not necessarily representative of the overall cancer surgery population, since study patients are selected by strict inclusion and exclusion criteria. *Aim of the study*. To evaluate the incidence of clinically overt VTE and unexplained death in a wide-spectrum population of consecutive patients undergoing cancer surgery. Major and minor bleedings were recorded. To explore the feasibility of a clinical trial on VTE prophylaxis in cancer patients based on clinically overt events. *Methods*. ARISTOS was a prospective registry using an electronic data collection system that allows a continuous, real-time update and quality data control. Patients were evaluated for occurrence of VTE at discharge, at 30±5 days after surgery or earlier in case of signs and/or symptoms of VTE. At least 3 monitoring visits were performed at each center. Study events were assessed by an independent adjudication committee. The incidence of clinically overt VTE was estimated to be close to 2%, therefore the calculated sample size of ARISTOS was 2,000 patients. *Results*. From November 2000 to October 2001, ARISTOS included 2,367 patients in 31 Italian hospitals: 1,254 in general (abdominal or thoracic) surgery departments, 684 in urologic surgery, and 429 in gynecologic surgery. Follow-up was obtained in about 99.5% of patients. The registry allows a reliable estimate of the incidence of clinically overt VTE in patients undergoing cancer surgery and an evaluation of the risk factors for the development of VTE.

P105

GENETIC THROMBOPHILIA: ASYMPTOMATIC AND SYMPTOMATIC CARRIERS AND CLINICS

Bonifacio M,* Allegra C,* Carlizza A, Antonucci G°

**Dept. of Angiology; °Thromboembolic and Hemorrhagic Diseases Diagnostic Dept.; S. Giovanni-Addolorata Hospital, Rome, Italy*

Background. The aim of our study was to assess the prevalence of the most frequent hereditary thrombophilic factors (APC-R, factor II mutation, protein C deficiency, protein S deficiency and AT III deficiency, hyperhomocysteinemia) in patients and their blood relatives and their correlations with clinics. *Methods*. We examined 315 consecutive patients affected by peripheral vascular diseases, attending our Day Hospital, and 213 controls, free from overt arterial or venous pathologies, recruited among outpatients. The investigation included: patient's history, clinical examination, arterial/venous Duplex scan. *Results*. The prevalence of thrombophilic factors among patients was 45,3% (143 out of 315). In 18 patients (12.5%) an association of thrombophilic disorders was observed. A peculiar correlation was found between mild and moderate hyperhomocysteinemia and cholesterol emboli disease, a disease poorly mentioned in literature, found in 21 out of 32 patients affected by POAD (65.6%). Among asymptomatic blood relatives, 48 carriers of at least one thrombophilic factor, most frequently hyperhomocysteinemia, were found. *Conclusions*. The collected data highlighted: a) a high prevalence of heterozygous mutant factor II, almost as frequent as APC-R, and associated mostly with VT; b) a relevant prevalence of mild and moderate hyperhomocysteinemia, associated with VTE and arterial diseases. Greatest interest concerns the correlation with a severe ischemic picture which can be referred to as *blue toe syndrome* and cholesterol embolism, of which the association with primary thrombophilia does not seem to be reported in literature; c) the contrast between the association of hyperhomocysteinemia and severe clinical pictures and high prevalence of carriers, asymptomatic at clinical and Duplex scan investigation.

P106

FACTOR V LEIDEN BUT NOT PROTHROMBIN G20210A MUTATION IS A RISK FACTOR FOR THROMBOSIS IN PATIENTS WITH LYMPHOPROLIFERATIVE DISEASE

Siragusa S, Barbera V, Iannitto E, Di Trapani R, Malato A, Grimaudo S, Capone F, Mariani G

Cattedra di Ematologia, Università di Palermo, Italy

The association between VTE (venous thromboembolism) and lymphoproliferative disease (LPD) is controversial and the role of congenital risk factors for thrombosis remains undetermined. The knowledge of such a condition may have an important clinical impact for choosing an appropriate antithrombotic approach both in terms of prophylaxis and treatment. Two hundred and twenty consecutive patients with a LPD observed between 1996-2001 were analyzed with reference to the occurrence of venous thromboembolic (VT) events. Events were objectively confirmed

by compression ultrasonography (in case of suspected deep vein thrombosis) or by lung scanning and/or lung CT (in case of suspected pulmonary embolism). In one hundred and twenty patients the most common genetic risk factors for thrombosis (factor V Leiden and prothrombin G20210A) were assessed. The overall prevalence of VTE (DVT and/or PE) in the population studied was 7.27% (16/220), no matter the disease and treatment; the comparison between VTE and non-VTE patients with reference to the prevalence of the studied thrombophilia markers is reported in the Table below.

	LPD+ VT- (n=94)	LPD+ VT+ (n=16)	Blood donors
Factor V Leiden	88 WT* (93.6%) 6 MT* (6.4%) 3 Hetero* + 3 Homo*	9 WT (64.3%) 5 MT (35.7%) All Hetero	7/90 (7.8%)
Protrombin G20210A	86 WT (91.5%) 8 MT (8.5%) All Hetero	13 WT (92.9%) 1 MT (7.1%) Hetero	6/100 (6.0%)

WT: wild type; MT: mutant; Hetero.: heterozygous; Homo.: homozygous

The relative risk for thrombosis was 5.5 concerning the FV Leiden mutation and 0.83 for the FII G20210A mutation. The prevalence of FVL and FII G20210A mutation in an unselected population of blood donors from the same area was not different from that found in the patients with LPD. In conclusion, FV Leiden appears to be an independent risk factor for venous thromboembolism in this category of patients.

P107

CO-EXISTENCE OF THROMBOPHILIC GENE POLYMORPHISMS AMONG 623 UNRELATED CONSECUTIVE PATIENTS WITH A HISTORY OF THROMBOSIS

Madonna P, Tufano A, Coppola A, De Stefano V, Varricchione N, Cirillo F, Cerbone AM, Di Minno G

Centro di Coordinamento Regionale per le Emocoagulopatie, Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Napoli "Federico II", Naples, Italy

The co-existence of predisposing factors has been associated with an abnormally high risk of thrombotic events. We screened 623 consecutive unselected and unrelated patients for factor V Leiden (FV Leiden), the G20210A mutation of the prothrombin gene (FII 20210A), and homozygosity for 677TT mutation of methylene-tetrahydrofolate reductase gene (MTHFR TT). Of the patients, 346 had a history of venous thrombosis (VT) (144 M, 202 F; first event at 41.9±15.3 years); 54 of venous and arterial thrombosis (VT+AT) (29 M, 25 F; first event at 41.4±12.8 years); and 223 of arterial thrombosis (AT) (135 M, 88 F; age at first event 40.9±13.2 years). As many as 291 healthy individuals (124 M, 167 F, mean age 37±13.2 years) served as controls. The coexistence of at least two of these polymorphisms was found in 5 (2.2%) patients with AT, in 3 (5.6%) patients with VT+AT, in 32 (9.2%) patients with VT and in 7 (2.4%) controls. A significant difference was only found when the controls were compared with VT (p=0.0006, OR 4.13, CI 1.7-10.4; χ^2 test). Among the 32 cases with VT and the co-existence of the polymorphisms, 9 (2.6%) had the association of FV+MTHFR; 9 (2.6%) cases of FV+FII and 12 (3.5%) cases of MTHFR+FII mutations. Two patients (0.6%) had coexistence of all the three polymorphisms. At variance with the other two combinations, the difference with controls was not statistically significant for the association MTHFR+FII (0.3% for FV+MTHFR, 0.3% for FV+FII; p=0.02, 2.4% for FII+MTHFR; p>0.05, χ^2 test). We conclude that, in our setting, the coexistence of genetic polymorphisms is associated with an increased risk of venous thrombosis but not of arterial thrombosis. For individual associations, the data were not statistically different from those found in controls only for the association MTHFR+F II.

P108

INHERITED PRO-THROMBOTIC CONDITIONS IN PATIENTS WITH A HISTORY OF VENOUS THROMBOEMBOLISM

Tufano A, Madonna P, Coppola A, Garofano T, Loffredo F, Cirillo F, Cerbone AM, Di Minno G

Centro di Coordinamento Regionale per le Emocoagulopatie, Clinica Medica, Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Napoli "Federico II", Naples, Italy

Factor V Leiden (FV Leiden), G20210A mutation of prothrombin gene (FII 20210A) and moderate hyperhomocysteinemia have been associated with an abnormally high risk of venous thromboembolism (VTE). The role of homozygosity for the 677TT mutation of the methylene-tetrahydrofolate reductase gene (MTHFR TT), often associated with moderate hyperhomocysteinemia, is still controversial. We evaluated the prevalence of FV Leiden, FII 20210A and MTHFR TT in 291 patients (136 M, 155 F; mean age at first event 38.6±14.9 years), with a history of VTE (deep vein thrombosis and/or pulmonary embolism) and in 291 apparently healthy matched controls (125 M, 166 F; mean age 37.2±13.3 years). The frequency of two of these polymorphisms was statistically different between VTE patients and controls, being 48/291 (16.5%) vs 17/291 (5.8%) for FV Leiden (OR 3.18, CI 1.7-5.9, p=0.00008; χ^2-test) and 40/291 (13.7%) vs 18/291 (6.2%) (OR 2.41, CI 1.3-4.5, p=0.004) for FII 20210A. The prevalence of MTHFR TT was higher in patients than in controls, being 68/291 (23.4%) and 49/291 (16.8%) respectively (p>0.05, χ^2-test). Despite the limitations of the sample size, these data argue against a role for MTHFR TT polymorphism, when analyzed alone, in venous thromboembolism.

P109

VENOUS THROMBOEMBOLISM, ORAL CONTRACEPTIVES AND HIGH PROTHROMBIN LEVELS

Legnani C, Palareti G, Boggian O, Cavallaroni K, Oca G, Lunghi B,* Bernardi F,* Coccheri S

*Unità di Ricerca Clinica sulla Trombofilia "Marino Golinelli" – Dipartimento Cardiovascolare, UO Angiologia, Azienda Ospedaliera di Bologna, Policlinico S. Orsola-Malpighi, Bologna; *Dipartimento di Biochimica e Biologia Molecolare, Università di Ferrara, Italy*

The G20210A prothrombin mutation is associated with elevated prothrombin activity levels that are a risk factor for venous thromboembolism (VTE). The G20210A mutation displays a strong interaction with oral contraceptives (OC); no data, however, are available on VTE risk of OC use in women with high prothrombin levels not carrying the G20210A mutation. In this study

we compared the prevalence of elevated prothrombin activity (chromogenic assay) in 140 women who suffered from VTE in reproductive age (30y, 14-49), to that of 286 healthy women (33y, 15-49). Subjects with other thrombophilic alterations were excluded. The interaction between elevated prothrombin levels and OC use was also investigated. Seventy-eight out of 140 patients had experienced VTE during OC; 127/286 healthy women had used OC for at least 6 months in the two years before presentation but had stopped such a treatment at least 3 months before the time of blood sampling. Prothrombin levels were stratified into quartiles. The OR in subjects with a prothrombin level higher than 1.09 IU/mL was 2.16 (95% CI: 1.18-3.98) as compared with those in the reference category (prothrombin level <0.94 IU/mL). For the interaction between OC and prothrombin levels only the two extreme strata of prothrombin were considered (> 1.09 vs ≤ 0.94 IU/mL). Women with low prothrombin (<0.94 IU/mL) who did not use OC were used as reference category. The VTE risk of using OC in the presence of low prothrombin levels was 1.17 (95% CI: 0.49-2.76). In non-OC users, the OR for high prothrombin levels was 1.57 (95% CI: 0.66-3.71). In OC users who presented prothrombin levels above 1.09 IU/mL, the OR increased 3.4-fold (95% CI:1.47-7.96). We conclude that VTE risk due to OC use is increased in women with high prothrombin levels not carrying the G20210A mutation and that these factors seem to have an additive effect.

P110

INFLUENCE OF WEATHER CHANGES IN ANNUAL VARIATIONS OF PULMONARY EMBOLISM

Ceccarelli E,* Masotti L,° Forconi S,* Cappelli R*

**Istituto di Medicina Interna e Geriatria, Università di Siena; °U. O. Medicina Interna Ospedale di Cecina, Livorno, Italy*

Background. Few data on seasonal variations in pulmonary embolism (PE) exist in literature. *Aims of the study*. To determine the yearly variations in incidence and mortality of PE and the possible relation between seasonal changes in weather and PE. *Methods*. We analyzed the yearly distribution of 457 hospitalised patients (71% medical, 29% surgical), 358 (78.3%) of them 65 years old and over, discharged from the Policlinico Le Scotte University Hospital of Siena, Italy, with a diagnosis of pulmonary embolism (415.1 code of ICD-9-CM) in the last six years (1995-2000). We evaluated four meteorological parameters: monthly mean temperature (°C), monthly mean excursion of temperature in a day (°C), monthly mean barometric pressure (mb) and monthly mean humidity (%H2O). *Results*. We noticed a higher incidence of PE in cold months from October to March with a peak in March than in warm months from April to September with a trough in August (54% vs 46%, p=ns). The seasonal distribution of the cases was: Winter 134, Spring 127, Summer 97, Autumn 109. While in medical patients we did not find significant difference in seasonal incidence (cold months 49.8%, warm months 50.2%, p=ns), in surgical patients we found a significantly higher incidence in cold months (61.5% vs 38.5%, p<0.05). 24.2% of patients with PE died. Mortality from PE was significant higher in cold months than in warm months (64% vs 36%, p<0.05). Considering the examined meteorological parameters we found an inverse correlation between monthly mean temperature and humidity and incidence and of mortality from PE, while we did not find significant relation between monthly mean temperature excursion and barometric pressure and PE. *Conclusions*. Our study could be considered a pilot study. With the limitations of the retrospective studies, our report identifies some meteorolgical parameters that could influence the incidence of and mortality from PE.

P111

HEPATIC VEIN THROMBOSIS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

Randi ML, Sartori MT, Ruzzon E, Pacquola E, Girolami A

Department of Medical and Surgical Sciencies, University of Padua Medical School, Padua, Italy

Myeloproliferative disorders (MPD) are the cause of about two thirds of hepatic venous outflow block, even before the MPD is clearly demonstrated. Over the last 10 years, we have prospectively followed (clinical and laboratory control at least twice a year) 10 cases of MPD (2 polycythemia vera PV and 8 essential thrombocythemia ET; 7 females and 3 males, mean age 28.9±14.8 years at the time of the diagnosis) with hepatic vein thrombosis (HVT). The median follow-up of our patients is 10.5 years (range 2.5-12.5). In 4 patients HVT was the presenting feature of MPD while in the remaining 6 patients HVT occurred 2.5-12.5 years after diagnosis. In all patients we have evaluated serotonin platelet content (5HT), serum thrombopoietin (TPO), plasma levels of antithrombin, protein C, protein S, plasminogen, PAI-1, lupus anticoagulant, anti-cardiolipin and anti β2-glycoprotein antibodies and genetic analysis for factor V Leiden and G20210A prothrombin mutation. All patients had low 5HT (1.1±0.9 nM/10^9plts) and normal TPO (53.7±34 pg/mL) values. No prothrombotic congenital condition was observed in 9 cases. One female with ET was homozygous for factor V. Another female with ET was heterozygous for factor V deficiency and she died from hepatic failure 10.5 years after the diagnosis. Five out of our 10 patients underwent liver transplantation (median post- transplatation follow-up = 1.89 years, range 1 month-5 years) and are now well. Six patients received hydroxyurea achieving a good control of the platelet number (lower than 600×10^9/L), 9 patients received heparin followed by low-molecular weight heparin in 3 cases and warfarin in 6. In conclusion, while the association of two prothrombotic conditions are often considered to be necessary in order to develop thrombosis, 90% of our patients had an isolated MPD; the correct management of patients with MPD and HVT is still poorly defined.

P111a

FACTOR IX ACTIVATION PEPTIDE AND SOLUBLE SELECTINS IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISORDERS

Randi ML, Cella G, Luzzatto G, Bauer KA,° Rosenberg RD*

*University of Padova, Medical School, Padova, Italy, °West Roxbury VA Hospital, Boston, Mass and *Massachusetts Institute of Technology, Cambridge, Mass, USA*

Essential thrombocythemia (ET) and polycythemia vera (PV) are chronic myeloproliferative disorders (CMD) characterized by a high incidence of thromboembolic complications. Increased markers of platelet activation and of activated coagulation system have been reported in patients affected by such disorders. We evaluated factor IX activation peptide (FIXP) and soluble

adhesion molecules of the selectin class (endothelial sES, leukocyte sLS and platelet sPS) in 31 healthy volunteers and in 26 patients with CMD and high platelet count (platelets 818.9±226, range 610-1,610×10⁹/L). Nineteen were affected by ET (mean age 53.73, range 22-87 years) and 7 by PV (mean age 69.28, range 51-84 years). All patients were in stable condition, none of them had thrombotic complications within one year before the study and during a two years follow-up. Patients and controls were divided in 3 groups of age: up to 44 years, from 45 to 59 years and from 60 to 87 years. In both patients and controls, FIXP plasma levels increased with the older age, although not significantly. No significant difference between patients and controls was noted. Moreover, no significant difference was observed in sES and sLS. In contrast, sPS was significantly higher in CMD than in controls. However, the concentration of sPS per platelet was significantly reduced in CMD as compared with controls (CMD 0.28±0.11 fg/platelet, controls 0.59±0. 24 fg/platelet $p < 0.0001$). In conclusion, we did not find a hypercoagulable state nor a leukocyte and platelet activation in these patients using FIXP, sLS and sPS as markers. No systemic dysfunction/damage of endothelial cells seems to be present in our patients with normal level of sES.

P112

SEVERE INCREASE OF PROTHROMBIN FRAGMENT 1+2 DURING PERITONEAL METASTASIS. A CASE REPORT

Di Micco P,* Niglio A,* Federico A,° Romano M,° De Sio I,° Torella R*

**V Divisione Medicina Interna, Dipartimento di Geriatria, Gerontologia e Malattie del Metabolismo; °Divisione di Gastroenterologia, Seconda Università di Napoli, Italy*

An acquired thrombophilia is present during oncological disease. The mechanism whereby cancer induces activation of hemostasis is still not completely understood, but many pathways are involved. We describe a case of a 58-year old man admitted to our Institute with ascites and alteration of hemostasis. Physical examination showed hepatomegaly and splenomegaly, but laboratory findings did not reveal chronic infection with B and/or C virus hepatitis. Oncologic markers revealed increase in gastrointestinal cancer antigen (GICA, 93U/mL) and carcinoembryonal antigen (CEA, 72U/mL). Moreover, ultrasound examination showed septated ascites of neoplastic nature due to peritoneal metastasis; CT scan examination confirmed the presence of peritoneal metastasis from pancreatic tumor with multiple abdominal lymph nodes swelling. Finally, cytology on ascitic fluid confirmed the presence of malignant cells of neuroendocrine origin. Chemotherapy was administered. In order to evaluate the acquired hemostasis alterations of the patient affected by peritoneal metastasis, we determined PT (1.14,INR), aPTT (1.01, ratio), fibrinogen (414mg/dl), protein C (77%), protein S antigen (81%), ATIII (72%), APC resistance (0.93, Bertina method), prothrombin fragment 1+2 (F1+2) (16.23 nM), D-dimer (4.2 ng/dL). The acquired hemostasis alterations were looked for because of the high incidence of deep venous thromboembolism or other thrombotic complications such as DIC in oncological patients, specially affected by pancreatic tumor. In particular, microthrombi are often founded in patients affected by peritoneal metastasis during post-mortem examination. A thrombotic event, in fact, seems to be related to bad prognosis of any cancer. Moreover, the 15-fold increase in F1+2 plasma levels testifies the marked increase in thrombin generation and ATIII reduced plasma levels suggests its consumption to counteract the thrombin generation. Furthermore, the increase in F1+2 could be related to the cancer procoagulants, as heparanase production reducing prothrombinase (f.Xa) inactivation by ATIII and heparan sulphate, or ectopic factor X localization in pancreatic tissue. However in this case the hypercoagulable state was treated by prophylactic use of low molecular weight heparin (reviparin 4200 UI/die) because of its selected inhibition of prothrombinase (f.Xa).

P113

PROPHYLAXIS OF DEEP VENOUS THROMBOSIS AND PULMONARY EMBOLISM IN THE SURGICAL DEPARTMENT OF "A.S.L.4 CHIAVARESE" (LIGURIA REGION, ITALY)

Lo Pinto G, Fiorini B, Giordano C, Prencipe E, Palareti G*

*Medical and Surgical Dept. ASL 4 "Chiavarese", Liguria Region and *Angiology Policlinico S.Orsola-Malpighi, Bologna, Italy*

Geerts *et al.*[1] suggest that each hospital should write down an evaluation proceeding of both thromboembolic risk and prophylaxis specially in high risk patients. First, we have written and delivered to the directors of the surgical divisions (surgery, orthopedics, day-surgery, gynecology, otorhinolaryngology, urology and critical care unit) a list of questions which enables evaluation of the importance of this problem in daily activity. Second, we had a meeting with all physicians and nurses involved, in which we showed the most up-to-date clinical trials according to *Evidence Based Medicine*. Third, we had a meeting with each surgical division in order to write down the very schedule by using a basic scheme drawn out by Policlinico S. Orsola-Malpighi of Bologna. The schedule allows calculation of risk score for each patient to be operated on the basis of personal risk (history of venous thromboembolism, thrombophilia, cancer, recent chemotherapy or radiotherapy, estroprogestinic drugs, age, obesity, severe venous varices, atherosclerosis, sepsis, impaired walking), and surgical risk. The score divides the patients into three risk classes (low, medium and high). The necessary prophylaxis (none, elastic stockings, low molecular weight heparin) is applied after the evaluation of the bleeding risk and the anesthesia method (especially spinal). Nowaday the schedule is used in each of our surgical divisions. Another important goal of this work is to validate our risk score.

References

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P114

THROMBOTIC PATHOLOGY: FROM THE LABORATORY TO CLINICAL ASSESSMENT OF THE RISK FACTORS. OUR EXPERIENCE

Stipa E,* Meo P,* Oliva F,* Olivieri M,* Forte V,° Amadori S°

**S. Eugenio Hospital, Rome; °Hematology Chair and Department, Tor Vergata University, Rome, Italy*

Over a six-month period we saw 300 patients in our department (99 males and 201 females with a median age 44); 180 patients had arterial and/or venous thrombosis while the remain-

ing 120 patients appeared to be healthy and homogeneous with respect to their demographic traits. Sixty percent of the thrombosis cases involved the central nervous system and the retina, 35% the peripheral vessels, 12% the heart, while 7% were multi-site thromboses. A case-control study was carried out in order to assess the significance of the association of some hereditary thrombophilic factors (i.e. factor V Leiden, prothrombin G20210A) with the thrombotic condition. The association of the concomitant presence of acquired risk factors (birth control pill, diabetes, pregnancy, hypertension, dyslipidemia, cancer), hereditary risk factors and thrombotic condition was also assessed. The results obtained were as follows:

Factor V Leiden	Acquired factor	OR
+	–	1.2
+	+	4.1

Prothrombin G20210A	Acquired factor	OR
+	–	1.1
+	+	3.2

The odds ratio (OR) for the genetic risk factor alone is not significantly deviated from unity. This fact seems to confirm the multifactorial origin of the thrombotic event and of the acquired risk factor importance. Factor V Leiden was found in 18% of patients with venous thromboembolism while prothrombin G20210A was found in 21%. The association between these mutations and arterial thrombosis was weaker (8% and 10% respectively). The association between APC-R (present in 14% of the patients) and FVL (present in 84%) was confirmed. 38% of patients presented with hyper-homocysteinemia, 70% of which were associated with thrombosis. Only 27% of patients with hyper-homocysteinemia were homozygote carriers of MTHFR polymorphism. In addition, 38% of patients showed marked platelet hyper-aggregability, confirmed over time. The percentage of patients with a family history of thrombotic events was significant (63%); only 24% of patients were dyslipidemic and 36% tested positive for antiphospholipid antibodies. In conclusion the epidemiology of hereditary thrombophilia points out the multiple causes of the thrombotic event and that pharmacologic prophylaxis may not, therefore, be appropriate for patients who are carriers of the thrombogenic defect, unless they are exposed to increased risk situations.

P115

A NET-SUPPORTED SCORING SYSTEM TO ESTIMATE THROMBOTIC RISK AND TO ADDRESS A TARGETED PREVENTION

Minozzi M

Centro Trasfusionale, Azienda Ospedaliera di Merano, Italy

Thrombotic events experienced by some individuals do not always correlate with the risk value generally expected from the association with the abnormalities discovered and assessed during the last decade. Fast identification and correct stratification of the cumulative effect of these associations could favor a targeted and more successful prophylactic strategy. *Study design*. We checked the cumulative probability of recurrent venous thromboembolism in all the patients coming to our hospital with a first episode of DVT, through the compilation of an electronic data sheet. The patients recruited in the study and monitored for a period of 5 years received differentiated lifestyle recommendations according to their score number, (based on *PAR* criteria). Our aim was to create a statistically oriented database to reproduce a multicenter, pragmatic, population-oriented clinical trial, which had to start on the following main feature: 1. shift from the medical logic, based on the treatment of single risk factors, to one centred on the patient's global risk condition, 2. statistical orientation and reproducibility; 3. visibility of the patient's risk status to all the connected and not connected sites and situations which can contribute to predict the risk (hospital departments, specialist, family doctors); 4. alarms for clinical-laboratory and instrumental controls; 5. a system for alerting about newly recognized or varied/increased risk conditions; 6. integration with the software for management of anticoagulation therapy; 7. print-out of the above mentioned data on an *individual risk passport*. *Conclusions*. We aim to provide an individual evidence-based guide for a prevention policy for a large number of non-selected patients by a large number of medical figures and structures (data visibility). These data will be also used to quantify the maximum benefit derived from modified risk factors.

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PREVALENCE OF THROMBOPHILIC GENOTYPES IN PATIENTS WITH DEFICIENCY OF NATURAL ANTICOAGULANTS. A STUDY OF 34 CASES

Brancaccio V, Ames PRJ, Iannaccone L, Guardascione MA,* Scenna G, Margaglione M

*Unità Emostasi-Trombosi e *Divisione Gastroenterologia, Ospedale "A. Cardarelli", Napoli; °Unità Aterosclerosi e Trombosi, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy*

Background. Overall the deficiencies of antithrombin (AT), protein C (PC) and protein S (PS) account for only 10% of causes of genetic thrombophilia. The identification of novel defects - factor V Leiden (FVL), the mutation A20210 of prothrombin gene (A20210/FII) and the genotype TT677 of methylene tetrahydrofolate reductase (TT677MTHFR) with its associated hyperhomocysteinemia – broadened the field of genetic thrombophilia. The association of AT, PC and PS defects and FVL, A20210/FII and TT677MTHFR genotypes could further increase the thrombotic risk. *Methods*. Thirty-four patients (M/F 24/10; median age 39 years, range 15-64), 16 with PS deficiency, 13 with PC deficiency and 5 with AT deficiency were studied for FVL, A20210/FII and TT677MTHFR genotypes. All patients (but one with ishemic stroke) have had previous episodes of deep vein thrombosis (DVT) and/or pulmonary embolism. *ResuIts*. Ten patients (29%) had TT677MTHFR genotype (1 AT, 5 PS and 4 PC deficiency). Seven of these patients had high plasma levels of homocysteine; 2 (6%) had FVL (both with PS deficiency); 2 (6%) had A20210/FII mutation (1 PS and 1 PC deficiency). Among patients with PS deficiency and associated defects: 1 (with both FVL and TT677MTHFR) had onset of DVTs as a child; 2 had relapsing DVT starting at 18 and 24 years and 1 more had a bilateral DVT with cava occlusion after an appendectomy. Among PC deficiency and associated thrombophilic genotypes group two patients had a portomesenteric thrombosis and a DVT at 27 years followed by myocardial infarction at 36 yrs, respectively. *Conclusions*. Genetic thrombophilic genotypes are associated in 44% and 38% of our patients with PS and PC deficiency. Thrombotic patients with PS deficiency and associated thrombophilic genotypes had a severe clinical course.

P117

ABDOMINAL COLOR-DOPPLER ULTRASOUND FINDINGS IN A PATIENT WITH THE BUDD-CHIARI SYNDROME ASSOCIATED WITH MYELOPROLIFERATIVE DISEASE, ANTITHROMBIN III DEFICIENCY AND ANTIPHOSPHOLIPID ANTIBODIES

Carnevale Maffè G, Invernizzi R, Ruga A, Oriani E, Gnocchi M, Nava A, Armellini E, Ascari E

Medicina Interna e Oncologia Medica, IRCCS Policlinico San Matteo, Pavia, Italy

The authors describe the sonographic (US) findings of a case of Budd-Chiari syndrome (BCS), occurring in a 44-year old woman, diagnosed one year earlier with polycythemia vera. On the admission there was jaundice, increase of the abdomen volume due to ascites, hepato-splenomegaly and visible cutaneous varices on the anterior abdomen wall. There was laboratory evidence of polyglobulia and hyperbilirubinemia with hepatic function tests within mean values. Lupus-type anticoagulants and low values of antithrombin III (43%) were found. A US scan of the abdomen showed a large liver with inhomogeneous pattern. Hepatic veins were not visible; the diagnosis of thrombosis of all hepatic veins was suspected. The portal vein was enlarged with the lumen of the right branch partially filled with anechoic thrombus, which at the color-Doppler study showed no signal; color-Doppler signal was present in the other part of the vessel. The color-Doppler findings at the study of the portal vein and its branches were consistent with portal hypertension and showed evidence of slow anterograde flow, with low velocity (15 cm/sec). There was splenomegaly (16.5 cm), with non-homogeneous echo-pattern as a consequence of multiple infarctions of the spleen; ascites was confirmed. Such findings were confirmed on the CT scan of the abdomen, therefore the patient was treated with transjugular intrahepatic portosystemic shunt (TIPS) and oral anticoagulants and ATIII iv. The US color-Doppler findings here described are typical of BCS, with the peculiar association of both the main causes of hepatic vein thrombosis (myeloproliferative disease and LAC syndrome).

P118

ACQUIRED AND INHERITED PREDISPOSING CONDITIONS IN PATIENTS WITH RETINAL VEIN OCCLUSION

Albisinni R, Cirillo F, Loffredo M,* Coppola A, Varricchione N, Greco GM,* Cerbone AM, Di Minno G

*Centro di Coordinamento Regionale per le Emocoagulopatie, Dip. di Medicina Clinica e Sperimentale and *Dipartimento di Scienze Oftalmologiche, Università "Federico II", Naples, Italy*

We have screened 86 consecutive patients (51 men, 35 women, mean age 56±11.2 years, mean age at first event 52.4±12.5 referred to our Center for a history of recent retinal vein occlusion (RVO). Fluorangiography showed that 38 patients (20 men, 18 women, mean age 53.8±13.7, age at first event 49.3±15.1) had experienced central retinal vein occlusion (CRVO); 43 (27 men, 16 women, mean age 57.86±8.4, age at first event 54.6±9.8), had branch retinal vein occlusion (BRVO), and 5 (4 men, 1 woman, mean age 61.2±10.1, age at first event 57.2±8.1) had both events in different eyes. In this setting, the prevalence of arterial hypertension was 69.5%, cigarette smoking 53.9%, diabetes mellitus 25.5%, obesity in 82.5%. All these conditions were significantly more prevalent in RVO patients than in a population of 90 control individuals, comparable for sex, age and genetic background and without history of thrombotic events. The prevalence of acquired conditions did not show any statistically difference when CRVO and BRVO patients were compared to each other; however, hypertension showed a trend towards a statistically higher prevalence in BRVO group. Both in patients and in controls, no antithrombin, protein C or portein S deficiency, nor lupus anticoagulant positivity was detected. As to prevalence of gene polymorphisms associated with venous thrombosis, factor V Leiden mutation was found in 7% of RVO patients, the prothrombin G20210A mutation in 4.7%, and the 5,10 methylenetetrahydrofolate reductase homozygous thermolabile variant in 17.4%, without statistically significant differences when compared to the control group. Moreover, mutation frequency was comparable in CRVO and BRVO patients. Our data suggest that, at variance with venous thrombosis in other sites, inherited thrombophilia is not involved in RVO, whereas acquired predisposing conditions to arterial thrombosis are maximally present. These data provide a rationale for the use of antiplatelet drugs in this setting.

P119

UTILITY OF A PRE-TEST PROBABILITY SCORE IN THE DIAGNOSIS OF PULMONARY EMBOLISM

Porro F, Schinco G, Graziadei G, Cappelletti M,* Tarsia P, Ceraldi T, Guariglia A

*Emergency Medicine Department, and * Radiology Department, Ospedale Maggiore di Milan, IRCCS, Milan, Italy*

A simple and objective clinical probability score for pulmonary embolism (PE) is needed in the emergency ward. We retrospectively applied a fast pre-test probability score, recently proposed by Wicki *et al.* (*Arch Intern Med, 2001*), to 70 consecutive patients (34 males, mean age 69.4±14 years) admitted to our emergency ward between November 2000 and June 2001 with symptoms suggestive of PE. All patients underwent physical examination, blood gas analysis, chest X-ray, ECG, and spiral multislice highspeed CT angiography. PE was confirmed by CT scan in 41/70 patients (58.5%). On the basis of the clinical probability score the 70 patients were distributed as follows: 23 to the low probability group, 36 patients to the intermediate probability group, and 11 to the high probability group.

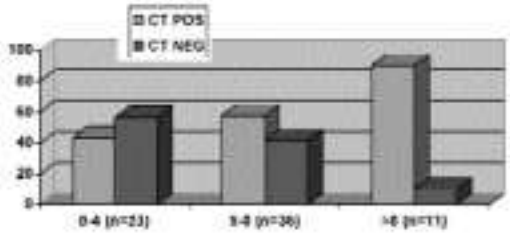

Figure 1. Distribution of CT confirmed pulmonary embolism according to clinical probability score group. 0-4 = low probability; 5-8 = intermediate probability; > 8 = high probability.

Probability group analysis showed a high degree of agreement between clinical score and CT response in the high probability group (positive predictive value = 90%) but a much lower degree of agreement in the low probability group (negative predictive value = 57%) (Figure 1).

P120

"CATASTROPHIC" ANTIPHOSPHOLIPID SYNDROME ASSOCIATED WITH TYPE II HEPARIN-INDUCED THROMBOCYTOPENIA: REPORT OF A CASE AND OUTCOME

Tamponi G, Schinco PC, Borchiellini A, Pollio B

Department of Onco-Hematology, University of Turin, Molinette Hospital, Turin, Italy

A 68-year old patient, admitted to hospital for post-traumatic multiple skeletal fractures, underwent prophylactic antithrombotic administration of subcutaneous UF heparin. Two days later thrombotic occlusion of the left humeral artery occurred, which was disobstructed with a Fogarty catheter under UF heparin continuous administration. Forty-eight hours later thrombotic reocclusion of the same artery was detected: heparin dosage was increased, but two days later thrombocytopenia developed and thrombotic occlusion of the left popliteal artery was detected. Type II HIT was diagnosed and heparin immediately withdrawn. Hirudin, Refludan™ (lepirudin [rDNA] for injection) (continuous infusion), was started at recommended dosage and seven days later OAT was started; when INR reached 2.5, hirudin was stopped. Immediately after withdrawal, a new humeral arterial occlusion occurred, which was disobstructed once more. Hirudin treatment was resumed and warfarin administered for 7 days until INR was equal to 3.5; hirudin was stopped again and OAT continued. INR was aimed around 3.5. The remaining clinical follow up of the patient was uneventful; she is alive and well and still on OAT. Serologic tests performed during illness showed heterozygous prothrombin G20210A mutation, high positivity of anti α2 glycoprotein Ib Ab (IgM type) and strong LA positivity. LA positivity and high titer antiphospholipid antibody positivity persists one year after onset. No underlying disease has so far been discovered which could justify the catastrophic clinical course and/or serological findings of this patient.

P121

HOMOCYSTEINE PLASMA LEVELS IN PATIENTS WITH MTHFR C677T MUTATION

Capezzera M, Bertolino G,*Rizzo V, Gamba A, Noris P, Gamba G

*Departments of Internal Medicine and *Biochemistry, University of Pavia, IRCCS Policlinico San Matteo, Pavia, Italy*

MTHFR C677T mutation affects the remethylation pathways of homocysteine (HCY) metabolism, and can contribute to the enhancement of HCY plasma levels. On the other hand, HyperHCY has been associated with arterial and venous thrombotic diseases. Relationships between MTHFR C677T mutation, HyperHCY and thromboses are controversial. The aim of this study was to evaluate HCY plasma levels in relation to the MTHFR C677T mutation and the response to the treatment with folic acid supplemented by vitamins B6 and B12 in HyperHYC patients *Patients and Methods*. In 95 patients aged between 19 and 79 yrs HCY levels and MTHFR C677T mutation were detected. 57 patients (60%) suffered from thromboembolic diseases. *ResuIts*. MTHFR C677T mutation was observed in 69 patients (73%) (19 homozygous and 50 heterozygous mutation). Values of HCY>15 microM/L were detected in 38 patients (40%), of whom 9 patients were negative for MTHFR C677T. The mean values of HCY were higher in homozygous MTHFR C677T patients, although without significant differences in comparison to heterozygous and negative patients (HCY μM/L mean: homo 27.62; Hetero 14.88; negative 17.81). Furthermore among HyperHCY patients homozygous mutation was associated with higher but not significant enhancement of HCY (HCY μM/L mean: homo 41.39; hetero 24.37; negative 29.03). 21 out of the 29 patients (70%) with HyperHYC and thromboses showed MTHFR C677T mutation. The treatment of HyperHCY with folic acid, vitamins B6 and B12 was generally able to normalize the HCY levels in heterozygous MTHFR C677T patients and in negative patients and to lower the levels in homozygous MTHFR C677T patients. *Conclusions*. The research for MTHFR C677T mutation do not add useful information over the HCY plasma level detection for the correction of HyperHCY in patient at risk of thrombosis.

P122

GENETIC MARKERS OF THROMBOPHILIA IN PATIENTS WITH THROMBOSIS OR FAMILIAL HISTORY OF THROMBOSIS

Gamba G, Capezzera M, Gamba A, Montani N, Soldavini E, Noris P, Balduini CL

Department of Internal Medicine, University of Pavia, IRCCS Policlinico San Matteo, Pavia, Italy

Mutations of FV (G1691A), FII (G20210A) and MTHFR (C677T) are reported to be associated with enhanced risk of thrombosis. The aim of this study was to detect FV G1691A, F II G20210A and MTHFR C677C mutations in selected patients with personal and/or familial history of thromboses. Seventy-three patients (44 females and 29 males) aged between 19 and 85 years were enrolled within four months. 46 suffered for DVT, 14 presented arterial thromboses (AT), 7 patients had both venous and arterial thromboses, 3 patients presented recurrent abortions and 3 patients were at thrombotic risk because of strong familial history of thromboses. Only in 16 patients (22%) was no mutation observed, although in 8 patients other markers of thrombophilia were detected: antiphospholipid antibodies in 2 patients, high HCY levels in 5 patients and PC deficiency in one patient. In the other 47 patients mutations were present as shown in the table below.

	MTHFR C677T			II G20210A			FV G1691A		
Patients (no.)	hetero	homo	tot%	hetero	homo	tot%	hetero	homo	Tot%
Total (73)	37	15	70	6	0	8	15	0	20
AT° (14)	6	4	71	2	0	14	1	0	7
DVT* (46)	25	8	72	3	0	6	12	0	26
Both (7)	2	3	71	0	0	0	0	0	0
Abortion^ (3)	2	0	56	1	0	33	2	0	66
Familial history	24	9	64	3	0	50	8	0	53

°1 MTHFR+FII and 1 MTHFR+FV+ FII mutations; *7 MTHFR+FV, 2 MTHFR+FII and 1 MTHFR+FII+FV mutations; ^ 1 MTHFR+FII and 1MTHFR+FV mutations.

Conclusions. The prevalence of the mutations studied in our selected patients was higher than those observed in general population and it could enhance the recurrences of thromboses in clinical conditions defined at thrombotic risk. Therefore the search for the above mentioned mutations in these patients is indicated for planning prophylaxis and treatment of the thrombotic events.

P123

HOMOCYSTEINEMIA AND THERMOLABILE METHYLENE TETRAHYDROFOLATE REDUCTASE VARIANT IN CANCER PATIENTS

Battistelli S,* Stefanoni M,* De Stefano A,° Sabatini L,# Fineschi D,# Lorenzi M*

**Istituto di Chirurgia Generale, °U.O.Chirurgia Oncologica, #U.O. Ematologia e Coagulazione, Università di Siena, Italy*

Cancer patients have an increased risk of venous thromboembolism. The mechanisms involved in cancer thrombogenesis are not entirely understood but an important role is played by acquired thrombophilia. Little is known about the relation between hypercoagulability of neoplastic disease and the common risk factors associated with venous thromboembolism such as hyperhomocysteinemia and inherited alterations of its metabolism. *Purpose*. To investigate in cancer patients plasma homocysteine levels and their relation with thermolabile methylene tetrahydrofolate reductase variant (C677T MTHFR polymorphism). *Methods*. Fifty-seven consecutive patients affected by gastrointestinal or pelvic primary solid neoplasms (mean age 69 years) and 56 healthy controls were enrolled in the study. Plasma homocysteine levels were measured by a fluorescence polarization immunoassay (FPIA). C677T MTHFR polymorphism was determined by polymerase chain reaction and restriction analysis. *Results*. In cancer patients plasma homocysteine levels (μm/L) were 11.1±3.72 in CC genotype (31.57%), 13.5±5.48 in CT genotype (43.65%) and 18.3±10.6 in TT genotype (24.56%). In control subjects homocysteinaemia (μm/L) was 9.7±2.69 in CC genotype (21.44%), 11.1±4.52 in CT genotype (48.21%) and 15±9.80 in TT genotype (30.35%). Plasma homocysteine levels were statistically different between CC and TT genotype both in cancer patients (p=0.028) and in control group (p=0.038), while they were not statistically different between the same genotypes of the two groups. *Conclusions*. In our study homocysteinaemia is significantly higher in TT genotype than in CC genotype both in cancer patients and in the control group and it is not different between the same genotypes of the two groups. The study suggests that in cancer patients thermolabile methylene tetrahydrofolate reductase variant contributes to hyperhomocysteinemia but its relation with venous thromboembolism has to be established.

P124

HEPARINS COUNTERACT ENDOTHELIAL CELL TISSUE FACTOR EXPRESSION INDUCED BY INTERLEUKIN-1 β

Marchetti M, Suardi S, Vignoli A, Barbui T, Falanga A

Hematology Division, Ospedali Riuniti, Bergamo, Italy

The mechanisms of anticoagulant actions of heparins in blood have been extensively studied: however, their effects on the hemostatic properties of the endothelium are still under investigation. In this study we evaluated the activity of two LMWH (i.e.: dalteparin, DLT, and enoxaparin, ENX) and unfractionated heparin (UFH) on tissue factor (TF) expression by two types of endothelial cells (EC): the micro-vascular cell line, HMEC-1, and macro-vascular EC, HUVEC. EC were incubated with IL-1β (100 IU/mL)±heparins (0.01-10 IU/mL) or the vehicle (control cells). After 4h, TF expression was evaluated both as activity (TF:Act) by the one-stage clotting assay and as antigen (TF:Ag) by ELISA. The results show that, in HMEC-1, the three heparins dose-dipendently counteracted the IL-1β-induced expression of TF:Act (% reduction, 10 IU/mL: ENX=53±5; DLT=43±4; UFH=37±3; p<0.05). ENX tended to be more effective than DLT and UFH on decreasing IL-1β-induced TF:Act, without reaching statistical significance. These results were confirmed by TF:Ag analysis (% reduction, 10 IU/mL: ENX=52±5; DLT=43±4; UFH=33±3; p<0.05). Similarly in HUVEC, all three heparins significantly inhibited the IL-1β-induced TF expression, both as activity (% reduction, 10 IU/mL: ENX=23±2, DLT=24±2, UFH=44±4) and antigen (% reduction, 10 IU/mL: ENX=20±2, DLT=41±4, UFH=63±5). However, UFH was significantly more effective (p<0.05) than the two LMWH in counteracting the IL-1β action on TF of HUVEC. In conclusion, ENX, DLT, and UFH are all able to significantly inhibit the IL-1β procoagulant stimulus on EC, but differences exist between the heparin types depending on the different origins of EC.

P125

CASE REPORT: IS ALWAYS COAGULATION STUDY SUFFICIENT TO SCREENING THE YOUNG PATIENTS WITH DVT?

Ruocco L,* Pellegrini G,* Pavia T,* Bianchi M,** Giannini D, Castiglioni M,** Balbarini A, Iofaro A*

***U.O. Angiologia Universitaria,*U.O Laboratorio Analisi Cliniche, U.O. Medicina Interna, Azienda Ospedaliera Pisana, Ospedale Cisanello, Pisa, Italy*

The congenital anomalies of the inferior vena cava (IVC) are always underestimated by ultrasound-scan and commonly reported as a fortuitous finding in 3-5% of young patients with DVT. The case of an 18-year old man with an idiopathic femoral-popliteal DVT was diagnosed by colorDoppler ultrasonography that revealed the presence of a thrombus above the femoral ligament. MNR scan angiography with intravenous contrast showed an iliac vein thrombosis and a congenital anomaly (hypoplasia) of the inferior vena cava with a dilation of the venus azygous system. Coagulation studies for predisposing factors to thrombophilia (antithrombin, protein C, S, plasminogen, thrombin time, fibrinogen, lupus anticoagulant, antiphospholipid antibodies, homocysteinemia, factor V Leiden) showed a qualitative defeciency of antithrombin III. The patient with DVT was given subcutaneus fractioned heparin followed by warfarin treatment. No DVT recurrences were recorded in the subsequent 6 months. The same methodology was applied to study of the others members of the family for a total of 5 patients: the presence of hypoplasia of the IVC with partial supplence of the azygous system was observed, by RMN-scan angiography, in one of the patients (sister, 16 years old). Coagulation studies revealed a deficit in antithrombin III in all the members of the family. None of the patients had acquired risk factors except the sister who presented the combination of hypoplasia of the IVC (HIVC) with a deficiency in AT III and the use of an oral contraceptive with-

out evidence of previous or present deep vein thrombosis. The present report suggests that the presence of HIVC is an important predisposing genetic factor that should be taken into account in young patients with DVT. The presence of clotting defects predisposing to thrombophilia could be associated with a congenital malformation of the abdominal veins. This association could be due to common genetic polymorphism. Gender seems to play an important role in the time of appearance of the initial thromboembolic manifestation.

P126

SUCCESSFUL PREGNANCIES IN A WOMAN WITH HOMOZYGOUS FACTOR V LEIDEN AND HETEROZYGOUS G20210 MUTATION, ANTI-RO/SSA/SSB ANTIBODIES AND COMPLETE ATRIO-VENTRICULAR HEART BLOCK

Brambilla G, Brucato A, Castellino G, Mostarda G,* Pisoni MP,^ Canesi B, Redaelli R,* Morra E,* Baudo F*

*Departments of Rheumatology, ^Obstetrics, *Hematology-Thrombosis Hemostasis Unit, Niguarda Hospital, Milan, Italy*

A 24-year old woman with a positive family history for diabetes, hypothyroidism, arterial and venous thrombosis was referred to our department in November 1994 because of polyarthralgia, photosensitivity, Raynaud's phenomenon, non-scarring alopecia, and xerophthalmia. She had been taking the oral contraceptive pill for 3 years. *Laboratory tests*: rheumatoid factor 63 U/mL, Waaler-Rose reaction 1:320, antinuclear antibodies titer 1:1280 speckled, anti-Ro/SSA/SSB antibodies 168 U, low FT3 with high TSH and positive anti-thyroglobulin antibodies. A diagnosis of undifferentiated connective tissue disease and hypothyroidism was made and treatment with deflazacort (6 mg/daily), hydroxychloroquine (200 mg/daily) and thyroxin (100 μg/daily) was started. During the following 2 years she was in good health notwithstanding deflazacort tapering until 3 mg/day. In 1995 and 1997 she had two pregnancies. As prophylaxis for pregnancies she received fluocortolone (10 mg/5 days/week) + acetylsalicylic acid (100 mg/day) and folic acid with successful deliveries and full-term babies. On September 1999 she experienced a syncopal episode; electrocardiogram (ECG) was diagnostic for complete atrio-ventricular heart block (CAVHB). A pace-maker was implanted and she was discharged with prednisone 50 mg/day. She was in a good health until August 2000 when she was referred to our department again for a superficial venous thrombosis of the left leg. Thrombophilic screening tests: absence of lupus anticoagulant and antiphospholipid antibodies, presence of homozygous factor V Leiden and heterozygous factor II G20210A mutations. Three months later a routine ECG showed QT interval prolongation. Patient is now well on therapy with prednisone (5 mg/daily), acetylsalicylic acid (100 mg/daily) and folic acid. In the babies ECG was normal and anti-Ro/SSA/SSB antibodies negative. *Conclusions*. In pregnancy the presence of inherited thrombophilic factors, particularly in homozygous form, is a significant risk factor for venous thromboembolism and for miscarriages and might require anticoagulant prophylaxis. Our patient delivered two alive babies without anticoagulant therapy and with an excellent maternal and fetal outcome.

P127

HIGH PREVALENCE OF A20210 MUTATION OF PROTHROMBIN IN PATIENTS WITH LIVER CIRRHOSIS AND NON-NEOPLASTIC PORTAL VEIN THROMBOSIS

Brancaccio V, Guardascione MA,* Iannaccone L, Margaglione M,° Amitrano L,* Ames PRJ, Balzano A*

*Unità Emostasi-Trombosi e *Divisione Gastroenterologia, Ospedale "A. Cardarelli", Napoli; °Unità Aterosclerosi e Trombosi, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy*

Background. Non-neoplastic portal vein thrombosis (PVT) is rare in patients with liver cirrhosis. Its prevalence ranges from 8% and 16% in different series and local and systemic thrombophilic factors have been implicated in the pathogenesis. *Patients and Methods*. We studied 53 patients with non-neoplastic PVT (M/F 33/20; mean age 58±10.2 yrs). The etiology of cirrhosis was: HCV 41.5%, HBV 11.3%, alcohol 17%, cryptogenic 5.1%, other 15%. Six patients had Child-Pugh score A, 28 B and 19 C. Patients with previous porto-systemic shunts, TIPS or splenectomy were excluded. PVT was diagnosed by Doppler ultrasound and confirmed by CT scan. The patients were tested for: antithrombin, protein C, Protein S deficiency, activated protein C resistance, lupus anticoagulant and anticardiolipin antibodies; factor V Leiden (FVL), prothrombin A20210 and TT677 genotype of MTHFR. *Results*. Acquired thrombophilic factors: abdominal surgery 15 (28.3%) patients; pancreatitis 1 (1.8%); previous sclerotherapy 18 (33.9%). Congenital thrombophilic factors: FVL 6 (11.3%)patients; prothrombin A20210 14 (26.4%); TT677/MTHFR 16 (30%). *Conclusions*. Most patients with PVT and liver cirrhosis have acquired and genetic thrombophilic risk factors. Among the latter the mutation A20210 of prothrombin gene is highly prevalent.

P128

THROMBOPHILIC FACTORS IN CENTRAL RETINAL VEIN THROMBOSIS. A STUDY OF 66 CASES

Brancaccio V, Scenna G, Margaglione L, Ames PRJ*

*Unità Emostasi e Trombosi, Ospedale " A. Cardarelli", Naples; * Unità Aterosclerosi e Trombosi, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy*

Background. Central retinal vein thrombosis (CRVT) is a rare event whose pathogenetic mechanisms are not well understood. The role of known thrombophilic factors and therapeutic strategies of this severe disease often affecting young people with invalidating consequences need to be further clarified. *Patients and Methods*. We studied 66 patients with CRVT (M/F 36/30; median age 49 years, range 16-80); in 6 patients CRVT was relapsing. Thirteen patients (20%) were smokers, 19 (29%) hypertensive, 7 (11%) hyperlipidemic, two patients (3%) had ischemic cardiac disease and 2 (3%) diffuse atherosclerosis. Three patients (10%) of the females were on oral contraception. Eleven patients (17%) had 2 or more risk factors. Three patients had had a previous deep vein thrombosis. All patients were tested for: antithrombin, protein C, protein S deficiency, activated protein C resistance, lupus anticoagulant (LA) and anticardiolipin antibodies (aCL); factor V Leiden (FVL), prothrombin (FII) A20210, TT677/MTHFR mutations.

Results. No defects of natural anticoagulants or LA/aCL positivity were found. Three patients (5%) had activated protein C resistance and FVL heterozygous (not significant vs control group of 431 healthy subjects); A20210/FII was present in 7 patients (5 <50 years and 1 with relapsing CRVT)(11%) and 20 controls (4.7%,NS); TT677/MTHFR. *Conclusions*. A20210/FII genotype is associated with CRVT in patients <50 years. Acquired thrombophilic risk factors have a more relevant role than genetic thrombophilia in CVRT.

P129

HIGH PREVALENCE OF GENETIC THROMBOPHILIA IN DEEP VEIN THROMBOSIS FOLLOWING ARTHROSCOPIC KNEE SURGERY

Brancaccio V, Iannaccone L, Scenna G, Ames PRJ, Margaglione M*

*Unità Emostasi-Trombosi, Ospedale "A. Cardarelli", Napoli; *Unità Aterosclerosi e Trombosi, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy*

Venous thromboembolism is a frequent complication of orthopedic surgery performed without adequate thromboprophylaxis. Arthroscopic knee surgery (AKS) requires the preliminary application of a tourniquet to limit blood flow to the operating area. It is generally felt that this procedure is associated with a low risk of postoperative thromboembolism. We were referred 15 patients (10M, 5F, median age 33 years, age 21-61) for investigation of deep vein thrombosis of the leg (proximal in 12 cases) and/or pulmonary embolism (6 cases, isolated in 2) diagnosed by ultrasonography and/or angio-CT following AKS. Thrombophilia investigation included plasma measurement of protein C, free and total protein S, antithrombin, activated protein C resistance, homocysteine, anticardiolipin antibodies (aCL), lupus anticoagulant (LA), factor V Leiden (FVL), prothrombin (FII) A20210 and the methylene tetrahydrofolate reductase (MTHFR) C677T polymorphysm. There was a striking 80% frequency of thrombophilic genotypes, isolated or in combination, in this patient group (12/15) and no deficiencies of natural anticoagulants or positivity for LA/aCL. Frequencies of detected thrombophilic genotypes were as follows; heterozygous FVL 27% (4/15), heterozygous FII/A20210 7% (1/15), heterozygous FVL plus heterozygous FII/A20210 7% (1/15), homozygous MTHFR C677T with hyperhomocystinemia 27% (4/15), heterozygous FII/A20210 plus homozygous MTHFR C677T 13% (2/15). All patients with pulmonary embolism had at least one thrombophilic genotype. The only patient with a history of deep vein thrombosis had hyperhomocystinemia with homozygous MTHFR C677T. AKS (probably because of endothelial damage and interrupted blood flow) may predispose carriers of thrombophilic genotypes to an elevated risk of post-operative deep vein thrombosis.

P130

SUPERIOR OPHTHALMIC VEIN THROMBOSIS IN PATIENT WITH CAVERNOUS SINUS DURAL ARTERIOVENOUS FISTULA. A CASE REPORT

Cappelli R,* Polito E,^ Bracco S,° Gennari P,° Zalaffi A,^ Loffredo A,^ Pichierri P^

**Istituto di Medicina Interna e Geriatria;^Dipartimento di Scienze Oftalmologiche e Neurochirurgiche, Università di Siena; ° U. O. Neuroradiologia, Azienda Ospedaliera Senese, Siena, Italy*

A 43-year old woman with a controlled post-operative hypoparathyroidism-hypothyroidism (thyroidectomized seven years before for a multinodular goiter) and pituitary microadenoma with increased prolactin production, presented in the June 2000 left side (I branch of V nerve) short lasting grip, followed after some weeks by left hemifacial numbness and ocular pain, ptosis with diplopia and proptosis, chemosis and conjutival edema in the left eye. No trauma or intracranial bruit were referred. A magnetic resonance followed by cerebral angiography performed at the end of September revealed a cavernous sinus dural arteriovenous fistula, the superior ophthalmic vein resulted dilated with an inversion of blood flow. The fistula was treated by transvenous embolization of the left cavernous sinus with platinum coils. A progressive improvement of symptoms (exophthalmos and ocular motility) was observed. Nine months later, the left exophthalmos newly increased. A cerebral angiogram revealed a partial superior vein thrombosis with a residual small fistula. Treatment was started with enoxaparin 6000 U. twice a day for three months and successively 6000 U. once a day. The personal and family history were negative for thromboembolic events. Prothrombin time and INR, antithrombin III, protein C, protein S, APC resistance, anticardiolipin and anti B2 glycoprotein antibodies were within the normal ranges. Partial thromboplastin time 40 sec, plasma homocysteine 18.4 μmol/L, positive lupus anticoagulant test. In October 2001, the last selective angiography showed a correct fistula closure without superior ophthalmic vein dilatation. A moderate exophthalmos lasted in the left eye but with ocular motility significantly improved. In dural cavernous fistulas the superior ophthalmic vein can represent the principal venous drainage route and sometime this vein is involved in a thrombosis. In this case the thrombophilic condition of the patient represents a risk factor. An antithrombotic strategy should be considered in the fistulas treatment.

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CANDIDATE GENE POLYMORPHISMS IN THROMBOTIC DISEASE

Penco S,* Grow M,° Baglietto L,# Lando G,* Patrosso MC,* Redaelli R,~ Caimi MT,~ Mostarda G,~ Cheng S,° Baudo F,~ Marocchi A*

**Dipartimento di Diagnostica/Prestazioni Intermedie; Struttura Complessa di Laboratorio Analisi Chimico Cliniche Patologia Clinica; Settore Analisi Genetiche, Ospedale Niguarda Ca' Granda, Milan; °Department of Human Genetics, Roche, MS, Usa; #Divisione di Epidemiologia e Biostatistica, Istituto Europeo di Oncologia, Milan, ~Modulo di Trombosi e Emostasi, Divisione di Ematologia, Ospedale Niguarda Ca' Granda, Milan, Italy*

Venous thromboembolism (VTE) is a multifactorial disease that depends on variable combinations of acquired and genetic risk factors. Well established risk factors include advancing age, prolonged bed rest and surgery. Genetic risk factors are also common and may play a role in approximately 25% of the individuals who develop VTE. Factor V, factor II, MTHFR and CBS polymorphisms are genetic markers associated with VTE; their single contribution might not be so evident to allow identification. It has been postulated that more than one genetic risk factor may co-segregate with a consequent cumulative or synergistic effect on thrombotic risk.[1] A multilocus assay was used to genotype 65 biallelic polymorphisms or mutations within 36 genes[2] in an Italian population (638 individuals) affected (323) or not affected (315) by venous thrombotic events. These genes are involved in lipid metabolism,

homocysteine metabolism, blood viscosity, platelet aggregation, leukocyte adhesion and the renin-angiotensin system. Genotype frequencies for all the markers were compared between the two groups. For each locus the association between genotype and VTE event has been evaluated by means of a logistic model, assuming that event risk depends on the genotype. Markers showing an association with VTE, with a *p* value <0.05 at univariate level, are reported.

Marker	likelihood ratio test p-value
Factor II.G20210A	0.004
Factor V.arg506gln	<0.001
ICAM.gly214arg	0.039
Angiotensin Receptor 1.A1166C	0.047

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RISK FACTORS FOR THROMBOSIS IN PATIENTS WITH THE NEPHROTIC SYNDROME

Podda G, Lussana F, Moroni G,* Zighetti ML, Faioni E, Ponticelli C,* Mannucci PM, Cattaneo M

*A. Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine; *Division of Nephrology and Dialysis, IRCCS Ospedale Maggiore and University of Milan, Milano*

Arterial and venous thrombosis are frequent complications in patients with the nephrotic syndrome (NS). Although some abnormalities of the hemostatic system have been described, the causes for the high thrombotic risk in these patients are only partially understood. The aim of the study was to investigate whether two common risk factors for thrombosis, resistance to activated protein C (RACP) and hyperhomocysteinemia are frequent in patients with NS. Sixty-four NS patients and 63 healthy subjects were studied. The following variables were investigated: plasma levels of total homocysteine (tHcy), protein C, protein S, antithrombin, fibrinogen, factor VIII, creatinine; the anticoagulant response to activated protein C (APC); the glomerular filtration rate (GFR). tHcy was significantly higher (14.5 vs 9 mmol/L, $p<0.001$) and the anticoagulant response to APC was significantly lower (ratio: 0.88 vs 1, $p<0.001$) in NS patients than in controls; NS patients had significantly ($p<0.001$) lower GFR (48 vs 104 mL/min) and higher plasma levels of factor VIII (190% vs 104%), protein C (133% vs 112%) and protein S (139% vs 107%) than controls. Four NS patients (6%) and no controls had RAPC. In NS patients, there was a statistically significant correlation between the APC ratio and factor VIII levels ($r= -0.46$, $p<0.001$) and between tHcy and GFR ($r = -0.469$, $p<0.001$). A subgroup analysis showed that the plasma levels of tHcy in NS patients with normal GFR were not different from those of controls (9 vs 9.3). Therefore, hyperhomocysteinemia and RAPC were frequent in NS patients. However: 1) high tHcy levels were found only in patients with abnormal GFR, indicating that decreased renal function is responsible for the abnormality; 2) the reduced response to APC is probably due to the high plasma levels of factor VIII.

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PREGNANCY IN INHERITED THROMBOPHILIA: FOLLOW-UP OF A SERIES OF 53 PATIENTS

Donvito V, Maina A, Vaccarino A,* Bazzan M*

*Servizio di Medicina Interna, Azienda Ospedaliera OIRM, Sant'Anna, Turin; *Servizio di Ematologia, Ospedale Evangelico Valdese, Turin, Italy*

Introduction. Congenital defects of natural inhibitors of hemostasis are well-known risk factors for arterial and venous thromboembolism in carriers. There is also growing but still controversial evidence that thrombophilia may predispose to *vascular* complications of pregnancy such as severe pre-eclampsia, IUGR, fetal death and *abruptio placentae*. For the same reason women affected by repeated fetal loss syndrome are being investigated. *Aim of the study*. To evaluate pregnancy outcome and vascular complications in patients affected by hereditary thrombophilia. *Subjects and methods*. Fifty-three women affected by inherited thrombophilia with a previous obstetric adverse outcome (n.23) and/or thromboembolism (n.18) were followed-up in pregnancy and puerperium in the years 1997-2001. Fourteen patients had a positive family history without thromboembolic events. During pregnancy patients were treated with low- dose aspirin and /or subcutaneous heparin (UH or LMWH). Choice of treatment and dose of heparin were adapted for each patient considering the clinical history and the biological response (aPTT, antiXa activity, when assayed). *Results*. Fifty-five pregnancies were followed and we observed: n.2 early miscarriages; 9 vascular complications of pregnancy (pre-eclampsia, IUGR) and preterm birth; 44 uncomplicated pregnancies; 53 alive healthy babies; 3 venous thromboembolisms (2 out of 3 after Cesarean section). *Discussion*. A thrombophilic defect should be suspected in all women with a history of (repeated) pregnancy loss. Risk of pregnancies complications is still higher than normal (about 20%) and a careful obstetric assistance and intensive neonatal care unit are required.

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THROMBOEMBOLIC EVENTS IN CHILDREN ADMITTED TO "OSPEDALE INFANTILE REGINA MARGHERITA" OF TURIN BETWEEN SEPTEMBER 1998 AND DECEMBER 2001

Messina M, Ferrero NM, Albiani R, Pagliarino M, Saracco P,* Perugini L

*Service of Immunohematology and *Division of Hematology, Azienda Ospedaliera OIRM, Sant'Anna, Turin, Italy*

Thromboembolic events in children are a rare, but increasing pathology. In 3 years we had 65 cases of thromboembolism (39 male and 26 female) in patients aged 1day-18 years, of which 27 cases in the first year of life (18 in the first month) and respectively 22, 6, 10 cases between 1-6 years, 6-10 years and over 10

years; (average age 3.3 years). The thrombosis developed in the venous vessels and in the right heart in 28 cases (43%), in the arterial vessels and in the left heart in 37 cases (57%); of these, 30 cases, were stroke and it was more frequent in the first year of life (12). We checked the deficiency of protein C, S, antithrombin, APCR and mutations of factor V, II and MTHFR. In 77% of patients we identified a pathologic prothrombotic condition: cardiovascular congenital malformation (18), prematurity, sepsis and perinatal asphyxia (9), otomastoiditis, immobility (4), liver or kidney disease (9), cancer (6), LAC (3), central venous line (27). In 38% of patients the thrombosis was secondary and associated to only one risk factor: pathologic prothrombotic condition or family history of thrombosis, TIA, ictus. In 15% of patients there was only genetic risk factor. In 42% of cases there was genetic and acquired risk factor together and in 5% there was not any prothrombotic risk factor. These data confirm that in children thrombosis occurs when there are more risk factors together, and that the acquired factor is prevalent over the genetic factor; thrombosis is more frequent in the first year of life and it is a stroke; the main prothrombotic risk factor is a central venous line.

P135

THROMBOPHILIC SCREENING IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Dragoni F,* Iori AP,** Avvisati G,*** Pignoloni P,* Minotti C,* Arcese W,** Mazzucconi MG*

Thrombosis Center, Dept. "Biotecnologie Cellulari ed Ematologia", Univ. "La Sapienza", Rome, Italy. Dept."Biotecnologie Cellulari ed Ematologia", Univ. "La Sapienza", Rome, ***Libera Università "Campus Biomedico", Rome, Italy*

Background. Paroxysmal nocturnal hemoglobinuria (PNH) in an acquired disorder characterized by intravascular hemolysis and life-threatening venous thrombosis. Prophylactic anticoagulation treatment in all patients could improve survival and reduce morbidity. *Patients and Methods*. Thirteen patients (males = 2, females = 11; mean age 46 years, range 26-65) with well documented PNH underwent prospective evaluation of antithrombin, protein C, free and total protein S and activated protein C resistance. In all patients the presence of antiphospholipid antibodies was investigated by kaolin clotting time (KCT), diluted Russel's viper venom time (dRVVT) and by the research of anticardiolipin antibodies IgG and IgM (ACA-G and ACA-M. Prevalence of factor V Leiden, prothrombin variant G20210A and thermolabile variant C677T of methyletetrahydrofolate-reductase was evaluated. The same parameters were tested in 100 normal subjects (males = 50, females = 50; mean age 45 years, range 24-69) who constituted the control group. *Results*. Five patients (38.4%) had a history of thrombotic events vs no case in control group (Fisher's test = $p < 0.0001$). Antithrombin, protein C and protein S were normal in all cases. No patients had factor V Leiden and prothrombin variant G20210A. Only one patients was homozygous for the thermolabile variant C677T of methylenetetrahydrofolate-reductase. Antiphospholipid antibodies were found in eight patients (61.5%) and in one (1%) normal control (OR=158.4, 95% CI = 14.4-4159.6). All five patients with PNH and a history of thrombotic events had antiphospholipid antibodies. *Conclusions*. A high rate of patients with paroxysmal nocturnal hemoglobinuria have antiphospholipid antibodies. An evaluation of the presence of antiphospholipid antibodies should be performed in all patients with paroxysmal nocturnal hemoglobinuria in order to identify the patients with thrombotic risk factors.

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CLINICAL SIGNIFICANCE OF ANTI-PROTHROMBIN ANTIBODIES IN PATIENTS WITH A HISTORY OF VENOUS AND/OR ARTERIAL THROMBOSIS

Montaruli B,* Vaccarino A,° Foli C,° Plateroti S,* Rous C,° Saitta M,* Bazzan M°

**Laboratorio Analisi,° Servizio di Ematologia e Malattie Trombotiche, CIOV - Ospedale Evangelico Valdese, Turin, Italy*

Arterial and venous thromboses are the most frequent and clinically relevant complication of patients with antiphospholipid antibodies (aPL). The role of anti-prothrombin antibodies (aPT) as risk factors of thrombosis has not yet been clearly established. In the present study we investigated the association of IgG and IgM aPT with venous and/or arterial thrombosis. We determined the prevalence of aPT IgG and IgM in 105 patients [63 females and 42 males (16-84 years; median: 45 years)] with a history of arterial thrombosis (35 cases), venous thrombosis (66 cases) and both arterial and venous thromboses (4 cases) and in 105 sex and age matched controls. IgG and IgM aPT titers were measured by commercial immunosorbent assay (Orgentec,kindly supply by Bouty, Sesto San Giovanni, Italy). aPT IgG and IgM were detected more in patients with arterial thrombosis than in controls (aPT IgG 34% vs 6%, $p < 0.0002$; aPT IgM 28% vs 4%,$p < 0.0004$). Furthermore, we observed that the prevalence of aPT IgG and aPT IgM were significantly higher in patients with venous thrmobosis than in controls (aPT IgG 21% vs 6 %, $p < 0,02$; aPT IgM 22% vs 4%, $p < 0.0001$). In thr group of subjects found negative for Lupus Anticoagulant activity (LA) and aCL and anti β2 glicoprotein I autoantibodies, the prevalence of aPT IgG and IgM were significantly higher in patients with arterial and/or venous thrombosis than in control subjects (aPT IgG 16% vs 3%, $p < 0.0005$; aPT IgM 11% vs 1%, $p < 0.008$). Univariate analysis revealed that aPT IgG and IgM were significant risk factors for arterial thrombosis [odds ratio (OR) 8.6 and 4.8; 95% Confidence Interval (CIs) 2.9-25.3 and 1.3-13.2 respectively] and venous thrombosis [OR 9.9 and 6.7; 95% CIs 2.8-34.1 and 2.1-21.3 respectively]. In conclusion, these results showed that aPT IgG and IgM are correlated with a history of venous and/or arterial thrombosis in patients with or without LA activity.

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THE NEED FOR A STRUCTURED TEACHING PROGRAM FOR NURSES INVOLVED IN ORAL ANTICOAGULANT MANAGEMENT (A PROJECT OF EMILIA ROMAGNA REGION TO UNIFORM ORAL ANTICOAGULANT TREATMENT IN THE PARMA AREA)

Tagliaferri A,* Manotti C,* Pattacini C,* Zurlini C#

**Dip. Medicina 3, Centro per le Malattie dell'Emostasi e Cura dell'Emofilia, Azienda Ospedaliera di Parma, #Dip. Patologia Clinica, Azienda USL di Parma, Italy*

The number of patients receiving oral anticoagulants has been increasing constantly all over the world during the last year, due to both *new* indications for common pathology, such as atrial fibrillation and safer dosage derived by the use of ISI-INR system for laboratory control. Laboratory control in Anticoagulant Clinics (AC) will remain a difficult task particularly when many patients have to been checked every day. In the Parma area several thousands of patients are on anticoagulant treatment so different models of delivering service are been developed and includes AC and community-based service (CBS) in both all the role of nurses is of pivotal importance. We carried out a survey to verify if nurses involved in anticoagulation management have an adequate information and education on problems concerning this difficult task. All nurses working in the AC or CBS of Parma area are invited to participate to a two day training educational course, 46 out 51 attended to this. Before the training we provide them a questionnaire with 22 questions concerning oral anticoagulant therapy management. Seventy six percent of the answers are wrong or nurses are non-able to replay and only thirty-three are right. After the course they replay exactly to sixty three percent of the questions. In conclusion many of Parma area nurses involved in oral anticoagulation management have a non-adequate specific education. Doctors who have responsibility for AC or CBS must arrange structured teaching programs to improve specific education of nurses involved to achieve an efficient quality of anticoagulant therapy.

(The project is totally supported by Emilia Romagna Regional Research Program, Azienda Ospedaliera and Azienda USL di Parma).

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AN ALTERNATIVE MODEL FOR MANAGEMENT OF ORAL ANTICOAGULANT THERAPY: THE ROLE OF GENERAL PRACTITIONERS (A PILOT PROJECT OF EMILIA ROMAGNA REGION TO UNIFORM ORAL ANTICOAGULANT TREATMENT IN THE PARMA AREA)

Pattacini C,* Tagliaferri A,* Manotti C,* Zurlini C#

**Dip. Medicina 3, Centro per le Malattie dell'Emostasi e Cura dell'Emofilia, Azienda Ospedaliera di Parma, #Dip. Patologia Clinica, Azienda USL di Parma, Italy*

The clinical indications for anticoagulation are increasing leading to ever-greater numbers of patients attending anticoagulant clinics (AC). The pressure on AC intensifies and alternative models of service delivery have to be developed. The proposed model provides a decentralization of delivery services through General Practitioners (GP) who are directly involved in complete management of their own patients. Two important tools allowed this great degree of decentralization: a) near-patients test devices; b) computerized decision support systems. The aim of the project is to maintain the treatment efficacy, to reduce access of stabilized patients to AC and to improve the patient's life quality. The project started on 01/01/2002: twenty GP of the Parma area were recruited on voluntary basis to manage their own patients (about 10-15 per GP). In synthesis every GP work as an AC: a) patients attend to the GP office; b) capillary blood is taken and analyzed with a near-patient testing device; c) dosing is provided directly by GP using a computerised decision support system (P.A.R.M.A. system version 4.2). d) all patient data are stored in the central database of our AC and GP can see only data of his own patients. e) at present the GP may communicate with AO specialists about complicated or unstable patients by phone, but we are planning to provide a on line link with reference AC. All GP participant were trained and instructed to use of near-patient testing device and computer program. Dedicated educational training and regular audits have been carried out and planned. To assess the efficacy of this model oral anticoagulant treatment analyses will be carried out before and after its implementation. Also clinical end points (hemorrhagic and thromboembolic complications) will be checked. The cost effectiveness of implementation of this decentralized model will be assessed in comparison with the traditional model (AC).

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HEPARIN, ENOXAPARIN, PEG-HIRUDIN AND TWO NOVEL ORAL THROMBIN INHIBITORS, BSF 208791 AND BSF 411693 IN THE INHIBITION OF FLUID PHASE AND FIBRIN-BOUND THROMBIN

Filippucci E, Agnelli G, Hornberger W*

*Sezione di Medicina Interna e Cardiovascolare; Dipartimento di Medicina Interna; Università di Perugia, Italy; *Knoll AG, Research and Development, Dept. of Angiology, Ludwigshafen, Germany*

Background. Fibrin-bound thrombin promotes thrombus extension by catalyzing the formation of new fibrin. To achieve optimal inhibition of thrombus extension, an antithrombotic agent should inhibit both free and fibrin-bound thrombin. *Aim of the study.* To compare the ability of five different antithrombin agents to inhibit fluid-phase and fibrin-bound thrombin. *Methods.* The following agents were tested: unfractionated heparin (UFH), enoxaparin, PEG-hirudin and two novel oral synthetic thrombin inhibitors, BSF 208791 and BSF 411693. FPA generation (ELISA) was used as an index of thrombin activity. The effect of increasing concentrations of the five inhibitors was tested both in fluid-phase and in fibrin-bound system. The concentrations of the five thrombin inhibitors able to produce 10, 25, 50, 75 and >75% inhibition of fluid-phase thrombin were identified and then tested in the fibrin-bound system. In addition, the maximal inhibition of fibrin-bound thrombin produced by the five agents was identified.

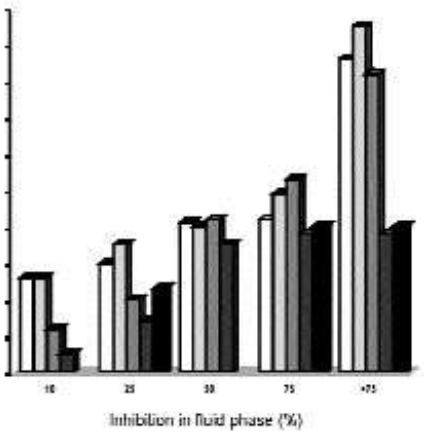

Figure 1. Correlation between inhibition of fluid phase and fibrin-bound thrombin by the five agents at concentrations able to produce 10, 25, 50, 75 and >75 % inhibition of fluid phase T.

Results. At the lowest concentrations BSF 208791 and BSF 411693 were the most effective in inhibiting fibrin-bound thrombin. The maximal inhibition of fibrin-bound thrombin was higher than 90% for BSF 208791 and BSF 411693, 80% for PEG-hirudin and 40% for over-therapeutic concentrations (aPTT ratio >10.0) of UFH and enoxaparin. The aPTT ratio for concentrations of BSF 208791 and BSF 411693 producing maximal inhibition was 2.4±0.6 and 2.3±0.5, respectively, and 1.9±0.1 for PEG-hirudin. *Conclusions.* BSF 208791 and BSF 411693, as well as PEG-hirudin, are able to induce a maximal inhibition of fibrin-bound thrombin while this is not the case for UFH and enoxaparin. The maximal effect of BSF 208791 and BSF 411693 on fibrin-bound thrombin is achieved at concentrations producing a *therapeutic* aPTT prolongation. The direct thrombin inhibitors could be more effective than heparins in the prevention of thrombus extension and rethrombosis.

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PLASMA LEVELS OF ENDOTHELIAL PROTEIN C RECEPTOR RESPOND TO ANTICOAGULANT TREATMENT

Stearns-Kurosawa DJ,* Swindle K,* D'Angelo A,° Della Valle P,° Fattorini A,° Caron N,# Grimaux M,# Woodhams B,# Kurosawa S*

**Free Radical Biology & Aging Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; °Coagulation Service & Thrombosis Research Unit, IRCCS H S. Raffaele, Milan, Italy; #Diagnostica Stago, Gennevilliers, France*

The endothelial protein C receptor (EPCR) facilitates protein C activation and plays a protective role in the response to *E. coli*-mediated sepsis in primates. A soluble form of EPCR (sEPCR) circulates in plasma whose generation is regulated by inflammatory mediators, including thrombin-mediated up-regulation of surface metalloproteolytic activity *in vitro*. In this study we address the question of whether plasma sEPCR levels reflect changes in thrombin generation in individual undergoing anticoagulant treatment. Plasma sEPCR levels of patients treated with coumarin-type oral anticoagulants (n = 55) had significantly lower sEPCR levels (105.3±70.8 ng/mL, p <0.0001) than 200 normal controls (165.8±115.8 ng/mL). Within this normal population, sEPCR levels revealed a bimodal distribution and they were slightly higher in males (184.51±129.2 ng/mL; n=100) than in females (147.1±97.8 ng/mL;n=100, p<0.01). In a small sample of patients undergoing intravenous unfractioned heparin therapy (n = 10) the reduction in sEPCR levels (147.1±39.3 ng/mL) followed the trend observed above in patients undergoing oral anticoagulant therapy. The effect of warfarin treatment on plasma sEPCR levels was studied in 6 adult volunteers for 7 days with a additional follow-up period to day 15. There was a lag time of 24-48 hours before sEPCR declined and subsequently 24-48 hours after cessation of warfarin, sEPCR levels began to increase again in all subject. The INR values for these samples were essentially a mirror image of the sEPCR levels. The changes in sEPCR, protein C antigen and Factor II coagulant activity were compared in this population. A significant decrease in sEPCR was observed on day 3 and persisting 48 hours after last warfarin administration. Protein C antigen levels dropped to a 46% of baseline values by day 2 and returned to a normal levels 96 hours after last administration. Factor II:C levels decreased to 61% of baseline by day 2, reached a nadir by day 8 and then increased, being still lower than baseline 7 days after last administration. These data suggest that the decrease in sEPCR is not dependent on reduced circulating levels of protein C, but rather mirrors the inhibition of *in vivo* thrombin generation. A similar decline in sEPCR level over time was observed in seven patients initiating warfarin treatment for thrombotic disorders, including deep vein thrombosis, atrial and dilatative cardiomyopathy. These studies demonstrate that plasma levels of sEPCR decline in response to treatment with anticoagulants whose mechanism of action is known to decrease *in vivo* thrombin generation.

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THROMBOPROPHYLAXIS FOR PREVENTING ADVERSE OBSTETRIC OUTCOMES IN WOMEN WITH INHERITED THROMBOPHILIA

Grandone E, Colaizzo D, Brancaccio V, Vecchione G, Iannaccone L, Sciannamé N, Pavone G, Di Minno G, Margaglione M

"Unità di Aterosclerosi e Trombosi", IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, (FG), Coagulation Unit of "A. Cardarelli" Hospital, Naples, Italy

Information is lacking regarding the best treatment for genetic causes of thrombophilia associated with the occurrence of obstetric complications. To improve feto-maternal outcomes in women with obstetric complications and inherited causes of thrombophilia, we treated 25 women, who carried 31 pregnancies from January 1999 to March 2001. Over a period of about two years, in 31 pregnancies of 25 women with inherited thrombophilia and previous severe outcomes, we used thromboprophylaxis with low fixed doses of heparin or aspirin and recorded all obstetric and fetal outcomes. Two (6.5%) pregnancies ended in an early pregnancy loss, and 29 (93.5%) ended in the delivery of a live newborn, whereas in 50 out of 55 (90.9%) of previous pregnancies a poor obstetric outcome was registered (p<0.001). One woman showed a severe fetal growth retarda-

tion. All pregnancies reached the term, except five, the most severe ending at 30 weeks. One newborn was <3 centile, while all the remaining were >10 centile. All the babies were discharged in good clinical status. Overall, 7 (22.6%) pregnancies were treated with aspirin and all with good outcomes. No patient showed thrombosis during the current pregnancies. No side-effects were registered. Heparin prophylaxis at fixed low doses and possibly aspirin could be efficacious in preventing adverse outcomes in women carrying inherited thrombophilia with previous poor obstetric outcomes.

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QUALITY AND SAFETY OF ORAL ANTICOAGULANT THERAPY IN ELDERLY OUTPATIENTS WITH NON VALVULAR ATRIAL FIBRILLATION

Cerè E, Lombardi A, Serafini F, Maccaferri A, Grande S, Maini EP, Martelli A,* Rovinetti C,* Rosafalco M,° Di Pasquale G

*Cardiology Division; *Laboratory, Bentivoglio Hospital, Bologna; °Instrumentation Laboratory, Milan; Italy*

Non-valvular atrial fibrillation (NVAF) is a risk factor for stroke and its prevalence increases with age. Oral anticoagulant therapy (OAT) is the drug of choice for stroke prevention, but is still underused in elderly patients with NVAF for the fear of increased bleeding complications, and difficulties in OAT management. *Methods*: The aim of this study was to evaluate possible differences in quality and safety of OAT [percentage of time (days) within, above, below the target range, INR 2.0-3.0] in two groups (Group 1 age < 75 years vs Group 2 age > 75 years) of outpatients with NVAF followed at the Anticoagulation Clinic of the Cardiology Division of Bentivoglio Hospital. The protocol of *co-ordinated* OAT management comprises: extensive instruction to all new patients; computer-aided management with a software program (PARMA 3.3, Instrumentation Laboratory, MI) with algorithm able to suggest OAT dosing and schedule appointments for follow-up; prothrombin time expressed as INR; external laboratory quality-control. *Results.* In the year 2001 a total of 719 patients attended our Anticoagulation Clinic (mean age 71±11 yrs), 291 (40%) were pts with NVAF; 226 patients (132 M/94 F), mean age 74±9 yrs (37-90), with a minimun follow-up in OAT > 60 days (mean follow-up 258.3 days) were considered for evaluation (Table 1). Complications during OAT: Group 1: 2 minor bleedings, 2 major bleedings; Group 2: 3 minor bleedings, 2 major bleedings, 1 thromboembolic event (NS).

Table 1.

	Patients	Mean age yrs (range)	Sex	% INR within range	% INR below range	% INR above range
Group 1 (age < 75 years)	102 (45%)	67±7 (37-74)	65M/37F	73.2% *	18.5% **	8.3% °
Group 2 (age > 75 years)	124 (55%)	80±4 (75-90)	67M/57F	76.7% *	12.2% **	11.1% °
				* NS	** NS	°NS

Conclusions. In our experience the overall quality of OAT was good, without significant differences between the two groups and complications rate was low. Our results suggest that in NVAF elderly patients, OAT can be practised with quality and safety, provided that OAT management is *co-ordinated* as in an Anticoagulation Clinic.

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ORAL ANTICOAGULANT THERAPY AND THROMBOCYTOPENIA

Sottilotta G, Oriana V, Latella C, Musitano P, Lombardo VT

Centro Emofilia Servizio Emostasi Trombosi, Azienda Ospedaliera " Bianchi–Melacrino–Morelli", Reggio Calabria, Italy

Introduction. Oral anticoagulant therapy represents usual treatment of different pathologies. The improvement of heart surgery determined a great increase of patients with valvular prosthesis or other types of surgical operations that require an anticoagulation for a long time or for the whole of the patients' life. Recent knowledge about thromboembolism associated with genetic thrombophilic defects determined new therapeutic practices. In fact, great attention must be given to prophylaxis in patients carriers of genetic thrombophilic alterations to protect the subjects from more serious events. Antiphospholipid syndrome is the most common acquired condition associated with vein or arterial thrombosis. The result of these studies is the continuous increase in the number of patients in OAT. There have been various studies aimed at assessing the hemorrhagic risk during OAT. *Aim of study*. To determine the frequency of thrombocytopenia in a cohort of 1126 patients on oral anticoagulant therapy (OAT), and to compare the grade of thrombocytopenia and the severity of bleeding complications. *Results*. We observed severe thrombocytopenia in 5 patients, and moderate and light thrombocytopenia in 208 patients. Thrombocytopenic patients on OAT presented 5 major and 6 minor hemorrhages. We evaluated the positiveness to the hepatitis markers and autoantibodies. All patients at the time of bleeding complication were in a therapeutic range of anticoagulation.

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LOW-MOLECULAR-WEIGHT HEPARIN TREATMENT AND CANCER: POOLED ANALYSIS OF 908 PATIENTS TREATED FOR THREE MONTHS

Guercini F, Pini M,* Iorio A

*Sezione di Medicina Interna e Cardiovascolare, Università di Perugia; *Dipartimento di Medicina Interna, Ospedale di Fidenza, Italy*

Background. A mortality reduction in patients treated with low-molecular-weight heparin (LMWH) for the initial treatment of venous thromboembolism (VTE) was observed, and it reached statistically significance in the subgroup of cancer patients in meta-nalysis evaluating death rate at 3 months. Then, the hypothesis has been raised that a short period of LMWH treatment could reduce cancer mortality, through some still unknown mechanism. We wanted to check whether a similar result was achieved in cancer patients treated with LMWH for a longer period of time. *Aim of the study*. The objective of this review was to evaluate the effect on cancer mortality of a three-month treatment with LMWH, by performing a meta-analysis of studies comparing LMWH and oral anticoagulants in the prevention of recurrences after an episode of VTE. *Materials and Methods*. Computerized searches of MEDLINE and EMBASE were performed; clinical trials were also located through colleagues and

hand-scanning of meeting proceedings and reference lists. Two reviewers extracted data independently using a standard form. The analysis was performed with a fixed-effect model. *Results*. Six studies were identified that fulfilled our predefined criteria, for a total of 908 patients. After one year of follow up, 51 patients died of cancer, 27 in the LMWH group and 24 in the oral anticoagulant control group (OR 1.14 95% CI 0.64 – 2.02; z=0.39; p=0.7). Considering cancer mortality in cancer patients the figures were 27/65 in the LMWH group and 24/61 in the oral anticoagulant control group (OR 1.13 95% CI 0.54 – 2.38; z=0.33; p=0.7). The test for study heterogeneity was negative. Five studies showed an individual non significant OR between 1.12 and 1.43. *Conclusions*. In the 908 patients randomized to receive a three month course of LMWH or oral anticoagulants for the treatment of VTE, no effect on cancer mortality was found.

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INTRACRANIAL BLEEDING IN REGGIO EMILIA: EPIDEMIOLOGY AND RELATIONSHIPS WITH ANTITHROMBOTIC TREATMENT

Nicolini A, Ghirarduzzi A, Silingardi M, Iorio A, Baldi G

Dept. Medicina Interna 1^, Dept. Patologia Clinica, Centro Emostasi e Trombosi, Dept Medicina d'Urgenza, Azienda Ospedaliera S. Maria Nuova, Reggio Emilia, Italy. Sezione di Medicina Interna e Cardiovascolare Dipartimento di Medicina Interna Università di Perugia, Italy

Background. Anticoagulants (AC) and antiplatelets (AP) drugs are effectively used in the prevention of thromboembolic events, with the tradeoff of bleeding side effects, particularly intracranial (IB). *Aim of the study*. To determine IB incidence and to investigate the potential effect of AC and AP. *Materials and Methods.* We reviewed all the patients admitted for IB at our hospital between 4/1998 and 9/2000. Data were collected with a standard form. All the patients were recalled to estimate long-term mortality. Comparisons were performed with χ^2 and t-test, as appropriate. Logistic regression analysis was performed to test predictors of mortality. Pharmacy data were employed to estimate the total number of AC and AP patients. *Results.* We found 241 cases (107/134 female/male, mean age 61 years, 133/107 spontaneous/traumatic events, 0.32 /1000 /year). Twenty-nine and 47 patients were given AC or AP, respectively (4.9 /1000 /year and 3.7 /1000 /year). The relative risk of IB is 11 in AP and 15 in AC treated patients. Mortality was 17/27 (62.9%), 26/47 (55.3%) and 57/157(36.3%) in AC, AP and untreated patients, respectively (p = 0.015). This increased risk was mainly confined to traumatic events (p = 0.0009), without difference between AC and AP. At the time of the event, mean duration of AC treatment was 26.3 months (range 1 to 120). Mean INR was 1.94 (1.6–8.8). Overall mortality was 100/241 (41.8%), 25/104 (24%) in traumatic versus 75/132 (58%) in spontaneous events (p < 0.0001). The mortality was significatively predicted by GCS (p < 0.0001), by the type of bleeding (spontaneous versus traumatic) (p = 0.0026) and by age (p< 0.0001). *Conclusions*. Accurate patient selection and traumatic event prevention are the main candidate mechanisms to reduce IB in AC and AP patients.

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LOW DOSE ORAL VITAMIN K REVERTS WARFARIN-INDUCED COAGULOPATHY MORE EFFECTIVELY THAN SUBCUTANEOUS VITAMIN K

Ageno W, Crowther M, Ultori C, Mera V, Squizzato A, Marchesi C, Dentali F, Steidl L, Douketis J, Schnurr T, Venco A

Department of Medicine, University of Insubria, Varese, Italy; Department of Medicine, Mcmaster University, Hamilton, ON, Canada

Background. Excessive anticoagulation due to warfarin is a frequently encountered clinical problem. Vitamin K is effective in the reversal of warfarin-induced coagulopathy. Currently, the most widely used methods of vitamin K administration are orally and subcutaneously. This study was designed to determine whether oral vitamin K is more effective than subcutaneous vitamin K in the treatment of patients with warfarin-induced coagulopathy. *Methods.* We performed a multi-center randomized trial in two tertiary care hospitals. In this study, patients receiving warfarin who had an INR value between 4.5 and 10.0, and who did not have an indication for immediate normalization of their INR, had their warfarin withheld and were randomly allocated to receive 1 mg of vitamin K either orally, or by the subcutaneous route. The primary outcome measure was the INR value on the day after study drug administration. Secondary outcome measures included INR values on subsequent days, and the risk of hemorrhage and recurrent thrombosis over a one month follow-up period. *Results*. Patients given oral vitamin K had more rapid reduction in their INR than those given subcutaneous vitamin K: 15 of 26 compared with 6 of 25 patients had INR values of 1.8 to 3.2 on the first day following study drug, respectively [p = 0.015 odds ratio (OR) 4.32, 95% confidence interval (CI): 1.13, 17.44]. The INR values were higher in the subcutaneous vitamin K group than the oral vitamin K group on the second and third days. After 1 month, there were no episodes of thromboembolism or bleeding. *Conclusions*. When compared with oral vitamin K, subcutaneous vitamin K is more slowly acting than oral vitamin K for the treatment of warfarin-associated coagulopathy.

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LOW MOLECULAR WEIGHT HEPARIN (PARNAPARIN) VS ASPIRIN IN THE TREATMENT OF RETINAL VEIN THROMBOSIS: A PROTOCOL FOR A MULTICENTER, RANDOMIZED, DOUBLE BLIND, CONTROLLED CLINICAL TRIAL

Ageno W,* Cattaneo R,° Imberti D,# Ferrari P^ for the Study Investigators

**Department of Internal Medicine, University of Insubria, Varese, °Emergency Department, Hospital of Busto Arsizio; #Department of Internal Medicine, Hospital of Piacenza and ^Alfa Wassermann, Bologna, Italy*

Background. Retinal vein thrombosis is a severe disease potentially leading to blindness that affects approximately 1.6% of patients aged over 49 years and 4.6% of patients aged over 80 years. Despite its clinical relevance, there are no available treatment options of proven efficacy, and the lack of randomized, controlled trials led some consensus guidelines to conclude that there is no evidence to recommend the use of antithrombotic

drugs. *Objectives.* The aim of this study is to compare with a methodologically rigorous design the efficacy and safety of parnaparin, a low molecular weight heparin (Fluxum, Alfa Wassermann, Bologna, Italy) versus aspirin in the treatment of retinal vein thrombosis. *Study design*. Randomized, double blind, double dummy. *Population.* Consecutive patients aged between 18 and 85 with objectively documented retinal vein thrombosis and symptoms lasting for no more than 15 days. Patients will be excluded if their body weight is less than 50 kg, if they have contraindications to the administration of heparin or aspirin, if they are affected by ophthalmological disorders impairing the objective diagnosis of retinal vein thrombosis, if they fail to give informed consent. *Treatment*. Parnaparin will be administered in a fixed (6400 IU), twice daily dose for 7 days followed by a once daily dose starting on day 8 until day 90. Aspirin will be administered in a 100 mg daily dose for 90 days. *Efficacy*. Primary endpoint will be based on the number of patients whose visual function worsens after 6 months from the event. Secondary endpoints are recurrent thrombosis and need for laser therapy. *Safety.* The rate of major and minor bleedings, heparin induced thrombocytopenia. *Sample size:* overall, 172 patients. We hypothesize that parnaparin will reduce the rate of visual function worsening from 72% to 48%.

Participating centers: Varese, Busto Arsizio, Castellanza, Genova, Padova, Perugia, Piacenza.

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ORAL ANTICOAGULANT TREATMENT INDUCES PERSISTENT HIGH PLASMA LEVELS OF FACTOR VIII ACTIVITY IN PATIENTS WITH DIFFERENT DISEASES, POTENTIALLY ASSOCIATED WITH AN INCREASED RISK OF THROMBOSIS

Cascio A, Rao Camemi A, Zito L, Baleste F, Siciliano RS, Gioia M, Valenti G, Galbo L, Arcoleo F, Cillari E

Clinical Pathology Laboratory, F.C.S.A. Centre, "V. Cervello" Hospital, Palermo, Italy

Recent literature has suggested that elevated plasma levels of factor VIII activity (FVIIIc) are associated with increased risk of venous thromboembolic disease (VTED) independently of their involvement in acute phase reaction. Less clear is the role of FVIIIc levels during oral anticoagulant treatment (OAT). In attempt to address this question we studied three groups of patients at our Center receiving OAT, 68 with atrial fibrillation (AF) without previous thromboembolic accidents, 32 with aortic or mitral prosthetic valves (PV), 18 with VTED and 40 healthy controls. Patients included in this study did not have evidence of acute phase responses. Measurements of FVIII, FV, FIX and protein C (PC) were performed. Raised FVIII levels were detected in all groups compared within the normal controls: 175.4±60% in AF, 187±90% in PV, 206±72% in VTED, 90±12% in controls. Even though the mean value of FVIIIc levels detected in VTED patients was higher than that observed in other two groups the difference was not significant. The levels of FVIIIc were not related to the INR and to stability of the patients into the optimal INR range. In agreement with the OAT, FIX and PC were significantly reduced in all subjects. In all groups mean value of plasma factor V levels was in the range of normal controls (80 to 120%). The increase in plasma FVIIIc levels in AF patients on oral anticoagulation similarly to that observed in VTED and in PV appears to indicate that OAT can cause a positive feedback on FVIII synthesis. The biological meaning of this increase is discussed on the basis of the complex interactions between coagulant and anticoagulant proteins and of the follow-up of these patients looking at the possible development of thrombosis.

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INTERACTION OF HERBAL MEDICINE WITH ACENOCOUMAROL

Cancellieri E, Mainardi E, Vagni A,* Brambilla L, Montanelli A

*Dipartimento di Patologia Clinica e *Servizio di Farmacia, Ospedale Maggiore di Crema, Italy*

Concurrent use of herbs may mimic, magnify or counteract the effect of drugs. We report a case concerning a young woman, 39 years old, undergoing regular acenocoumarol treatment for atrial fibrillation who, since some months, had reached a steady level of anticoagulation (INR therapeutic range =2.0–3.0). In the last month a remarkable instability of INR values, up to reaching a prothrombin activity with INR=1.0, was observed. The recent medical history referred by patient resulted negative for pharmacological or dietetic variations or intercurrent disease. However, in successive inquiries, the patient admitted oral assumption, for laxative purpose, of a herbal remedy (2 tablets/evening containing 24 mg of sennosides A+B, anthranoides derived from *Cassia Senna*). Seven days after herbal medicine suspension, a restored in anticoagulation level (INR=2.1) was noted. Our observation suggests a possible impaired absorbption of the anticoagulant produced by a simultaneous intake of *Senna* derivates. The limited knowledge of herbal action mechanism on target organs and the possible interaction between herbal remedies and conventional drugs, makes alternative medicine management extremely complicated and difficult. The use of herbal medicine imposes the acquisition of new medical knowledge and a critical evaluation with regard to uncontrolled use of herbal medicine often taken as self-treatment. Health-care practitioners should warn patients against uncontrolled assumption of herbs and pharmaceutical drugs.

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LONG-TERM ANTICOAGULATION IN NON-CIRRHOTIC PORTAL AND MESENTERIC THROMBOSIS

Brancaccio V, Iannaccone L, Ames PRJ, Guardascione MA,* Scenna G, Amitrano L,* Margaglione M,° Balzano A*

*Unità Emostasi-Trombosi e *Divisione Gastroenterologia, Ospedale "A. Cardarelli", Napoli; °Unità Aterosclerosi e Trombosi, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy*

Background. Splanchnic venous thrombosis (portal and mesenteric) (SVT) is an unfrequent occurrence; its natural history is not well known, nor are the needs and effects of long-term anticoagulation. *Methods.* Twenty-seven patients with SVT (10 males and 17 females) with a mean age of 43.6±17.4 years have been followed for an average of 51.9±71.6 months (median 40, range 7-324) starting from the first diagnosis of SVT. Fourteen patients had portal thrombosis, 8 mesenteric thrombosis and 5 portal and mesenteric thrombosis. The diagnosis of SVT was made by ultrasound and confirmed by CT. All patients were tested for: antithrombin, protein C, protein S deficiency, activated protein C resistance, factor V Leiden (FVL), prothrombin A20210, TT677 genotype of MTHFR, lupus anticoagulant and cardiolipin antibodies. Hematologic, hepatic and neoplastic diseases were inves-

tigated. *Results*:SVT was an occasional finding at imaging in 9 patients while had an acute onset in the further 18 patients: 3 with bleeding esophageal varices, 1 with ascites and 1 with tender splenomegaly. The remaining 13 patients had acute abdominal pain (8 with mesenteric and 5 with portal and mesenteric thrombosis); 9 underwent intestinal resection because of intestinal infarction, 1 splenectomy for a splenic infarction and 3 were treated conservatively. The following thrombophilic factors were found: myeloproliferative disease (22%), protein C deficiency (4%), FVL heterozygous (11%), MTHFRTT677 (18%), PTHRA20210 + MTHFRTT677 (4%), PTHRA20210 + FVL (4%), MTHFRTT677+ protein C deficiency (4%). Oral anticoagulation was given for six months in all patients with acute SVT onset and continued life-long in those with thrombophilic factors and previous or relapsing episodes of thrombosis. During the follow-up 2 patients had bleeding from esophageal varices (at 18 and 240 months) 1 required splenectomy for splenic infarction; no further episodes of thrombosis occurred in the remaining patients. No complications were observed in patients on life-long anticoagulation. *Conclusions*: Acquired or inherited and local or systemic causes of thrombosis have been identified in more than 60% of our SVT patients. Relapse or extension of thrombosis seems to be prevented in SVT patients with known thrombophilic factors with life-long anticoagulation.

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A BIDIRECTIONAL SYSTEM TO INTEGRATE AND SUPPORT TERRITORIAL SITES IN PRESCRIBING ORAL ANTICOAGULATION THERAPY

Minozzi M

Centro Trasfusionale, Azienda Ospedaliera di Merano, Italy

Devolution of management to community-based control is becoming ever more common. The only possible way of maintaining the present quality standards achieved in specialist centres relies on a bi-directional connection of all the prescribing sites to the anticoagulation clinic. *Objectives*: To demonstrate the efficiency of a net supported system integrated with territorial sites and with mobile monitoring units to support computer assisted anticoagulant prescription under the control of experienced medical staff. *Interventions*: using on Oracle standard project and development method we realized a program with the following main features: 1) link to the central anagraphic data-base; 2) link to the central laboratory data-base; 3) application of a new computerized *multimodular* algorithm for the initiation and control of anticoagulant treatment; 4) a statistically oriented data-base for the assessment of the venous and arterial thrombotic risk; 5) differentiated procedures for different patient's requirements. The patient can rely on the hospital, peripheral districts, community sites (pharmacy), family doctor, or he can refer directly to the hospital by sending his own clinical and history data and the INR value (portable monitor) through the compilation of an electronic data-sheet and by receiving the adjusted dosage through the informatic support he choosed (*anticoagulation passport*, SMS, E-mail, ...). *Results*. Evaluated 1200 patients managed with this procedure during the last 16 months: 1. lower incidence of minor events (hemorrhagic and thrombotic) in the group of patients managed by mobile monitoring unit (minor events percentage p/y from 21 to 13.5 respectively); 2. higher rate of days within the target range (from 40% to 72%) with significantly comparable data between the Hospital and the territorial setting; 3. higher proportion of patients directly referring to their community medical services, (better distribution of the work entailed).

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A NEW MULTIMODULAR ALGORITHM FOR SUPPORT THE TERRITORIAL COMPUTER ASSISTED PRESCRIPTION

Minozzi M, Mitterer M

Centro Trasfusionale, Azienda Ospedaliera di Merano, Italy

The worldwide increased demand for anticoagulant treatment is leading to the devolution of management of this treatment from specialized centres to territorial settings, where the use of mobile prothrombine check systems and prescriptions are becoming always more common. The only possible way to reach and maintain comparable quality standards between Hospital and territorial settings is by supporting them with a centralized net supported program which provides the automatic dose-adjustment and, if necessary, the advice of trainee doctors of the anticoagulation clinics. *Objectives.* We want to determine the efficiency of the computer assisted prescription based on the application of a new multimodular algorithm, which generates different dosage proposals according to different patient's categories and situations:1) initiation dosage (two modules); 2) adjusting dosage; 3) maintaining dosage. *Subjects*. 1072 unselected patients (stable and unstable patients during initiation and maintaining therapy). *Methods.* We compared 3 months management by trainee doctors without assistance from computerized support system (effectively prescribed doses) to a simulated computer supported management (computer suggested doses). *Main Outcome Measures*. Comparison between computer assisted decision to unassisted decision of trainee doctors, to evaluate the correlation of the computer proposal with the effectively prescribed dosage. *Results.* Computer unassisted dose adjusted group: 1)number of patients: 1072; 2)number of INRs: 3639; 3)proportion of time in range (days): 71.9%; Mean time between visits: 1,2/month; Proportion of days with low INRs: 16.3%; Proportion of days with high INRs: 11.7%; Proportion of days with INR >5.0: 0.5%. Significance of the correlation between the effectively prescribed doses and the computer suggested doses: r2 = 0.96.

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DIFFERENCE IN MORTALITY AFTER FRACTURE OF THE HIP IS ASSOCIATED WITH POST-DISCHARGE PRESCRIPTION OF ANTI-THROMBOTIC PROPHYLAXIS

Grion AM,* Gallo U,* Bano F,* Ragazzi M,* Cestrone A,* Orsini A,* Salomoni M,* Gaion R,° Pengo V#

**Pharmaceutical Department ASL 16 Padova, °Department of Pharmacology and Anestesiology University of Padua, #Clinical Cardiology, Thombosis Centre, University of Padua, Italy*

The aim of the present study was to determine whether a prolongation of pharmaceutical antithrombotic prophylaxis beyond hospitalization for hip fracture is associated with a reduced mortality rate. Among the 407 patients over 50 years of age admitted to local general hospitals in 1999, we identifyed 179 cases who received a post-discharge prescription of any anti-thrombotic

agent (heparin, oral anticoagulants, antiplatelet drugs) and 179 age and-sex matched patients with hip fracture who did not. The main outcome measure was post-discharge mortality at 90 days. Compared with patients who did not receive post-discharge prescription of any anti-thrombotic agent, those who did had an odds ratio of 0.22 (95% confidence interval 0.08-0.59) for all causes mortality. This result did not change after excluding non-vascular mortality (odds ratio=0.17, confidence interval 0.03-0.73, p=0.011). In conclusion, patients admitted to the hospital for hip fracture are at high risk of death after discharge if they are not prescribed antithrombotic treatment. To substantiate these data, *ad hoc* prospective randomized trials are needed.

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PREGNANCY AND RISK OF ABORTION IN WOMEN WITH ESSENTIAL THROMBOCYTHEMIA

Candoni A, Damiani D, Michelutti T, Russo D, Fanin R, Baccarani M*

*Division of Hematology and Bone Marrow Transplantation, University Hospital, Udine; *Institute of Hematology and Medical Oncology "Seragnoli", University of Bologna, Italy*

Essential thrombocythemia (ET) is a chronic myeloproliferative disease associated with an increased risk of thrombotic complications.Here we report our experience in a specific group of young patients with ET: women in pregnancy. Between 1989 and 2000, 50 women younger than 45 years with ET were seen at our Institution. Diagnosis was made according to the *Polycythemia Vera Study Group* criteria. Among the 50 ET women, 17 pregnancies occured in 12 patients; these cases were retrospectively analyzed. The median age at diagnosis of ET was 27 years (range 17-39) and 28 years (range 19-40) at the beginning of pregnancy; in 7/12 women the disease was discovered incidentally and only 5/12 patients were symptomatic. Platelet counts at diagnosis ranged from 650 to 1,750×10^9/L (median 800×10^9/L). Of the 17 pregnancies 7 (41%) resulted in live birth and 10 (59%) ended in spontaneous abortion (8/10 in the first trimester); all seven deliveries were uncomplicated. Only three patients had episodes of mild vaginal bleeding; no preeclampsia, preterm delivery and complications, during post partum period, were reported. Preconception platelet counts of pregnancies carried to term (median 850×10^9/L; range 650 to 1.300×10^9/L) were similar to those ending in abortion (median 900×10^9/L; range 600 to 1.300×10^9/L); however platelet counts before pregnancy (median 850×10^9/L) were significantly higher as compared with the lowest platelet counts during pregnancy (median 510×10^9/L) (p=0.01). During the post-partum period platelet counts gradually returned to pre-pregnancy levels. No specific therapy was administred in nine pregnancies (seven abortions and two live births); in other eight pregnancies acetylsalicylic acid (ASA 100 mg/day) alone was used (three abortions and five live births). Our experience, in accordance with other authors, confirme that: a) women with ET have an increase risk of spontaneous abortion (59% compared with 15% expected in the general population) especially during the first trimester of pregnancy (80% of all abortions), this risk is not predictable by the pre-conception platelet count; b) the outcome of pregnancy in ET (live birth vs abortion) seems to be positively influenced by anti-aggregant therapy with ASA; c)a significant and spontaneous decrase or normalization in platelet count during pregnancy in ET has been observed; this phenomenon should be related to the placental/fetal production of interferon like substances. The outcome and management of pregnancy in young women with ET is an increasing problem. On the basis of our findings and of the literature data, we believe that large, multicentric and prospective studies are necessary in order to establish the optimal therapeutic approach in this particular subgroup of patients with ET.

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PREGNANCIES AT RISK: FACTOR XA ASSAY DURING LOW MOLECULAR WEIGHT HEPARIN TREATMENT

Vaccarino A,° Montaruli B,° Donvito V,* Foli C,° Maina A,* Rus C,° Saitta M,° Bazzan M°

*°U.O.A. Ematologia e Malattie Trombotiche, Ospedale Evangelico Valdese, Turin; *O.I.R.M. S. Anna, Turin, Italy*

Laboratory monitoring is generally not required during treatment with low molecular weight heparins (LMWHs). Unfractionated heparin (UH) has been reported to lack a linear dose-response (assayed as anti-Xa activity) during pregnacy. LMWHs are replacing UH in antithrombotic prophylaxis and treatment, also during pregnancy. In a small percentage of pregnant women (i.e. in patients at high risk, or with previous thromboembolic events, or with severe chronic renal failure) monitoring LMWH effect (as anti-Xa activity) could be useful to obtain a correct anticoagulant action. To evaluate how many women were in prophylactic or therapeutic anticoagulant range under LMWH treatment during pregnancy we studied 15 pregnant women at high trombotic risk. Thrombotic risk was defined as a previous thromboembolic event (12 patients) or an inherited thrombophilic defect, or serious obstetric complications. Anti-Xa activity was assayed in 38 blood samples; 15 were obtained in the 1st, 13 in the 2nd and 10 in the 3rd trimester of pregnancy. Blood samples were all obtained 3 hours after LMWH injection. Factor Xa assay were repeated twice in each sample. Xa activity was performed using a commercial kit (IL- Test heparin), modified according to our previous experience. Profilaxis range were 0.1-0.3 antiXa IU/mL for prophylaxis, 0.3-0.8 for full anticoagulant tratment respectively. Anti-Xa activities were always in range during the first trimester of pregnancy. In contrast, 6 out of 13 were not in range during the second, and 3 out of 10 during the third. LMWH treatment during pregnancy, when used according to standardized schedules (fixed doses or weight-adjusted doses) have a good dose-responce during the first trimester of pregnancy. On the contrary, in a significant number of patients this was not observed in the second and third trimester.

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ORAL ANTICOAGULANT THERAPY IN PATIENTS WITH ACTIVE CANCER

Poli D, Antonucci E, Lombardi A, Marcucci R, Fatini C, Corsini I, Falciani M, Abbate R, Prisco D

Dipartimento di Area Critica Medico-Chirurgica, Università degli Studi di Firenze, Centro di Riferimento Regionale per la Trombosi, AO Careggi, Florence, Italy

In the last years the use of oral anticoagulant treatment (OAT) has increased also in patients with active cancer . Our study was aimed to evaluate the efficacy and safety of OAT in patients with active cancer referred for the control to the Anticoagula-

tion Clinic of the University of Florence. From June 1995 to September 2001, 49 patients have been enrolled (21 females, 28 males). Their mean age was 66.2±10.1 years and total follow-up period was 79 pt/yrs. Thirty-three out of 49 patients were in OAT for a previous venous thromboembolic event, 9 had mechanical heart valves and 7 suffered from cardiovascular disease. Nine patients were affected by breast cancer, 3 by uterine carcinoma, 13 by large intestine cancer, 7 by hematologic cancer, 6 by lung cancer, 5 by prostatic carcinoma, 3 by urinary tract cancer, 2 by liver carcinoma and 1 by rhinopharyngeal cancer. We observed that this group of patients spent a shorter time in intended therapeutic range in comparison to the whole population referred to our Centre (54.7% vs 66% respectively). The time spent above and below the intended INR was respectively 21.2% and 24.1% vs 16% and 18% of the whole population. Two patients experienced major bleedings (both non fatal uterine bleeding). The rate of major bleedings in neoplastic patients was higher than in the whole population (2.5 vs 1.1 per 100 pt/yrs respectively) [relative risk (RR) was 2.3 (95% CI 1.4-4.1), p=0.000]. We observed 9 thrombotic events (5 DVT and 4 arterial thrombosis); the rate of thrombotic complications was 11.3 per 100 pt/yrs, in comparison to the 3.8 per 100 pt/yrs of our population [RR 3.3 (95% CI 2.6-4.5) p=0.000)]. Our results show that OAT in cancer patients causes an increase of bleeding and thrombotic complications. Careful surveillance of OAT in this group of patients is needed.

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LOW RATE OF BLEEDING AND THROMBOTIC COMPLICATIONS OF ORAL ANTICOAGULANT THERAPY INDEPENDENTLY OF AGE IN THE REAL-PRACTICE OF AN ANTICOAGULATION CLINIC

Poli D, Antonucci E, Lombardi A, Marcucci R, Rizzuti G, Falciani M, Gensini GF, Abbate R, Prisco D

Dipartimento di Area Critica Medico-Chirurgica, Università degli Studi di Firenze,Centro di Riferimento Regionale per la Trombosi, AO Careggi, Florence, Italy

Over the last years, there has been a world-wide increase in oral anticoagulant treatment (OAT). This study was aimed at evaluating the efficacy and safety of OAT managing in a real-practice situation. 903 consecutive unselected patients referred for the control of OAT to the Anticoagulation Clinic of the University of Florence were studied. The total follow-up period was 1,679 patient-years. The rate of total, major and fatal bleeding events was 5.0, 1.1 and 0.06 per 100 patient years, respectively. In patients with a target INR ≥ 3 a significantly higher rate of bleeding (p = 0.02) with respect to patients with a target INR < 3 was observed. The rate of all thrombotic events was 3.8 per 100 patient years. The rate of major and fatal thrombotic events were 2.4 and 0.4 per 100 patient years, respectively. These results indicate that a low incidence of complications may be obtained even in elderly outpatients on OAT followed in an anticoagulation clinic specifically devoted to this management.

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A COMPARISON BETWEEN ROUTINE PLASMA INR AND FINGERSTICK WHOLE BLOOD INR DETERMINATIONS, USING THREE PORTABLE SELF-TESTING DEVICES (COAGUCHECK®, PRO-TIME®, GEM-PLC®) AND A NEAR-PATIENT WHOLE BLOOD DEVICE (THROMBOTRACK®)

Filippucci E, De Monte P, Cavalieri G, Boschetti E, Gresele P

Section of Internal and Cardiovascular Medicine, Department of Internal Medicine, University of Perugia, Italy

Background. Oral anticoagulant therapy is conventionally monitored by the International Normalized Ratio (INR) on plasma samples. In alternative, capillary blood monitoring has been used for years by adopting the Thrombotest® reagent that, differently from the prothrombin time (PT), detects oral anticoagulant-induced deficiency of factor IX. More recently, several portable monitors for INR determination on capillary whole blood have been introduced for the self-testing of oral anticoagulation. No systematic simultaneous comparison of the Thrombotest near-patient and of portable self-testing whole blood devices, has been performed so far, to the best of our knowledge. *Objective*. To compare the accuracy of four whole blood coagulation monitors (Thrombotrack, Hyland Baxter Immuno; CoaguCheck S, Roche; GEM PLC, IL; Pro-time, IL, USA) in reference to standard PT (IL Recombiplastin) for INR determination on a wide range of anticoagulation levels. *Materials and methods*. Dual INR measurements were performed in patients on long-term oral anticoagulation, stabilized for at least 6 months both from finger capillary blood, by one of the four monitors, and from venous blood by the plasma PT. The accuracy of the whole blood coagulation monitors was assessed by evaluating, in comparison to laboratory PT: correlation and agreement; the absolute difference in INR; the proportion of whole blood monitor results within 0.5 INR units of laboratory PT. Results are reported in the table below.

Parameter compared to plasma INR	Thrombotest N=230	Coagucheck N=167	GEM PCL N=99	Pro-Time N=97
Correlation (r^2) – all INRs	0.80	0.77	0.61	0.68
Concordance limits (CL; 95% CI)	–0.63;+0.54	–0.34; +1.22	– 1.7; +1.17	–0.64; +0.54
Agreement (% of results within CL)	83	80	81	72
Mean of differences (INR monitor – INR lab)	–0.03	0.44#	–0.55#	–0.05
% of results within 0.5 INR units				
– all INRs*	79	65	51	70
– INR <2	91	90	97	85
– INR 2-3	88	65	52	82
– INR >3	62	43	6	55

*r^2 = 26.8, p<0.001; # p<0.001

Discussion. Whole blood monitors showed a good correlation with plasma PT. However significant differences concerning agreement and the mean of differences as compared to plasma PT exist between the various whole blood monitors. In particular, some of the whole blood monitors showed a very low concordance and agreement with PT values in the high therapeutic range.

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SUCCESSFUL TREATMENT WITH DERMATAN SULPHATE AND PROTEIN C CONCENTRATE OF A PEDIATRIC PATIENT WITH SEVERE PROTEIN C DEFICIENCY, HEPARIN-INDUCED THROMBOCYTOPENIA AND WARFARIN SKIN NECROSIS

Gatti L,^ Carnelli V,° Rusconi R,° Moia M*

*^Servizio Immunoematologia Trasfusionale, ICP di Milano, °Clinica Pediatrica II Università degli Studi di Milano, *Centro Emofilia e Trombosi, IRCCS Ospedale Maggiore di Milano, Italy*

A eight-year old boy developed DVT of his right leg without evident triggering factors. At admission the patient had very low protein C levels (6 U/mL, amidolytic assay). We were able to test only the patient's mother, who has moderate protein C deficiency. The patient was initially treated with intravenous (IV) unfractionated heparin (about 28 IU/Kg/h) and warfarin, started at low doses on the third day. After ten days of combined treatment (heparin and warfarin), a sudden decrease of platelet count was recorded (from 320×10^9/L to 48×10^9/L) and was accompanied by worsening of the clinical symptoms of DVT (swelling of the leg, pain and cutaneous hemorrhagic effusions). Laboratory tests confirmed the hypothesis of heparin induced thrombocytopenia. Heparin was withdrawn and IV dermatan sulphate (DS, 0.6 mg/Kg/h) was started with the aim of maintaining the aPTT ratio between 1.5 and 2.0 times the basal value. Platelet count rapidly increased and clinical symptoms improved. Warfarin was started again at low doses together with protein C concentrate infusion (40 U/Kg/24h, for 8 days). DS was withdrawn after 10 days when INR values were above 2.0 for more than 3 days. After 4 days the patient developed a large post-traumatic hematoma of the contralateral thigh. Warfarin was only tapered (INR lowest value 1.8), but the patient developed worsening of the clinical symptoms of DVT and cutaneous hemorrhagic effusions, with laboratory signs of consumption coagulopathy. Warfarin was stopped, protein C concentrate (for 2 days only) and DS were resumed. After 10 days, DS was shifted to intramuscular route,11 mg/Kg/day, by single injection. The patient (who lives in a Country with poor medical facilities) is now continuing intramuscular DS. In this patient DS was an effective treatment in the acute phase of HIT and warfarin skin necrosis, and a reasonable choice for secondary prophylaxis of venous thromboembolism.

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DERMATAN SULPHATE AS A TREATMENT FOR HEPARIN-INDUCED THROMBOCYTOPENIA WITH VENOUS AXILLARY AND SUBCLAVIAN THROMBOSIS AT SITE OF INTRAVENOUS CATHETER INSERTION

Schinco PC, Tamponi G, Borchiellini A, Pollio B, Cristofori R^

Department of Onco-Hematology and ^Thoracic Surgery, University of Turin, San Giovanni Battista Hospital, Turin, Italy

A 60-year old patient with thymoma underwent surgery while on prophylaxis with LMW heparin and developed heparin-induced thrombocytopenia (HIT) on day 4 postoperatively, as shown by an ELISA serological antibody detection test and platelet count. US scan revealed axillary and subclavian venous thrombosis around the indwelling catheter. Serological sceening tests for hereditary thrombophilia were negative. APA were not detected in serum. LMW heparin administration was immediately stopped and dermatan sulphate therapy started (300 mg IV bolus followed by 0.6 mg/kg/h continuous infusion) (Mistral, Mediolanum Farmaceutici S.p.A.). Therapy was regularly monitored by aPTT evaluation, which was kept at 1.5-2 times the control normal value. Twenty-four hours later OAT was started with warfarin and dermatan sulphate stopped when INR settled around 2.5 for two consecutive days. Therapy was well tolerated, no hemorrhagic or other complication occurred and prompt recanalization of the venous axis was observed. OAT was administered for six months (INR around 2.5) and the catheter removed after three months of therapy. The patient underwent surgery once more six months later under prophylactic administration of i.m. dermatan sulphate, 300 mg b.i.d i.m. on day 1 and 300 mg/d i.m. from day 2. The post-operative clinical course was uneventful.

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TREATMENT OF PULMONARY EMBOLISM SECONDARY TO ESSENTIAL THROMBOCYTHEMIA: A CASE REPORT

Alatri A,* Testa S,* Bergonzi C,° D'Armini A,# Piovella F§

**Centro Emostasi e Trombosi e °Servizio di Ematologia, A.O. Istituti Ospitalieri di Cremona, Cremona; §Servizio Malattie Tromboemboliche e #Divisione di Cardiochirurgia, IRCCS Policlinico S.Matteo, Pavia, Italy*

We describe a case report of pulmonary embolism (PE) secondary to essential thrombocythemia (ET) not responsive to conventional antithrombotic therapy. A 50-years old woman was admitted to our hospital because of persisting fever, dyspnea and chest pain lasting for nearly 20 days. A perfusion lung scan showed high probability pulmonary embolism. Color-Doppler-ultrasonography ruled out a thrombosis both of inferior limbs veins and of cava and iliac veins. Anticoagulant therapy (intravenous unfractionated heparin and warfarin) was immediately started. Despite a good therapeutic management, the clinical conditions progressively worsened over the next few days with an increase of pulmonary pressure (from 60 mmHg to 72 mmHg). Rt-PA 40 mg was administered intravenously over 15 minutes without any benefit. The complete blood cell counts on admission showed thrombocytosis progressively increased up to 1.400.000 cell/m^3. A diagnosis of ET was formulated according to the Polycythemia Vera Study Group criteria. Ticlopidin 250 mg/die was started associated to hydroxyurea 1000mg/die, with a initial mild reduction of clinical conditions. After one year of medical treatment, despite an improving of general clinical conditions (pulmonary blood pressure was 40 mmHg; no dyspnea at rest, tachycardia and tachypnea are observed), chest angiography did not show any change in thrombi dimension. For this reason, patient was selected to undergo pulmonary thromboendoarterectomy (PTE), being included in the Pavia ThromboEndarterectomy Program for chronic thromboembolic pulmonary hypertension. The clinical general conditions 7 days after surgery were unchanged; pulmonary blood pressures were increased (from 40mmHg to 60mmHg). Histologic material showed macroscopic and microscopic differences with respect to typical fibrinous thrombus. Even if other studies are needed, this case report suggests that PE secondary to ET seems to have different characteristics in clinical course and therapeutic management. This statement also applies to the secondary pulmonary hypertension that follows. Probably it requires combined therapy in addition to conventional

antithrombotic treatment. PTE, a surgical procedure indicated in selected patients with chronic thromboembolic pulmonary hypertension, did not have show any advantage in this patient.

P160

PERIOPERATIVE MANAGEMENT WITH NADROPARIN OF PATIENTS ON LONG TERM ORAL ANTICOAGULANT THERAPY: A PROSPECTIVE STUDY

Mostarda G, Boniardi M,* Finzi M,° Grassi G,* Morra E, Baudo F

Departments of Hematology-Thrombosis Hemostasis Unit, Surgery Pizzamiglio° and Ponti, Ospedale Niguarda Cà Granda, Milan, Italy*

The perioperative management of the patients on long term oral anticoagulant therapy (OAT) is controversial. Therapeutic options are discontinuation of OAT with an INR target of <2.0 at the time of surgery and resumption of OAT as soon as possible; replacement of OAT with intravenous (iv) heparin; home treatment with low molecular weight heparin in order to reduce the time of the hospitalization. From January 2001 34 patients on OAT underwent elective surgery. Indications for OAT were mechanical prosthetic heart valve (MPHV), recent DVT/PE, two or more thromboembolic risk factors. *Treatment*. OAT discontinued 3-5 days prior to surgery and substituted by nadroparin (60 U/kg s.c. bid) at a INR value < the therapeutic range; last dose of nadroparin administered the evening before surgery; resumed 12 hrs after surgery. OAT restarted 12-24 hours after surgery. Nadroparin discontinued when the INR within the therapeutic range. Vitamin K is not given. *Study population*. Thity-four patients (24 males, 10 females), median age 67 years (47-83). OAT indication: atrial fibrillation (17 patients), MPHV (9 patients), DVT/PE (9 patients), mitral valve disease with AF (6 patients), congenital cardiopathy (1 patients), atrial thrombus (1 patient); in 9 patients more indications for OAT; in 12 patients concomitant pre-existent risk factors: acute inflammatory disease (1), neoplasia (7), bed rest > 3 days (3), CVC (1). Type of surgery: herniotomy (16 pts), hemicolectomy (3), cholecystectomy (6), gastrectomy 2, ileum resection 3, thyroidectomy, mastectomy, jejunostomy, hemorrhoidectomy (1 each). *Results*. Post-surgery median follow up 33 days (22-50). Duration time of heparinisation: median 11 days (-12;+17). Post operative INR in the therapeutic range in 6 days (1-17). No thrombotic or fatal events; 2 major bleedings (1 gastrectomy for neoplasia, 1 laparoscopic cholecystectomy); 2 minor bleedings (herniotomy). *Comments*. Nadroparin, initiated at home, is a convenient choice for thrombosis prophylaxis in patients on long term OAT submitted to surgical procedures.

P161

LOW MOLECULAR WEIGHT HEPARIN TREATMENT IN PREGNANT WOMEN WITH INHERITED AND ACQUIRED THROMBOPHILIA: A MULTIDISCIPLINARY APPROACH

Castellino G, Brambilla G, Mostarda G,* Brucato A, Pisoni MP,^ Solerte L,^ Muscarà M, Redaelli R,* Canesi B, Morra E,* Baudo F*

*Departments of Rheumatology, ^Obstetrics, *Hematology-Thrombosis Hemostasis Unit, Niguarda Hospital, Milan, Italy*

Prophylaxis with heparin and/or acetylsalicylic acid (ASA) improves pregnancy outcome and avoids thromboembolic complications in the antiphospholipids syndrome (APS). Nevertheless the treatment of pregnant women with inherited thrombophilia (IT) is still controversial. We report the pregnancy outcome of 21 women with IT and acquired thrombophilia (AT) followed by a multidisciplinary team (obstetric, haematology, rheumatology). *Patients and methods*. Twenty-five pregnancies in 11 women with antiphospholipid antibodies (aPL) or APS and 10 women with IT were prospectively followed. Four APS patients; 6 connective tissue disorders patients with aPL and/or lupus anticoagulant (LAC); 5 heterozygous and 1 homozygous carriers of factor V Leiden mutation; 5 heterozygous carriers of prothrombin gene mutation. Six patients had combined risk factors. Treatment protocol. Only laboratory abnormalities: no therapy, but ASA in AT. APS: ASA + Nadroparin 0.4-0.6 mL/day s.c. IT + previous clinical events: Nadroparin. The treatment was started from positive gravindex. In puerperium Nadroparin in all the patients. *Results*. AT patients: 14 pregnancies; 6 treated with LMWH + ASA (5 normal delivery, 1 premature membrane rupture); 8 with ASA alone (6 normal delivery and 2 early abortion). IT patients: 11 pregnancies (10 ended with a normal delivery and 1 with mild prematurity at 35th week); 7 treated with LMWH + ASA; 2 with only ASA; 2 without any drug. *Conclusion*. Thrombophilic women with high risk pregnancies may be successfully managed if closely followed by a multidisciplinary team. Even if our series is small, in this prospective study our protocol was effective and safe.

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MONITORING OF PT-INR WITH THE HELP OF A PORTABLE COAGULOMETER

Porcu A, Porcu A, Lochi MF, Pasciu D, Mulas R, Porcu PP, Sanna M

Service Of Laboratory Medicine And Microbiology, Oncology Hospital "A. Businco", Cagliari, Italy

Clinical indications for oral anticoagulant therapy (OAT) have increased steadily during the past few years. In addition to patients with DVT, ictus and cardiac valves prothesis, for which OAT is a standard therapy, it has been shown that other categories, such as patients with chronic atrial fibrillation, can also benefit from this type of treatment. As a consequence, the number of patients taking OAT has also increased (over 600,000 in Italy) and is predicted to further increase in the coming years. The health care system must therefore meet an increased public demand for the management of these patients, in terms of both space and time. To this end, a relevant improvement has been the introduction of a new technique to test PT-INR on whole blood, which can be performed with a portable coagulometer at the patient's home. Numerous studies have confirmed that results obtained with this technique are comparable to those of a standard hospital-based laboratory. The aim of the present study was to verify the reliability of one such instrument, Coag-Check from Roche, and to compare its performance to that of a standard automated method routinely used in our laboratory (CA-6000 from Dade Behring). *Materials and Methods*. A group of 76 patients (41 males, 35 females; age: 40 to 68 years), referred to our Institution (FCSA center 141) for chronic OAT, underwent double PT-INR test, using both the portable coagulometer and the standard laboratory method.

Results obtained are reported in the Table below.

	CA 6000	Coag-check
Reading interval	1 – 6.88	0.8 – 5.9
Mean values	3.19	3.01
Std. Dev.	1.23	1.05

The correlation coefficient was 0.88. *Conclusions.* The above data indicate that overall results obtained with Coag-Check are comparable to those of a standard laboratory instrument. However, differences were noticed between the two methods for PT-INR values above 5. The follow up of the latter patients should therefore be performed in standard laboratory setup.

P162a

CLINICAL USE OF DERMATAN SULPHATE IN HEMODIALYSIS PATIENTS WITH ANTI-HEPARIN ANTIBODIES

Borchiellini A, Schinco PC, Tamponi G, Martina G,* Pollio B

*Department of Onco-Hematology and *Nephrology, University of Turin; San Giovanni Battista Hospital, Turin, Italy*

Five hemodialysis patients, followed up at the University Department of Nephrology in our hospital, came to our observation for heparin-induced thrombocytopenia (HIT); clinical diagnosis was confirmed by positive antibody ELISA detection in sera (Diagnostica Asserachrom Roche). UF heparin was routinely administered as a bolus of 5.000 U.I. to patients before each hemodialytic session. None of these patients developed thrombocytopenia-associated thrombosis; however, in 3 of them, later lost in follow up, thrombocytopenia was reversed by heparin withdrawal and hemodialytic sessions were then carried out without heparin, whereas the remaining two developed rapidly worsening thrombocytopenia and were put on dermatan sulphate therapy, 6 mg/kg IV bolus before every hemodialytic procedure (Mistral, Mediolanum Farmaceutici S.p.A., Italy). Therapy with dermatan sulphate was safe and well tolerated and no clot formation in hemodialytic filters was observed. HIT antibodies became negative in the sera of the two subjects after approximately 30 days and persisted as such. One patient developed bilateral necrosis of femur heads after a few months therapy with dermatan sulphate.

P162b

AN EMILIA ROMAGNA REGION PROJECT TO UNIFORM ORAL ANTICOAGULANT TREATMENT IN PARMA AREA

Manotti C,* Pattacini C,* Tagliaferri A,* Zurlini C#

**Dip. Medicina 3, Centro per le Malattie dell'Emostasi e Cura dell'Emofilia, Azienda Ospedaliera di Parma; #Dip. Patologia Clinica Azienda USL di Parma, Italy*

Oral anticoagulant therapy is increasingly used for treatment of various thromboembolic diseases. In Parma area about 6,000 out of 500,000 inhabitants (1,25%) are managed by four Anticoagulation Clinics (AC), which deliver the service in different ways. One AC manages patients (n = 3,500) by P.A.R.M.A. SYSTEM a Local Area Network – LAN where all the operative units (patients' reception, laboratory, and specialist office) are directly connected to the same database: an automatic computerised program for dosage suggestion (validate by multicentre prospective trial, APROAT study, Haematologica, 2001) is implemented in the system. The other three ACs deliver service by traditional non-informatised archive and medical staff dosage. Patients in the same area are monitored in a very dissimilar way from AC to AC. It is well established that computerised dosage improve the quality of treatment in comparison with medical staff dosage; a LAN implementation may also allows personal staff saving time, reducing clerical work avoiding errors and also improve patient's quality life. The objective of the project is to uniform service delivered to anticoagulated patients in Parma area. It has been decided to implement PARMA SYSTEM in every AC and to connect each other with the same database, in this way it is possible to uniform service delivered of all patients and every AC may use computerised dosage for patient attending clinic. A register of all patients monitored in Parma area will be build. Every ACs must follow a common guideline derived by national and international bodies to uniform the delivered service. To assess the improving of delivered service several analyses will be performed before and after project implementation: a) statistical analysis on the quality of treatment. b) time spent by personnel involved in the service; c) patients' saved time; d) direct and indirect costs; e) calculation of optimal ratio between number of patients attending an AC and personal involved.

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Posters
Fibrinolysis

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DIAGNOSTIC ACCURACY OF D-DIMER TEST IS AFFECTED BY THROMBUS SITE, DURATION OF SYMPTOMS AND HEPARIN TREATMENT

Siragusa S,# Terulla V,* Pirrelli S,** Porta C, Falaschi F, Anastasio R, Guardone R, Scartabelli M, Odero A,** Bressan MA

*Servizio Pronto Soccorso Accettazione, *Servizio Analisi Microbiologiche and **Divisione di Chirurgia Vascolare, IRCCS Policlinico S. Matteo, Pavia, Italy*

The measurement of D-dimer is claimed to have potential value in excluding deep vein thrombosis (DVT). New rapid methods have been proposed, but few clinical trials have assessed their performance on an emergency basis. The different accuracy found between the D-dimer assays has been related to the test used (latex or ELISA), but other variables (such as the population investigated, thrombus extension, duration of symptoms or concurrent heparin treatment) may be important, and have not been investigated, so far. *Design and Methods*. We evaluated the accuracy of a rapid semi-quantitative D-dimer tests (Dimertest®, Dade Behring), with reference to: a) their use in an emergency unit; b) concomitant heparin administration; c) location of venous thrombosis (VT) (in the deep or superficial venous system limited to the greater saphenous vein) and d) symptoms older than 14 days. *Results*. Two hundred and ninety-eight patients suspected with DVT and 116 with suspected thrombosis of the great saphenous vein (GSV) were investigated. The diagnostic accuracy of Dimertest® according to clinical variables is reported in the table below.

	All patients	Excluded pts. on heparin	Excluded pts.with symptoms >15 days	Patients with suspected GSV thrombosis
Sensitivity (95% CI)	81.5% (69.1-93.9)	88.5% (78.5-98.5)	91.1% (81.5-100.7)	74% (57.9-90.5)
Specificty (95% CI)	78.4% (70-86.6%)	78.4% (70-86.6)	78.4% (70-86.6)	89.4% (79.5-99.3)
Positive PV* (95% CI)	60.7% (47.3-74.1)	60.7% (47.3-74.1)	60.7% (47.3-74.1)	83.3% (68.1-98.5)
Negative PV (95% CI)	91.2% (85-97.4)	94.8% (89.5-100.1)	96% (91.6-100.4)	82.9% (71.5-94.3)

*Predictive value.

Interpretation and Conclusions. Our results show that previous or concomitant heparin administration (either at therapeutic or prophylactic doses), non-acute symptoms and thrombosis localized to superficial veins reduces the clinical usefulness of the test due to the increase of the false negative result rate.

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AUTOLOGOUS PLASMIN IN SURGICAL MANAGEMENT OF DIABETIC RETINOPATHY

Della Valle P,* Azzolini C,° Codenotti M,° Maestranzi G,° Brancato R,° D'Angelo A

**Coagulation Service & Thrombosis Research Unit; °Dept of Ophthalmology and Visual Sciences, IRCCS H S. Raffaele, Milan, Italy*

Enzymatic manipulation of vitreous and vitreoretinal juncture is currently in the process of being actively evaluated. The goals of such manipulation are to disinsert the posterior hyaloid from the retinal surface in an atraumatic and clean cleavage plane or the peripheral vitreous from neurosensory retina. This is a pilot study to assess the efficacy of autologous plasmin (AP) in the surgical management of diabetic macular edema secondary to posterior hyaloid traction. Five days before surgery, 30 mL of venous blood were drawn from the patients' antecubital vein. Plasminogen was isolated from citrated plasma by affinity chromatography on a lysine-Sepharose column. The plasminogen was eluted with epsilon aminocaproic acid and washed with PBS on centrifugal filter unit. After concentratrion and activation by addition of streptokinase (50,000 IU/mg of plasminogen), the plasmin preparation was sterilized by passage through a 0.22 micron filter, calibrated versus IRP for plasmin and stored at -80°C until used. All preparations were negative at bacteriological assays. Eleven eyes of 10 consecutive patients were considered. Vitreoretinal surgery was performed 25 min after injection of 0.6-0.8 IU of AP in a volume of 100-200 μL. Patients were examined after one day, 1 week, 1 and 3 months. The entity of enzymatic posterior hyaloid detachment was classified by the surgeon as totally adherent, partially detached and totally detached. The surgical removal of posterior hyaloid was judged as difficult, easy, very easy. After the enzymatic vitreolysis, the posterior hyaloid was judged adherent in 3 eyes, partially detached in 6 eyes and totally detached in 2 eyes. The posterior hyaloid removal was judged difficult in 2 patients, easy in 3, and very easy in 6 eyes. These results suggest the efficacy of autologous plasmin in the removal of posterior hyaloid in these patients. Critical points are the exact autologous plasmin enzyme concentration, the enzyme-posterior pole contact and the potential for intravitreal enzyme neutralization.

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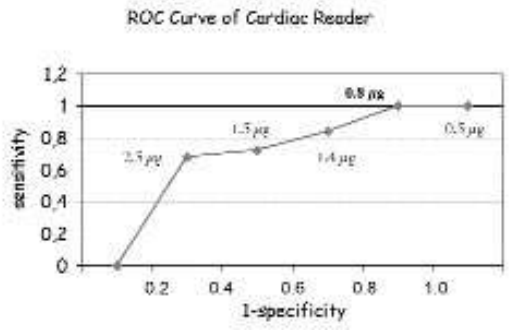
DIAGNOSTIC ACCURACY OF A NOVEL RAPID D-DIMER ASSAY (CARDIAC READER®) USED AS A SCREENING TEST IN PATIENTS SUSPECTED OF HAVING ACUTE DEEP VEIN THROMBOSIS IN THE EMERGENCY WARD

Granzow K, Anastasio R,* Buonanno C, Falaschi F, Bressan MA, Siragusa S*

*Servizio Pronto Soccorso ed Accettazione, IRCCS Policlinico S. Matteo, Pavia; *Unità Malattie Tromboemboliche ed Emorragiche, Cattedra di Ematologia, Università di Palermo, Italy*

The assay of D-dimer is considered a valuable tool in excluding the presence of an ongoing deep vein thrombosis (DVT); enzyme-linked immunosorbent assay (ELISA) techniques show a very high sensitivity and negative predictive value in the diagnosis of DVT, but are not suitable for immediate measurements.

New, rapid methods are available which are suitable for extensive use in critically ill patients with suspected DVT, but few clinical trials have assessed their performance in an emergency setting. During the period January-July 2001, we prospectively investigated 88 patients referred to our emergency ward (EW) with the clinical suspicion of DVT of the lower limbs. Six patients were excluded (2 because on oral anticoagulants, 4 because of a lack of objective diagnosis). All remaining patients were tested for D-dimer determination (Cardiac D-dimer, normal values 0.1-4.0 μgr/mL, time of performance 8 min.) and compression ultrasonography (CUS) of the symptomatic leg. D-dimer test was assayed in resting patients by an investigator unaware of the CUS result; the test was performed using an immune-enzymatic quantitative method (Cardiac Reader®, Roche Diagnostics) which also furnishes rapid determination of myoglobin and T-troponin levels. The accuracy of the test at different levels of D-dimer concentration is reported in Figure 1, based on the events that occurred in 3 months of follow-up. In the population investigated, the best cut-off of cardiac D-dimer® was obtained at a value higher (800 μg/mL) than that suggested by the manufacturer (500 μg/mL). In our opinion, this is the level of the D-dimer assay in question that may be appropriate in the population investigated; it should be used for obtaining the best predictive value for the screening of patients clinically suspected of having a DVT. Further investigation should corroborate these findings.



P166

A STUDY OF PLATELET FUNCTION DURING CARDIOPULMONARY BYPASS WITH TWO NEW "POINT OF CARE" ANALYZERS

Paniccia R, Ridolfi N,* Stefano PL,^ Bandinelli B, Attanasio M, Gori AM, Ilari I, Marcucci R, Gensini GF, Prisco D

*Dipartimento di Area Critica Medico-Chirurgica, Università degli Studi di Firenze; UU.OO. di *Centro Trombosi, Anestesiologia e Rianimazione 1, ^Cardiochirurgia, Azienda Ospedaliera Careggi, Florence, Italy*

Platelet dysfunction (PDF) can be the most frequent cause of bleeding complications during cardiopulmonary bypass (CPB). *Point-of-care* devices which identify PDF are under consideration. The PFA–100 system (DADE) records the closure time (CT) taken by platelets to occlude a membrane coated with collagen and either epinephrine (CT/EPI) or ADP (CT/ADP). The Sonoclot analyzer (SIENCO Inc., USA) measures changes in blood viscoelastic properties (clot impedance on a vibrating probe) recording these changes in the form of a graph. Sonoclot *signatures* show a lag period, corresponding to ACT, and a primary wave reflecting fibrin polymerization. Then, an inflection is produced as platelets are incorporated into the fibrin mesh and a secondary upslope leads to a peak, which occurs at completion of fibrin formation. The subsequent downslope is produced as platelets induce further clot retraction. The time to peak (minutes) (TP) reflects clot retraction, and is an indicator of platelet function (PF). This pilot study was designed to evaluate the usefulness of these devices as indicators of PF during CPB. One-hundred and eleven samples from 16 patients undergoing CPB were studied. PF assessment was performed pre and post-heparin bolus, during extracorporeal circulation (ECC) and post-protamine infusion. Pre-heparin: in 10/16 (63%) patients CT/EPI was prolonged. In 4 of these CT/EPI was also extended. TP was prolonged in 6/16 (38%) patients, corresponding to the 4 patients with both altered CT/EPI and CT/ADP and to 2 patients with only prolonged CT/EPI. Post-heparin bolus and during ECC: all CTs and TPs were prolonged. Post-protamine: CT/EPI remained prolonged in 9/10 patients who had baseline prolonged CT/EPI. Three of these showed both CT/ADP and TP prolonged and had significant bleeding following CPB (>500 mL/24hours). These preliminary results suggest that the evaluation of PFA-100 CTs and Sonoclot signature before and following CPB could be a useful screening tool to identify PDF, an important risk factor for bleeding complications after CPB.

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THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR ANTIGEN LEVELS IN POSTMENOPAUSAL WOMEN UNDER TRANSDERMAL HORMONE REPLACEMENT THERAPY

Papa MI,* Papasso F,* Albolino L,* Pudore L,* Torre S,* Russo V,* Papa R,° Cerasuolo L,° Tesorone M,° de Francesco F,#
De Lucia D,# Grandone E@

**Laboratorio di Emostasi e Trombosi, Ospedale San Giovanni Bosco, ASL1, Naples; °Consultori Famigliari, Unità Operative Materno Infantili, ASL1, Naples; #Istituto di Patologia Generale ed Oncologia, II Università, Naples; @Unità di Aterosclerosi e Trombosi, I.R.C.C.S. "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy*

The thrombin activatable fibrinolysis inhibitor (TAFI) is activated by the thrombin-thrombomodulin complex to TAFIa which inhibits fibrinolysis by cleaving lysine residues on fibrin then lowering its binding capacity for t-PA and plasminogen. High TAFI levels were observed in elderly persons and associated with higher cardiovascular ischemic risk factors. To know whether hormone replacement therapy (HRT) may influence such biochemical variations we studied 100 post-menopausal women, 50 under transdermal HRT and 50 non- users and compared them to 100 healthy pre-menopausal women. We assayed TAFI antigen by a new two site ELISA technique (Chromogenix, Malmö, Sweden). Results were expressed as percentages of a normal plasma. Our results show that both groups of post-menopausal women had high plasma levels of TAFI when compared to pre-menopausal women. However, HRT users exhibited lower levels of TAFI compared to those of non-users (83.7±225.21% vs 86.84±25.92 vs 62.68±18.10, respectively; $p<.001$). Our findings show that during the post-menopausal period there was an increase in TAFI plasma concentrations. In conclusion, these results confirmed that TAFI antigen could be influenced by hormonal status with greater levels after menopause. Instead transdermal HRT seems to limit the increased

concentrations found in the post-menopausal period lowering the cardiovascular ischemic risk observed in such a population.

P167a

FIBRINOLYTIC DERANGEMENTS IN PATIENTS WITH THROMBOSIS OF ARTERIOVENOUS FISTULAE

Molino D,* De Lucia D,° Sica G,° Ceccarelli M,° Carannante S,° Del Giudice V,° Marotta R,° de Francesco F,° Perricone F,° Lupone MR,# Anastasio P,* De Santo N*

**Chair of Nephrology, Second University of Naples; °Institute of General Pathology, Clinical Pathology and Laboratory of Haemostasis and Thrombosis, Second University of Naples; #Hemophilia and Thrombosis Center, PausiIlipon Hospital; Naples, Italy*

Vascular complications in patients on hemodialytic treatment are frequent, and thrombosis of fistulae often occurs since knowledge of pathogenesis of thrombotic complications in hemodialysis patients is incomplete, a study of coagulation mechanisms in patients with frequent thrombosis of fistulae was therefore undertaken. A total of 11 patients (P) treated with hemodialysis for a mean of 36 months and 400 healthy blood donors' (C) were enroled into the study. In both groups we recorded: prothrombin time (PT), fibrinogen (Fg), plasminogen (PLG), protein C (PC), protein S (PS), antithrombin III (AT III), APC resistance test (APC-r), D-Dimer (D-D), tissue plasminogen activator (t-PA), Ilasminogen activator inhibitor type-1 (PAI-1), prothrombin fragments 1+2 (F1+2), antibodies lupus anti-coagulant (ACLs), coagulation factors (FV, FVII, FVIII, FIX, FX, FXI, FXII). Our results show that PAI-1 (M±SD) was 46.8±26.3 ng/mL in P and 22.5±11.5 ng/mL in C (p<0.01). F 1+2 was 2.01±0.55 nM in P and 0.48±0.35 nM in C (p<0.01). Factor VIII was 149.4±17.85% in P and 100±55% in C (p<0.01). Factor X was 139.9±30.4% in P and 100±25% in C (p<0.01). The data demonstrate that in patients with frequent thrombosis of the fistulae a state of hypercoagulability is present and is characterised by elevated PAI-1, F 1+2, FVIII, FX plasma levels which are important elements in the control of fibrinolysis and coagulation cascade. The authors feel that derangements in blood coagulation may often occur in patients under hemodialytic treatment with thrombin formation and fibrin deposition associated to higher risk in thrombotic events.

P168

TAFI PLASMA ACTIVITY AND ANTIGEN LEVELS ARE NOT INCREASED IN ISCHEMIC HEART DISEASE PATIENTS ADMITTED TO A CORONARY CARE UNIT

Cellai AP, Alessandrello Liotta A, Fedi S, Rogolino A, Lombardi A, Rostagno C, Comeglio M, Abbate R, Prisco D, Gensini GF

Dipartimento di Area Critica Medico Chirurgica, Università degli Studi di Firenze, Centro di Riferimento Regionale per la Trombosi, AO Careggi, Florence, Italy

Thrombin activatable fibrinolysis inhibitor (TAFI) is a recently described carboxypeptidase, which decreases plasminogen binding to the fibrin surface, and is involved in the regulation of the balance between coagulation and fibrinolysis. High TAFI plasma levels may therefore contribute to an increased risk for thrombotic disorders. Hypofibrinolysis has been reported to occur frequently in patients with ischemic heart disease (IHD). Scarce and controversial data have been reported on TAFI plasma levels in IHD patients. The aims of this study were to measure TAFI activity (ac) and antigen (ag) levels in a group of IHD patients admitted to a Coronary Care Unit (CCU) and in a suitable group of matched controls: 1) to compare TAFI ac and ag levels and 2) to search for differences between IHD patients and controls. Fifty-three patients (40 males, 13 females; mean age 63.3±10 yrs), admitted to the CCU of our department for acute myocardial infarction (AMI) or unstable angina (UA) and fifty healthy controls (mean age 53.8±9.2 yrs) were studied. Plasma TAFI activity and antigen levels were measured by chromogenic assay, and ELISA respectively (American Diagnostica commercialised by IL Milan). No differences in TAFI levels were found between pts and controls (TAFI ac: median 6.9 (2.5-14.0) in patients and 6.3 (0.8-9.8) in c; TAFI ag: median 100 (34-201) in patients and 103 (57-233) in controls. Moreover, TAFI levels were similar in AMI and UA patients. Neither sex-related differences were observed. A significant correlation between TAFI ac and ag levels was found both in patients and in controls (p<0.05). These preliminary results do not support a role of TAFI plasma levels as a relevant risk factor for IHD.

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ACTIVATION OF COAGULATION AND FIBRINOLYSIS DURING MYOCARDIAL REVASCULARIZATION: ON-PUMP VERSUS OFF-PUMP TECHNIQUES

Casati V,* Gerli C,* Franco A,* Della Valle P,° Benussi S,# Alfieri O,# Torri G,* D'Angelo A°

**Department of Anesthesiology, °Coagulation Service and Thrombosis Research Unit, #Division of Cardiac Surgery, IRCCS H S. Raffaele, Milan, Italy*

Activation of coagulation and fibrinolysis pathways during cardiac surgery with extra-corporeal circulation and the potentially resulting hemorrhagic syndromes are well assessed. Recently, the interest in surgical techniques permitting heart operations without cardiopulmonary bypass has increased. This study compares the changes in selected hemostatic parameters in patients undergoing myocardial revascularization with on-pump (CABG) or off-pump (OPCAB) techniques. Platelet counts, antithrombin, fibrinogen, D-dimer, α2-antiplasmin and plasminogen, were measured pre-operatively, 5 minutes after the administration of heparin, 10 minutes after the arrival in ICU, and 24 hours after surgery in consecutive patients scheduled for OPCAB (n = 15) or CABG (n = 15). To correct for dilution, hemostatic parameters and platelet counts were adjusted for the changes in IgG plasma levels and hematocrit, respectively. By adjusting for dilution, there were no differences in the changes observed after surgery for antithrombin (- 4.5%), fibrinogen (-8%), plasminogen and α2-antiplasmin levels in patients submitted to OPCAB or CABG. Only the latter was however associated with platelet consumption (-24%, p = 0.0001) and D-dimer formation (+ 500%, p = 0.004). Twenty-four hours after surgery, platelet counts were still lower in patients submitted to CABG (p = 0.049), but all the investigated parameters were similar in the two groups of patients when adjusted for dilution. Heart revascularization surgery involves a net consumption of antithrombin and fibrinogen. Transient platelet consumption and D-dimer formation are, however, only observed with CABG. Twenty-four hours after surgery, the hemostatic pattern of patients submitted to OPCAB or CABG is similar.

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