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

Rome, May 9-12, 2002
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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
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.

<table>
<thead>
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<th>Family history of hemophilia</th>
<th>Children with inhibitor (n=18)</th>
<th>Children without inhibitor (n=16)</th>
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<tr>
<td>Family history of inhibitor</td>
<td>5 (28%)</td>
<td>5 (31%)</td>
</tr>
<tr>
<td>Median age (months) at 1st FVIII infusion</td>
<td>11 (5 days-6)</td>
<td>10 (1 day-6)</td>
</tr>
<tr>
<td>Surgical procedures</td>
<td>6 (63%)</td>
<td>6 (63%)</td>
</tr>
<tr>
<td>Port-a-cath placement</td>
<td>4 (22%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>Switch of recombinant products</td>
<td>7 (39%)</td>
<td>6 (37%)</td>
</tr>
<tr>
<td>Amniocentesis/viilcentesis</td>
<td>3 (17%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Premature birth</td>
<td>1 (6%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Caesarian birth</td>
<td>7 (39%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>Breast-feeding</td>
<td>14 (78%)</td>
<td>11 (69%)</td>
</tr>
<tr>
<td>FVIII infusions associated with infections or vaccinations</td>
<td>3 (17%)</td>
<td>4 (25%)</td>
</tr>
</tbody>
</table>

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, Radosso 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 (Alimafix DJ, 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/kg 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 cooperation 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) when patients were on 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 $6.9 \pm 3.3$ (range 4.3-13) per patient-month in this subgroup vs. $2.6 \pm 0.8$ 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 $36.1 \pm 12$, 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**


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

In contrast to the menorrhagia/menorrhagia 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 thrombocytopenies. 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 defined by a pictorial blood assessment chart (PBAC) when a score more than 100 was calculated. 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, FX I 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 menarche. 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 thrombocytopenies, which should be carefully researched in all young women with menorrhagia.

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 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.

**C009**

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

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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.
The prevalence of PVT was 24.6% in males and 23.8% in females. After exclusion of 27.6% of patients. The prevalence of PVT according to gender total thrombosis was present in one vessel of the portal tree in 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 were 0.46±0.02 vs. 0.48±0.08, R2: 0.31±0.01. 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.

CO12
PREVALENCE OF PORTAL VEIN THROMBOSIS AND ASSOCIATED FACTORS IN CIRRHOTIC PATIENTS: A PROSPECTIVE STUDY
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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 in patients 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 cryptogenic 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 23.8% 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 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.

CO13
SERUM D-DIMER TEST AND ASSESSMENT OF FIBRINOLYTIC CAPACITY BEFORE AND AFTER VENOUS OCCLUSION
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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, 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 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.

CO14
MEPACRINE RELEASE ASSAY: A NEW FUNCTIONAL METHOD FOR THE ASSAY OF HEPARIN-ASSOCIATED ANTIBODIES DURING HEPARIN-INDUCED THROMBOCYTOPENIA
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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 [14C] serotonin release method. The test samples included plasma from 24 patients with a clin-
tical diagnosis of HIT (true positive) and 24 patients with idio-
pathic thrombocytopenic purpura (ITP) (true negative). Platelet
pool from 3 patients with a history of HIT were washed by dif-
frential centrifugation, labeled with 4 \( \mu M \) mepacrine (37°C for
30min), and then resuspended in buffer containing 2 \( \mu M \) Ca
(200×10⁹ platelets/L). The test mixture consisted of 20 \( \mu L \) of test
plasma, 10 \( \mu L \) of buffer solution or heparin (0.3 IU/mL) and 100
IU/mL) and 70 \( \mu L \) 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 \( \mu M \)
onophore. 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 (\( \chi^2 \) 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 polyan-
ion/PF4 ELISA was 75%. Considering the immune diagnosis of
HIT (presence of Ab-anti polyanion/PF4) and polyanion/PF4
ELISA was 75%. Considering the immune diagnosis of
HIT (presence of Ab-anti polyanion/PF4), the percentage of sen-
sitivity and specificity was, respectively, 61% and 100% for MRA
and 78% and 83% for HIPAA. Statistical analysis showed a sig-
nificant correlation between MRA and HIPAA (p<0.007). In con-
cclusion, 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
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Chemistry Cleveland State University, Cleveland, OH, and Depart-
ment of Molecular Cardiology the Lerner Research Institute, the
Cleveland Clinic Foundation, Cleveland, OH, USA

Anti-factor V inhibitory antibodies have always been associ-
ated with bleeding disorders and impaired coagulant activi-
ty 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 antiphos-
pholipid antibodies were present in the patient’s plasma and
both PT and aPTT were normal. The total immunoglobulin frac-
tion was isolated from the patient’s plasma using protein G-
Sephase and found to induce severe APC-resistance when
added to normal plasma and to factor V-deficient plasma sup-
plemented with factor V. Thus, the immunoglobulin fraction
interferes with factor Va inactivation by APC. Immunoblotting
and immunoprecipitation experiments with the total immu-
oglobulin fraction purified from the patient’s plasma dem-
strated 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 inacti-
vation 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 CONTEXT 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 Hospi-
tal, Milan; *Fondazione Luigi Villa, Centro Studi di Patologia Mole-
colare 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 preclin-
ical aspects are not globally considered. We report a problemat-
ic 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)
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 muta-
tion localized in exon 7 which leads to a stop codon. This molec-
ular result was clearly in contrast with the phenotype. The moth-
er was heterozygous for the same mutation. Since results were
inconclusive, the whole genetic study was repeated for the sec-
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 pos-
sibility to have an error during amplification. All members of fam-
ily were carrying the deletion in the heterozygous state in two dif-
cent 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.
SICKLE CELL DISEASE, VASCULAR ENDOTHELIUM SUFFERING, HYPERHOMOCYSTEINEMIA AND THROMBOEMBOLIC RISK

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-RCB), platelets, plasma adhesive proteins and endothelial cells alterations might cause micro- and macro-vascular occlusions and multorgan damage (Lubin BH NEJM 1997; 27:1623). Previously, we reported that fibrenectin (FN), factor VIII von Willebrand factor (FVIII-vWF) and thrombospondin (TSP), well known adhesive glycoproteins which regulate the S-RCB 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 tropism in SCD would strictly depend on the vitamin status of these patients. Therefore, the vitamin B12, pyridoxine plus 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, 8 beta thalassemia/SA 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).

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 B12 should be warranted in SCD patients.

FASTING AND POSTMETHIONINE LOAD HYPERHOMOCYSTEINEMIA IN CENTRAL RETINAL VEIN OCCLUSION

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To evaluate the prevalence and potential implication of hyperhomocysteinemia (HHcy) in central 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 (n 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

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**p<0.001 vs controls; ****p<0.001 vs baseline.

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<tr>
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<tr>
<td>Post-Ml</td>
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**p<0.001 vs controls; ****p<0.001 vs baseline.

| Comparison | tHcy (µmol/L) | PML | CRVO | Controls |
However, in a generalized linear model there was no statistically significant dependence of HHcy 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 HHcy 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.

**C020**
HIGH PREVALENCE OF HYPERHOMOCYSTEINEMIA IN ATRIAL FIBRILLATION PATIENTS WITH HISTORY OF TRANSIENT ISCHEMIC ATTACKS/STROKE


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 µmol/L in males and <13 µmol/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


Dipartimento Area Critica Medico-Chirurgica, *Dipartimento di Scienze Neurologiche e Psichiatriche; **Dipartimento di Fisiopatologia Clinica, Sezione di Genetica Medica ed Etologia; *Medicina Generale 1*; **Dip.to Medicina Interna U.O. Patologia Medica 1* 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 Parkin-sonism with multifibratral encephalopathy. The patients were normotensive, normocolesterolemic, 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.

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 treat-

<table>
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<tr>
<th>Pt, sex, family yrs manifestations Clin Hcy tHcy PML Mutation Lp(a)</th>
<th>FA mg/L</th>
<th>V. B12 pg/mL</th>
<th>V. B6 pg/mL</th>
<th>MTHFR C677T mg/L</th>
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<tbody>
<tr>
<td>VE M, 1 44 Stroke</td>
<td>22.0</td>
<td>3.5</td>
<td>360</td>
<td>8.6</td>
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<tr>
<td>CG, F, 2 53 Stroke</td>
<td>15.6</td>
<td>2.4</td>
<td>267.0</td>
<td>12.6</td>
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<tr>
<td>CL F, 2 55 Stroke</td>
<td>14.4</td>
<td>4.8</td>
<td>647</td>
<td>5.7</td>
</tr>
<tr>
<td>VA M, 2 32 A*</td>
<td>10.7</td>
<td>3.3</td>
<td>196</td>
<td>4 Negative 26</td>
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<tr>
<td>BA F, 2 55 -</td>
<td>11.0</td>
<td>3.4</td>
<td>108</td>
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<tr>
<td>BN F, 2 48 -</td>
<td>10.8</td>
<td>6.1</td>
<td>339</td>
<td>4</td>
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<td>MS M, 2 33 Strokes A*</td>
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<td>2.9</td>
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<tr>
<td>RR M, 3 46 -</td>
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<td>83</td>
<td>829</td>
<td>53</td>
</tr>
<tr>
<td>A B0 R, 3 21 A*</td>
<td>10.3</td>
<td>3.9</td>
<td>864</td>
<td>6.6</td>
</tr>
</tbody>
</table>

* 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 ≤ 0.04), and vitamin B12 levels lower in patients from group 3 than in the other groups (p ≤ 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-methio-

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 hyperhomocyste-

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting HHcy (µmol/L)</th>
<th>PML Δ HHcy (µmol/L)</th>
<th>Folate (ng/mL)</th>
<th>Vit. B12 (pg/mL)</th>
<th>PLP (µmol/L)</th>
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<tbody>
<tr>
<td>Group 1 (n = 14)</td>
<td>27.2±.81</td>
<td>18.2±3.6</td>
<td>234±11</td>
<td>324±73</td>
<td>21±12.2</td>
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<td>Group 2 (n = 11)</td>
<td>8.4±1.7</td>
<td>37.5±13.7</td>
<td>5.4±2.8</td>
<td>309±148</td>
<td>30.1±18.5</td>
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<tr>
<td>Group 3 (n = 9)</td>
<td>43.2±29.5</td>
<td>35±21.6</td>
<td>3.7±2.6</td>
<td>18±40</td>
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<tr>
<td>p</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.036</td>
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Table 2.

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<tr>
<th>Fasting HHcy (µmol/L)</th>
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<tr>
<td>B6</td>
<td>Folate, B12</td>
<td>B6</td>
<td>Folate, B12</td>
<td>PLP</td>
</tr>
<tr>
<td>(n = 3)</td>
<td>(n = 9)</td>
<td>(n = 3)</td>
<td>(n = 6)</td>
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<tr>
<td>Fasting HHcy (µmol/L)</td>
<td>71±.04</td>
<td>45±.05</td>
<td>39±.05</td>
<td>21±.02</td>
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<tr>
<td>% of pretreatment levels</td>
<td>10±.05</td>
<td>45±.05</td>
<td>46±.05</td>
<td>47±.05</td>
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<tr>
<td>p</td>
<td>ns</td>
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<td>0.03</td>
<td>0.001</td>
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<tr>
<td>PML Δ HHcy (µmol/L)</td>
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<td>17±.05</td>
<td>40±.07</td>
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<tr>
<td>% of pretreatment levels</td>
<td>70±.05</td>
<td>8±.05</td>
<td>70±.05</td>
<td>70±.05</td>
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<tr>
<td>p</td>
<td>ns</td>
<td>0.002</td>
<td>0.002</td>
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Homocysteine I

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.

CO24

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


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 anniovascular risk factor assessment, 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 anniovascular risk factor assessment, 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 anniovascular risk factor assessment, 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 anniovascular risk factor assessment, the effect of vitamin supplementation on 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.
THE CB07T POLYMORPHISM IN THE PLATELET GLYCOPROTEIN IA GENE AND THE RISK OF ISCHEMIC STROKE IN THE YOUNG
De Stefano V, Chiussolo 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/IIIa mediates platelet adhesion to collagen; the CB07T 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 CB07T 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 FM R 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, dyslipidemia, 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 genotype being associated with a higher receptor density. The odds ratio for ischemic stroke among C-allele carriers and 3.0 (95% CI 1.5-6.0) in comparison with CC (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 CB07T polymorphism and the role of this polymorphism in coronary atherosclerosis 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 Thr715Pro 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.

INTERLEUKIN-1 GENOME CLUSTER POLYMORPHISMS AND RISK OF CORONARY ARTERY DISEASE
Vohnout B, Di Castelnuovo A, Trotta R,† D’Orazio A, Pannitteri 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 Imbarco, *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 (I LL-1RA) and the -511 C/T polymorphism of IL-1B 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- vessel disease (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-1RA and IL-1B -511 C/T polymorphisms between CAD+ cases and CAD-controls. However, there was a significant difference in IL-1RA 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 heterozygous 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 Thr715Pro 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.
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 Intrion 2 VNR and IL-1p -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.

CO28
ENDOTHELIAL NITRIC OXIDE SYNTHASE POLYMORPHISMS IN CORONARY ARTERY DISEASE PATIENTS
Dipartimento Area Critica Medico Chirurgica; *Dipartimento di Fisiopatologia Clinica, Unità di Genetica Medica; Università di Firenze; Centro Trombosi A.O. Careggi, Firenze, 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 maintain 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=0.0033). 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.

CO29
A TISSUE-FACTOR POLYMORPHISM REDUCES THE RISK OF FAMILIAL MYOCARDIAL INFARCTION IN SMOKERS
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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 smoking. 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 carrieship 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
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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; Fil G20210A 2.5 (1.1-5.9), p<0.05; Lp(a) >300 mg/L 3.1 (2.2-4.4), p<0.001; Hcy >95th percentile of controls 10.2 (6-17.4), p<0.001. 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<0.005; Hcy: 16.6 (6.4-43.1); p<0.001). 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
C031

ROLE OF GENETIC THROMBOPHILIA IN 254 CASES OF JUVENILE ISCHEMIC STROKE


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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 significantly higher in TT677/MTHFR subjects (19.3±15.1 vs 10.7±4.4 µmol/L, p<0.001). Among CVT patients, the frequency of heterozygous FII20210A was 3.5% that was always>0.05. (χ²-test). The frequency of FVL and of MTHFRTT in CVT patients was 7.1% (1/14) and 28.6% (4/14), respectively: χ²-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.

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

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Factor V Leiden (FVL), G20210A mutation of prothrombin gene (FII20210A), and homozygosity for 677TTT 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>0.05, χ²-test). Among CVT patients, the prevalence of 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: χ²-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.
C033
A TRIAGE TEST, BASED ON STANDARDIZED CLINICAL PROBABILITY AND D-DIMER, FOR EXCLUDING ACUTE VENOUS THROMBOEMBOLISM IN THE EMERGENCY WARDS

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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 order 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, Diertest®, 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 results test, acute VTE was confirmed in 114 patients (84 DVT, 30 PE, 25.5% of 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 SCP alone D-dimer alone SCP + D-dimer

<table>
<thead>
<tr>
<th>SCP alone</th>
<th>D-dimer alone</th>
<th>SCP + D-dimer</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>87.7% (61-93.8)</td>
<td>64.2% (61.4-67.7)</td>
</tr>
<tr>
<td>Specificity</td>
<td>46.5% (43.5-50.8)</td>
<td>71.7% (67.2-76.2)</td>
</tr>
<tr>
<td>Positive PP</td>
<td>64.4% (58.8-70)</td>
<td>49.4% (37.1-61.7)</td>
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<tr>
<td>Negative NP</td>
<td>89.9% (85.2-94.6)</td>
<td>52.9% (89.6-92.2)</td>
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*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, Moret P, Rosencher N, on behalf of the Methro Study Group

C035
THE INCIDENCE OF VENOUS THROMBOEMBOLISM IN ASYMPTOMATIC FAMILY MEMBERS WHO ARE CARRIERS OF FACTOR V LEIDEN. A PROSPECTIVE COHORT STUDY


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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 unscreened 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.

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.
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, FII G20210A 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; FV Leiden, FII G20210A gene mutations). Patients with super-

C038
INHERITED THROMBOPHILIA: DIFFERENT CONTRIBUTION TO DEEP VEIN THROMBOSIS OF UPPER EXTREMITIES AND LOWER LIMBS
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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 (16.6%). The investigations for the Outlet Thoracic Syndrome were 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.

C039
RISK FACTORS FOR UPPER EXTREMITY DEEP VEIN THROMBOSIS
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The diagnosis of UL-DVT 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 (16.6%). The investigations for the Outlet Thoracic Syndrome were 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.
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, β, and γ), CD2019 (selective for RAR α), 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), 40 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.

Retinoids are anti-tumor agents that can affect the procoagulant activity of human estrogen receptor (ER)-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^{-4} 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; 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.
ENOXAPARIN FOR PROLONGED THROMBOPROPHYLAXIS IN PATIENTS UNDERGOING ABDOMINAL CANCER SURGERY: THE ENOXACAN II STUDY RESULTS
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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=N.S.). 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.

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

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


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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.

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

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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). 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 UFH 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.
The interaction of platelet glycoprotein Ib\(\alpha\) (GpIb-\(\alpha\)) 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-\(\alpha\), 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 FVII 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-\(\alpha\) on thrombin-induced factor VIII activation, as evaluated by 1) a chromogenic assay of the generation of FVIIa cofactor activity in Factor X activation; 2) reversed-phase HPLC measurements of cleavage of FVIII 344-375 synthetic peptide (NH2-E-E-A-A-E-D-Y-D-D-L-T-D-S-E-M-D-V-R-F-D-D-N-S-P-S-F-I-Q-I-R-S-V-A-K-COOH). The 268-282 GpIb-\(\alpha\) 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-\(\alpha\) makes the enzyme not available for FVIII activation. The physiological implications of these findings need further studies to be fully unraveled.

**Oral Communications**

**Physiopathology of Platelet and Leukocyte Activation**

**C049**

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

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**C050**

**MECHANORECEPTOR FUNCTION OF GLYCOPROTEIN Ib\(\alpha\) LEADING TO SEQUENTIAL CALCIUM SIGNALS, INITIAL PLATELET ACTIVATION AND THROMBUS FORMATION IN ARTERIAL FLOW**

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Platelet activation, which modulates the ligand binding function of \(\alpha I b \beta 3\) and is required for stable adhesion and cohesion, may be coupled to the interaction between glycoprotein (GP) Ib\(\alpha\) and the von Willebrand factor A1 domain (A1VWF). We analyzed concurrently the instantaneous velocity and [Ca\(^{2+}\)]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) Ib\(\alpha\) with the A1 domain of immobilized VWF leads to Ca\(^{2+}\) release from intracellular stores (type \(\alpha\) peaks), which precedes stationary platelet adhesion. Type \(\alpha\) peaks appear to be directly modulated by tensile stress on GP Ib\(\alpha\) 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\(^{2+}\) response, suggesting that GP Ib\(\alpha\) may act as a proper mechanoreceptor. Raised cAMP/cGMP levels, as well as membrane permeable calcium chelators, inhibit these [Ca\(^{2+}\)]i oscillations and prevent stable adhesion without affecting the dynamic characteristics of the typical platelet translocation on VWF mediated by GP Ib\(\alpha\). Once adhesion is established through the integrin \(\alpha I b \beta 3\), new [Ca\(^{2+}\)]i oscillations (type \(\gamma\)) 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 Ib\(\alpha\)-VWF bonds activate \(\alpha I b \beta 3\) for localized adhesion, and outside-in signals from ADP receptors and ligand-occupied \(\alpha I b \beta 3\), 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?**

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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 G\(_\text{i}\)-coupled receptors (epinephrine; ADP interacting with P2Y12) [2]. The aim of the study was to evaluate whether the activation of human platelets by Asialo-vWF is mediated by the G\(_1\) 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 \(\alpha_2\)-adrenoceptors and AR-C69931MX (1
Convulxin induces platelet shape change through myosin light chain kinase and Rho kinase.

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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 Ca2+/calmodulin-dependent, the other subsequent to RhoA activation, mediated by Rho-kinase. The Ca2+-dependent pathway depends upon stimulation of a Gq-coupled receptor and phospholipase C (PLC) activation. Cyclic AMP exerts a potent action on cytoskeletal 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 assess 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 Ca2+-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 Ca2+-calmodulin pathway was inhibited by the Ca2+ chelator BAPTA or by the calmodulin inhibitor W-7. Taken together our findings suggest that convulxin-induced platelet shape change occurs via both a Ca2+-calmodulin-dependent and a Rho A-dependent mechanism.

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 ([Ca2+]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 [Ca2+]i induced by ADP. The aim of the study was to evaluate whether P2Y12 plays any role in the ADP-induced increase in platelet [Ca2+]i. We studied 13 normal subjects and 2 patients with P2Y12 deficiency (VR and MG). We studied the ADP-induced [Ca2+]i in washed human platelets in the presence of EDTA (1 mM) and in the presence or absence of the antagonists for P2Y1 (A2P5P 0.5 mM) or P2Y12 (AR-C69931MX 1 mM). The mean (±SD) increase in [Ca2+]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 [Ca2+]i in VR's and MG's platelets was borderline-low (130 and 262). 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 [Ca2+]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 [Ca2+]i increase. Like for platelet aggregation, P2Y1 triggers the initial platelet [Ca2+]i response, while P2Y12 amplifies it. The mechanism by which P2Y12 amplifies the [Ca2+]i increase is not mediated by inhibition of adenylyl cyclase.

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.

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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
dyne/cm² 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±3.3% of normal with 10 µM MRS 2216, 10±7.0% with 1 µM AR-C69931 IMX). In the presence of both antagonists, SIPA was 9±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
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Adhesion of PMN to activated platelets, requires a P-selectin-triggered, SRC-kinases- and cytoskeleton-dependent adhesive- ness 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 Rhô-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 aggregation and P110 phosphorylation (IC50 500 µM). IBMX-inhibited adhesion and P110 phosphorylation was restored by H89, a specific inhibitor of cAMP-P-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 Rhô proteins. Western blot analysis of the immunoprecipitated protein revealed that tyrosine phosphorylation of PYK2 was strongly increased in a SRC and β-2 integrin-dependant 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 PDE-4 and 5), blocked PMN-platelet adhesion (IC50s 0.1, 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
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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 (1-100 µM) 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±4.5% of PMN adhered to 60±10% of CHO-E by a β-2 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-Ig 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.
C057
SIMULTANEOUS DECREASE OF PROINFLAMMATORY CYTOKINES AND TISSUE FACTOR BY SIMVASTATIN
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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, USA) 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<0.0002) 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.

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
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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 coefficient (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 mm³; mean±SE) and in the simvastatin-treated rats (pre-treated: 40.9±9 mm³; post-treated: 32.2±4.4 mm³) 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
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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-
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 instability 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 radioimmunoassay for 6-keto-PGF1α production and by Western blotting analysis showed increased expression. Expression of COX-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 in blood from activated platelets in vitro, we measured MMP-2 levels in the blood emerging from a skin wound inflicted for the measurement of the bleeding time in 17 human healthy volunteers. In a subgroup of volunteers, the same measures were carried out before and 1 h 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 < 0.003) 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 in stimulated in vitro, 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/10⁶ 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 might represent one reason for the partial inefficacy of this drug.
C062

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

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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 alkalization, 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 vs. 77.4±4.3), while preventing the cytosolic alkalization, 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**

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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 dys-

function (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 02- production, serum TxB2, serum TNFα. In vitro studies were performed using TNFα as agonist, and the specific TNFα inhibitor, WP9QY. Results. An increased platelet 02- 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**

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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 (61.3±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.
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Molecular Basis of Inherited Coagulopathies

DYSFIBRINOGENEMIA: RESULTS FROM THE MOLECULAR ANALYSIS OF 7 PATIENTS
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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 α∥-chain gene, a C-to-T transition leading to a Pro/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.

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 24.7±3.3, 24.4±3.1, 24.9±4.1 and 20.6±0.6 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.
RESIDUAL FACTOR VII ACTIVITY AND DIFFERENT HEMORRHAGIC PHENOTYPES IN CRM+ FACTOR VII DEFICIENCIES (GLY331SER AND GLY283SER)

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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 c140 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.

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


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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 transition leads to a Arg/Ser substitution at position 110, whereas a G-to-T transversion leads to an Arg110Ser substitution at position 123. Her first-degree relatives, who had approximately half the normal FVII values and showed concordance between functional and immuno-logic levels, were heterozygotes, 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 antigen levels. A homozygous G-to-A substitution within exon 8 was identified that leads to a Gly5Ser 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 substitutions 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
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The development of inhibitors against transfused factor VIII still remains the main complication of replacement therapy of hemophilia A. 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 regulatory 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)
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Phenotype and genotype analysis was carried out in two patients with severe factor (FX) deficiency. Three coagulant assays (PT, FTT, 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
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Coagulation factor XIII (FXIII) is a plasma transglutaminase 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 B 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.
Incidence of newly diagnosed cancer after three months or one year of oral anticoagulation for a first episode of idiopathic venous thromboembolism


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 transient 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.

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

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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 hematomas, 3 spontaneous muscular hematomas, 9 gastrointestinal bleedings, 1 hematrosisis). 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 3.8±2.3 (n = 1681) and 3.0 (n = 962), and receiving acenocoumalone (4 mg, n = 797) or warfarin (5 mg, n = 1668) 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% CI.: 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.

C076

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

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We evaluated the laboratory quality of oral anticoagulant treatment by 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 = 1668) 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% CI.: 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

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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 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 had 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

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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

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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 non-valvular 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

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Bleeding and thrombotic complications of oral anticoagulant (OA) treatment are often associated with poor anticoagulation control. The FCISA 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.

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.

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. Chi-
anti 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 day 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 reduc-
tion of TF levels, in all but two subjects, by about 17.3%. In addi-
tion, 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 reduc-
tion, 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
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Many epidemiologic studies have evaluated whether different alcoholic beverages protect against vascular disease. We per-
formed a systematic review and a meta-analysis on the relation-
ship 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 estima-
tions of the vascular risk associated with either beverage con-
sumption. From 13 studies involving 209,418 subjects, the rel-
ative 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 sig-
nificant protective effect was found up to a daily dose of 150 mL wine. The overall effect of moderate beer consumption, mea-
sured 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. Sig-
nificant risk reduction was also obtained in studies which for-
mally excluded ex or light drinkers from the reference group or in studies that had adjusted for different types of alcoholic bev-
erages or indicators of social class level or to those that com-
pared both wine and beer drinking groups with the same refer-
ence group. These findings are strongly supportive of a signifi-
cantly inverse association between light to moderate wine con-
sumption and vascular risk. A similar, though smaller association was also apparent concerning beer consumption. The latter find-
ing however is difficult to interpret, as no dose-response rela-
tionship 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
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Background. PAI-1 4G/5G polymorphism is a predisposing fac-
tor to arterial thrombosis. Epidemiological studies have shown that environmental and genetic factors act in a synergetic way to determine PAI-1 plasma levels. In particular, the 4G poly-
morphism of PAI-1 gene promoter seems to enhance the expres-
sion 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 vit-
amin E (500 IU/die) for 10 weeks. PAI-1 antigen, PAI-1 activity, and the main fibrinolytic parameters were evaluated at baseline

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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
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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 (98±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 EXTRAVERGIN OLIVE OIL: STUDIES ON OLEuropeIN AND HYDROXYTYROSOL
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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 pharma-co kinetics 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-spectrometry. 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-glu curonidase 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.
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

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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 umbilical vein endothelial cells (HUVEC) and MN from healthy donors were stimulated with bacterial lipopolysaccharide (LPS), IL-1β 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 EM SA (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 IκBα. 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.

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

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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 abundant 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 lipopolysaccharide (LPS) for 4-6 h in the presence or absence of DHA (10-25-50 µM) for 72 h (or seerate 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.
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 VTE during idiopathic thrombophilia were 8.3% (95% CI: 2.2%-6.1%) and 1%-21%, 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 idiopathic thrombophilia 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.

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; χ²-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; χ²-test). Our data confirm a high prevalence of FV Leiden (27.1%) and FII G20210A (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 anti-coagulant strategies show that screening should be reconsidered at least in women with a family history of thrombosis during pregnancy.

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
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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
ROLE OF THROMBOPHILIA IN IMPLANTATION FAILURE AFTER IN VITRO FERTILIZATION
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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,
CARRYING THROMBOPHILIC GENE POLYMORPHISMS

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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 FII G20210A 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 FII G20210A 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

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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 U/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.
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).

### C097

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

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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.

<table>
<thead>
<tr>
<th>Promoter (CSL07)</th>
<th>Exon 3</th>
<th>Exon 7</th>
<th>Exon 8</th>
<th>Exon 8</th>
<th>Exon 9</th>
<th>Exon 9</th>
<th>Exon 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-11C</td>
<td>R-31C</td>
<td>R106Q</td>
<td>G197E</td>
<td>C222R</td>
<td>V297M</td>
<td>G381D</td>
<td>F279L</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Disruption of an HNF-1 binding site (loss of a negative charge)</td>
<td>Localized in the propeptide region</td>
<td>Loss of the thrombin cleavage site</td>
<td>Modification of the electrostatic potential in the active site (introduction of a negative charge)</td>
<td>Impairment of the correct binding (loss of C212-196C disulphide bond)</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
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<td>2</td>
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<tr>
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<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
</tr>
</tbody>
</table>

**Comments**

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**

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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**


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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 TM in 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.
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 DD and PAI-1 4G/4G genotype was associated with a further increase 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

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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 (APC-SR) independent from FV Leiden at the 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

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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 Laboratory 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

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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.

<table>
<thead>
<tr>
<th>Likelihood of thrombophilia</th>
<th>AT/PC/PS detect</th>
<th>FV Leiden</th>
<th>PT G20210A</th>
<th>Combined defects</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>23 (9.3%)</td>
<td>51 (18.9%)</td>
<td>10 (3.7%)</td>
<td>9 (3.3%)</td>
<td>95 (35.3%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>16 (4.9%)</td>
<td>57 (17.4%)</td>
<td>23 (7.0%)</td>
<td>10 (3.0%)</td>
<td>106 (32.4%)</td>
</tr>
<tr>
<td>Low</td>
<td>2 (3.4%)</td>
<td>7 (11.8%)</td>
<td>6 (10.2%)</td>
<td>1 (1.7%)</td>
<td>16 (27.1%)</td>
</tr>
</tbody>
</table>

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

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Previous reports suggested that chronic infection associated with Helicobacter 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) H. pylori. Diagnosis of chronic gastritis was done 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.), clarithromycin (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.

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

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*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 of 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 allaporphin (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 ubiquinone, 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.

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

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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/IκB-α 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
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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-1β, 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 EM SA (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
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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/3×10⁶ cells; TC=4.96±0.74 U/3×10⁶ cells; CC=1.51±0.54 U/3×10⁶ cells; p<0.003 vs TT; p=0.01 vs CT). Basal levels of either IL-1 β or TF were unaffected by -511C/T/T or IL-6 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 effect of the inflammation/hemostatic response of isolated monocytes contributes to explaining the different susceptibility to cardiovascular disease after exposure to inflammatory stimuli.
SUBCUTANEOUS ADJUSTED-DOSE UNFRACTIONATED HEPARIN VERSUS FIXED-DOSE LOW-MOLECULAR-WEIGHT HEPARIN IN THE TREATMENT OF VENOUS THROMBOEMBOLISM. A PROSPECTIVE CONTROLLED RANDOMIZED STUDY


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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.

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


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The optimal treatment of superficial thromboembolitis 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, 0.7 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.

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

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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 antiocoagulant treatment with INRs ranging from 1.2 to 6.84, 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 = 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.

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 plasma 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. 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.
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.

C115
HOMOCYSTEINE PLASMA LEVELS IN CHILDREN: DIFFERENT MEASUREMENT METHODS AND ROLE OF POLYMORPHISMS OF ENZYMES INVOLVED IN THE METABOLISM
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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/CBS. 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?
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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 response 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 fast- ing tHcy levels. ANOVA on log-transformed data showed a statistically significant difference between the means of subjects with COPD and those with stable COPD. Thirteen patients with acute exacerbation of 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.

**C117**

**HOMOCYSTEINEMIA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE**


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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 significant differences (p=0.537).

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**


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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 (15.66±5.18) but we did not observe significant differences between the means of subjects with COPD and those with stable COPD. 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 significant differences (p=0.537).
THE PLASMA LEVELS OF VITAMIN B6 ARE LOW IN WOMEN ON ORAL CONTRACEPTION

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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=0.008 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.

<table>
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<th>n</th>
<th>Men (%)</th>
<th>Age (yrs)</th>
<th>PLP (nmol/L)</th>
<th>Folate (nmol/L)</th>
<th>Vit. B12 (pmol/L)</th>
<th>Cystatin C (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy &lt; 15 µmol/L</td>
<td>683</td>
<td>37.5%</td>
<td>34±6</td>
<td>31.6</td>
<td>10.7±22</td>
<td>403±459</td>
<td>56.6±15</td>
</tr>
<tr>
<td>tHcy ≥15 µmol/L</td>
<td>306</td>
<td>65.3%</td>
<td>31±11</td>
<td>31.9</td>
<td>10.6±13</td>
<td>311±278</td>
<td>68.2±9</td>
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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 dilation <2.2 cm/m² body surface area) (n=45); C) major criteria (moderate to severe aortic dilatation >2.2 cm/m² 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 B 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 extra-cellular matrix involvement in vascular damage due to hyperhomocysteinemia.
NQO1 POLYMORPHISM AS A MODIFIER OF THE ORAL ANTICOAGULANT THERAPY DOSE REQUIREMENT

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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 \( \text{f} \)-carboxylation 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 Haematosis 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 28.7±12 mg/week) and 74 received warfarin as their anticoagulant drug (mean dose 14.3±7 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.

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 TAFI 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 BaSO4-adsorbed plasma (lacking vitamin K-dependent factors), or addition of a specific inhibitor of TAFIa (PTI, 50 µg/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 \( ^{125} \text{T} \)-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.

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 tCy levels were measured with an HPLC method with fluorometric detection. The plasma concentrations of tHcy and tCy did not change over 6 hours 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 6 h storage), while that of tCy tended to decrease slightly in samples with EDTA (5% decrease after 2 h storage). No changes in tCy concentrations were observed in samples in ACD stored at RT. Therefore, the plasma concentrations of both tHcy and tCy remain stable for at least 6 h, 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 HIBITS NITRIC OXIDE PRODUCTION BY HUMAN PLATELETS
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Elevated plasma homocysteine (Hcy) is associated with an enhanced risk of atherosclerosis, 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 aggregation was enhanced from 56.6±11% to 75±9% for ADP stimulation with ADP (10.5±5.5 vs 34.4±10.8 pmol/10⁸ 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
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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 antiphospholipid 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.
A SIMPLE TEST TO DETECT β2GPI-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 (β2GPI or prothrombin) complexes over the phospholipid surface, thus impeding normal assembling of coagulation factors. Recent data confirm the role of anti-β2GPI monoclonal antibodies and β2GPI deposition (clustering) on lipid vesicles 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 β2GPI 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 β2GPI 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-β2GPI antibodies and no increase (107%) in the group of patients testing negative for anti-β2GPI antibodies. Thus, a simple dilution of calcium ions in a modified PT assay, might be useful in identifying anti-β2GPI-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-β2GPI-independent LA.

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.

<table>
<thead>
<tr>
<th>Diagnosis of VWD types</th>
<th>1</th>
<th>2A</th>
<th>2B</th>
<th>2M</th>
<th>2N</th>
<th>3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 1992 Case n. (%)</td>
<td>218 (69)</td>
<td>45 (14)</td>
<td>30 (9.5)</td>
<td>n.i.</td>
<td>1 (0.3)</td>
<td>22 (7)</td>
<td>316 (100)</td>
</tr>
<tr>
<td>Family n. (%)</td>
<td>123 (69)</td>
<td>23 (13)</td>
<td>14 (8)</td>
<td>n.i.</td>
<td>1 (0.5)</td>
<td>18 (10)</td>
<td>179 (100)</td>
</tr>
<tr>
<td>January 2002 VWF:RCo/Ag (mean)</td>
<td>0.96</td>
<td>0.36</td>
<td>0.53</td>
<td>0.57</td>
<td>1.01</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
<tr>
<td>Mutation found (%)</td>
<td>30</td>
<td>90</td>
<td>94</td>
<td>46</td>
<td>100</td>
<td>92</td>
<td>53</td>
</tr>
<tr>
<td>Case n. (%)</td>
<td>105 (34)</td>
<td>45 (24)</td>
<td>35 (12)</td>
<td>105 (33)</td>
<td>1 (0.3)</td>
<td>22 (7)</td>
<td>315 (100)</td>
</tr>
<tr>
<td>Family n. (%)</td>
<td>85 (47)</td>
<td>23 (13)</td>
<td>15 (8)</td>
<td>37 (22)</td>
<td>1 (0.5)</td>
<td>18 (10)</td>
<td>179 (100)</td>
</tr>
</tbody>
</table>
 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. Multivariable 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.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Controls</th>
<th>Type 1 OC</th>
<th>Type 1 Aff.</th>
<th>Type 3 OC</th>
<th>Type 3 Aff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epistaxis</td>
<td>0.17</td>
<td>0.87</td>
<td>0.73</td>
<td>0.34</td>
<td>0.55</td>
</tr>
<tr>
<td>Cut bleed</td>
<td>0.05</td>
<td>1.00</td>
<td>1.10</td>
<td>0.31</td>
<td>1.61*</td>
</tr>
<tr>
<td>Tooth extraction</td>
<td>0.19</td>
<td>1.45</td>
<td>1.10</td>
<td>0.08</td>
<td>1.33</td>
</tr>
<tr>
<td>Surgery</td>
<td>0.14</td>
<td>1.29</td>
<td>1.03</td>
<td>0.08</td>
<td>1.33</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>0.58</td>
<td>1.52*</td>
<td>1.53*</td>
<td>0.46</td>
<td>1.5</td>
</tr>
<tr>
<td>Post-partum</td>
<td>0.06</td>
<td>1.30*</td>
<td>1.26</td>
<td>0.39</td>
<td>1.28</td>
</tr>
</tbody>
</table>

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 in controls (p=0.38 and 0.002, respectively). Bleeding 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.

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, 40 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(+2)g→a, IV545(+-7)c→t), 3 single nucleotide insertion (4664insC, 7375insC, 7387insT), 8 nonsense mutations observed. Thirty distinct mutations were identified, 40 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(+2)g→a, IV545(+-7)c→t), 3 single nucleotide insertion (4664insC, 7375insC, 7387insT), 8 nonsense mutations observed. Thirty distinct mutations were identified, 40 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(+2)g→a, IV545(+-7)c→t), 3 single nucleotide insertion (4664insC, 7375insC, 7387insT), 8 nonsense mutations.

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.
propertide (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 W222X. 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-WVFH, containing cDNA of the human VWF, was used as a template to make, by the site direct mutagenesis method, vector pSV-WVFHC275S. 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-VWFHC275S 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-VWF275S, 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 2B (P1337L) VON WILLEBRAND'S DISEASE ASSOCIATED WITH HETEROZYGOUS DEFECT OF TYPE 1 (C275R) IN A PATIENT PREVIOUSLY DIAGNOSED AS TYPE 2A: THE IMPORTANT ROLE OF MOLECULAR CHARACTERIZATION OF THE ENTIRE FAMILY
Canciani MT, Baronciani L, Forza I, Cozzi G, Siboni S, Federici AB
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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
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 ADAM TS family of metalloproteinasases. Deficiency of von Willebrand factor cleaving 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).

<table>
<thead>
<tr>
<th>VWF:CP</th>
<th>VWF:Ag</th>
<th>VWF:CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>HELLP Syndrome</td>
<td>30±6</td>
<td>30±6</td>
</tr>
<tr>
<td>(25-45)</td>
<td>(20-42)</td>
<td>(18-33)</td>
</tr>
<tr>
<td>HELLP in remission</td>
<td>107±11</td>
<td>119±53</td>
</tr>
<tr>
<td>(105-130)</td>
<td>(98-127)</td>
<td>(73-152)</td>
</tr>
<tr>
<td>Normal pregnancy</td>
<td>72±15</td>
<td>109±68</td>
</tr>
<tr>
<td>(60-105)</td>
<td>(102-320)</td>
<td>(102-320)</td>
</tr>
</tbody>
</table>

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.
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 VTE (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), PL20210GA 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.

<table>
<thead>
<tr>
<th>Sex (M/F)</th>
<th>177/13</th>
<th>31/36</th>
<th>307/409</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first thrombosis (median, range)</td>
<td>36 (26-70)</td>
<td>32 (6-74)</td>
<td>42 (6-86)</td>
</tr>
<tr>
<td>Interval from the first VTE (median, range - yr)</td>
<td>4.5 (1-27)</td>
<td>3.0 (40)</td>
<td>2 (1-40)</td>
</tr>
<tr>
<td>Unprovoked first DVT (%)</td>
<td>12 (40%)</td>
<td>26 (39%)</td>
<td>37 (43%)</td>
</tr>
<tr>
<td>Recurrent VTE (%)</td>
<td>15 (50%)</td>
<td>4 (63%)</td>
<td>20 (24%)</td>
</tr>
</tbody>
</table>
C135
HIGH PLASMA LEVELS OF FACTOR VIII AND RISK OF RECURRENCE OF VENOUS THROMBEMBOLISM
Unità di Ricerca Clinica sulla Trombofilia “Marino Golinelli” - Dipartimento Cardiovascolare, U.O. 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=0.0001). Recurrent VTE developed in 101 (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: 1.38-4.42). 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: 1.34-4.28; 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
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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 (1-1.497) mg/L vs 102 (9-695) mg/L; p<0.05). Lp(a) levels above 300 mg/L were detected in 146/603 (24.2%) patients and in 41/300 (31.6%) controls (p<0.005). 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<0.001). In 368/603 (61%) patients no circunstantial 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<0.001) 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<0.001), hyperhomocysteinemia (OR=5.0 (3.0-8.4); p<0.001) and the presence of both FV Leiden and FII polymorphisms (OR=9.7 (1.9-4.4); p<0.001). 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 RECURRENT IN PATIENTS WITH THROMBOPHILIC ALTERATIONS AND PREVIOUS VENOUS THROMBOEMBOLISM
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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

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C138
RESIDUAL VEIN THROMBOSIS AS A PREDICTIVE FACTOR OF RECURRENT VENOUS THROMBOEMBOLISM. A PROSPECTIVE COHORT STUDY
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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.

C139
RESISTANCE TO ACTIVATED PROTEIN C IS RELATED TO AN INCREASED INTIMA-MEDIA THICKNESS IN THE GENERAL POPULATION
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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
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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’sath, M etris, 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.

C142

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


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


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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 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 anti-coagulant 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 ×10^-6 vs 4.6±1×10^-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 anti-coagulant 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.

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


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Background. An increased risk of cardiovascular disease has been associated with the use of highly active antiretroviral 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. WVF 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±0.88 6.02±1.88 4.4±2.92 3.7±3.36 0.32±0.1
DD 0.83±0.14 0.32±0.37 0.64±0.34 0.37±0.3 0.37±0.1
TM 55.2±33.7 29.1±4.5 36.6±14.8 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.
C145
CHARACTERIZATION OF DYSFUNCTIONAL P2Y12 RECEPTOR IN A PATIENT WITH CONGENITAL BLEEDING
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The combined action of the two platelet ADP receptors, P2Y1 (coupled to Gq and PLCb) and P2Y12 (negatively coupled to Gq and PLCb) 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 aggregation of AC’s platelets was severely impaired, while that by epinephrine was normal. However, the number of binding sites and affinity for [33P]-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-COLLAGE INTERACTION AND REDUCED GP1A-IIA EXPRESSION
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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/L at light microscopy in a Bürker counting chamber). On arrival the patient presented a cutaneuous-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 4µm). 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 α2 gene defining three alleles: allele 1 (807C-873A/837T/Brl(e)b) associated with increased levels of GpIa-IIa; allele 2 (807C-873G/837T/Brl(e)b) associated with lower levels; allele 3 (807C-873G/837C/Brl(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 defined by severe deficiency of GpIa-IIa and defective platelet-collagen interaction.

C147
GLANZMANN’S THROMBOASTHENIA ITALIAN TEAM (GLATIT): A MULTICENTER ITALIAN STUDY FOR THE IDENTIFICATION OF CAUSAL MUTATIONS
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Glanzmann’s thrombasthenia (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 αIIb and 4 in the β3 gene have been found. Only two (c.1113G>T in β3 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 αIIb 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 αIIb and β3 genes.
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 non-muscle 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 uniformly distributed, and leukocyte myosin was clustered within Dohle-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.
We report our experience of long-term follow-up of essential thrombocythemia (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 x 10^9/L: complete response (C.R.); <600 x 10^9/L: partial response (P.R.)), 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 therapy (median follow-up: 97 months (50-136), mean platelet count: 506 x 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, anemia, gastric distress) must be seriously considered. Heart function monitoring is imperative. Four adverse thrombotic events occurred. However, no case of leukemic evolution was recorded.

### C150
**LONG-TERM FOLLOW-UP OF PATIENTS AFFECTED BY ESSENTIAL THROMBOCYTHEMIA TREATED WITH ANAGRELIDE**

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### C151
**THE WAPS TRIAL (WARFARIN IN THE ANTIPHOSPHOLIPID SYNDROME): STUDY POPULATION AND PRELIMINARY RESULTS**

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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 antiphospholipid 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 symptoms, (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) it 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=0.008) 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**

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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 (+) one were affected by primary antiphospholipid syndrome (APS), 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 2000 mg/day) for 4 weeks. APL (+) patients showed higher IPF-III (228 [80-386] vs 152 [76-218] pg/mg creatinin; p<0.001), isoprostane F-2-α-VI (1674 [634-2424] vs 1023 [426-1780] pg/mg creatinin; p<0.008) monocyte TF antigen (52 [20-78] vs 18 [10-26] pg/200,000 monocytes; p<0.0001) and activity (35 [20-48] vs 12 [6-20] U/2×10⁶ 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
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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) 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

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 50.1 y, 25-66 y; 17 males) who developed HAT (15 cases) or hepatic artery stenosis (9) after OLT, group B= 14 patients who underwent uncomplicated OLT (mean age 46.1 y, 36-62 y; 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 concentrations (Platelet Extract Reagent, Bio-Da; LA-Check, Organon Teknika). Anticardiolipin IgG and IgM and anti β2 glycoprotein I (IgG and IgM) levels were measured by ELISA assays (BEIA Boutu). 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.
ASPIRIN AND CALCIUM HEPARIN: A COMPARATIVE EVALUATION OF BOTH TREATMENTS IN ANTIPHOSPHOLIPID ANTIBODIES POSITIVE WOMEN WITH RECURRENT PREGNANCY LOSS

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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.

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

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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 fraction 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.
The combination between a thiopyridine and aspirin (ASA) represents the treatment or choice for patients undergoing coronary angioplasty with stent implantation. Moreover, recent trials 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 anti-thrombotic 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.

<table>
<thead>
<tr>
<th>Mortality (%)</th>
<th>Bleeding time (sec)</th>
<th>Plasma MDA MM (µM)</th>
<th>Serum TXB2 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mice</td>
<td>88</td>
<td>202±10 5</td>
<td>34±2.5</td>
</tr>
<tr>
<td>ASA 30 mg/kg</td>
<td>75</td>
<td>40±11.8</td>
<td>10.6±4</td>
</tr>
<tr>
<td>NCX 4016 60 mg/kg</td>
<td>74</td>
<td>45±12.5</td>
<td>36.7±3.12</td>
</tr>
<tr>
<td>Clopidogrel 0.5 mg/kg</td>
<td>78</td>
<td>45±12.7</td>
<td>18±6</td>
</tr>
<tr>
<td>ASA + Clopidogrel</td>
<td>72</td>
<td>80±6</td>
<td>-</td>
</tr>
<tr>
<td>NCX + Clopidogrel</td>
<td>73</td>
<td>48±4</td>
<td>12±8</td>
</tr>
<tr>
<td>ASA+N CX + Clopidogrel</td>
<td>73</td>
<td>73±5</td>
<td>36±2.13.9</td>
</tr>
</tbody>
</table>

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

The combination of aspirin and clopidogrel exerts a stronger anti-thrombotic 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+clopipodregel does not further increase anti-thrombotic 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.

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 idaripril. 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.
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 µmol/L) dose-dependently reduced PGE2 production (85% maximal inhibition) and dexamethasone (10 µmol/L) totally suppressed it, whereas aspirin (10-300 µmol/L) was almost ineffective, producing only 15% inhibition at 300 µmol/L. NCX4016 (50-300 µmol/L) inhibited dose-dependently, though only partially, PGE2 production (50 µmol/L = 19% inhibition, p =0.01; 300 µmol/L = 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 (IC50 0.02 and 0.01 µmol/L, respectively) while DUP697 and SNP were ineffective. Under the same experimental conditions COX-2 expression, measured by Western blot, was completely suppressed by dexamethasone in the same experimental conditions COX-2 expression, measured by Western blot, was completely suppressed by dexamethasone 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 of the controls had been taking aspirin in the previous 15 days. These volunteers 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
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Research carried out in the last few years has given new insights into the role of extracellular proteases, e.g. 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 a 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, 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

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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 758.27±28.50 at baseline and 667.27±29.17 <0.005 vs 0.875±0.04 (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 reduces 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.
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 apoE/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
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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
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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 (-746G→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 -746G→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 carriers of the A allele frequency was 0.07 (95%CI 0.05-0.08). No CC homozygous subjects were found. Also this variant was not associated with an 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.
ASSOCIATION BETWEEN FIBRINOGEN LEVELS AND FIBRINOGEN G-455A POLYMORPHISM AND INCREASED INTIMA-MEDIA THICKNESS IN THE GENERAL POPULATION

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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.

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

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

Human cathepsin G (h-CG) gene spans 2.7 kilobase pairs on chromosome 14q11.2 and consists 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 subjects 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 polymorphism of CG promoter introduces a new binding site for NF-κ-B. It does not, however, affect the risk of MI at young age.

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

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 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 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.
logistic regression model was used to evaluate the independent and cumulative effect of the gene polymorphisms 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.

The logistic regression model was highly significant (p < .0001) 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). 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.

**PO05**

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


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, Firenze, 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.

**PO04**

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


Dipartimento Area Critica Medico Chirurgica; Dipartimento di Fisiopatologia Clinica, Unità di Genetica Medica, Università di Firenze; Centro Trombosi A.O. Careggi, Firenze, 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 associated with the risk of disease. Our data show that G894T polymorphism, modulating eNOS activity, may influence mechanisms responsible for the pathogenesis of carotid atherosclerosis.
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 gestational age in the eye, such as regulation of aqueous humor 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 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 matched with 60 control subjects (35 males and 25 females). 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.

P007a
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 gestational age in the eye, such as regulation of aqueous humor 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.

P007
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WEIGHT LOSS REDUCES PERSISTENT PLATELET ACTIVATION IN WOMEN WITH ANDROID OBESITY
Falco A,* Guagnano MT,* Ciabattoni GJ, M arinopolicci M,* Nutini M,* Sensi S,* Patrono C,* Davì G*
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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/m²), 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.

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

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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, 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

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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

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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 anti-fibrinolytic 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^*
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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 (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 monocyte 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.
Homocysteine

**P016**

**HOMOCYSTEINE LEVELS IN PATIENTS WITH MIGRAINE WITH AURA**

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


Migraine with aura (MA) has been established as an possible risk for ischemic stroke, in particular in young adults. It has 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).

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<th>Patients</th>
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<td></td>
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<tr>
<td>X (µmol/L)</td>
<td>17.36</td>
<td>11.29</td>
<td></td>
</tr>
<tr>
<td>sd</td>
<td>16.55</td>
<td>4.69</td>
<td></td>
</tr>
</tbody>
</table>

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 Emotasi 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% (homozygosty 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 homozygosty 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 hyperhomocysteinemia 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 mobilization through the activation of Ca2+-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 diphenyllethiodonium (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.

VARIABILITY OF HOMOCYSTEINEMIA IN DIFFERENT POPULATIONS
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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 diseases and to evaluate the risk factors related with Hcy levels. The role of genetic factors in the variability of Hcy levels was also assessed.

RESULTS

<table>
<thead>
<tr>
<th>Group</th>
<th>Cardiovascular</th>
<th>Diabetes</th>
<th>Blood donors</th>
<th>Outpatients</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. patients</td>
<td>26</td>
<td>86</td>
<td>95</td>
<td>57</td>
</tr>
<tr>
<td>Mean age</td>
<td>69.6</td>
<td>64.9</td>
<td>73.6</td>
<td>76.6</td>
</tr>
<tr>
<td>% smokers</td>
<td>72.2%</td>
<td>76.2%</td>
<td>70.3%</td>
<td>78.3%</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>2.25</td>
<td>1.95</td>
<td>2.24</td>
<td>1.94</td>
</tr>
<tr>
<td>Hcy smokers (mean &amp; s.d.)</td>
<td>14.6±10.85</td>
<td>13.9±9.9</td>
<td>15.2±12.0</td>
<td>13.0±12.1</td>
</tr>
<tr>
<td>Hcy non smokers (mean &amp; s.d.)</td>
<td>14.1±8.28</td>
<td>13.9±7.5</td>
<td>15.1±10.1</td>
<td>13.3±11.1</td>
</tr>
<tr>
<td>Hcy males (mean &amp; s.d.)</td>
<td>15.1±10.3</td>
<td>14.7±10.4</td>
<td>15.2±10.3</td>
<td>14.3±10.3</td>
</tr>
<tr>
<td>Hcy females (mean &amp; s.d.)</td>
<td>13.9±8.9</td>
<td>13.4±7.4</td>
<td>14.1±9.3</td>
<td>13.5±9.3</td>
</tr>
<tr>
<td>Hcy total (mean &amp; s.d.)</td>
<td>14.4±10.7</td>
<td>13.9±10.4</td>
<td>14.2±10.7</td>
<td>13.7±10.7</td>
</tr>
<tr>
<td>Hcy &gt; 15 µmol/L (# and %)</td>
<td>6 (23.1%)</td>
<td>11 (12.8%)</td>
<td>17 (20.3%)</td>
<td>12 (21.1%)</td>
</tr>
<tr>
<td>Hcy &gt; 30 µmol/L (# and %)</td>
<td>1 (3.8%)</td>
<td>2 (2.3%)</td>
<td>9 (10.5%)</td>
<td>2 (3.5%)</td>
</tr>
</tbody>
</table>

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.

PLASMA HOMOCYSTEINE IN FAMILIES OF TYPE 1 DIABETIC PATIENTS
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Background and Aims. Elevated plasma homocysteine is an independent risk factor for atherosclerotic disease. After the demonstration 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±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<0.001). 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.
prevalence of HyperHcy and its genetic determinants in 55 con-
secutive 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<0.01). Hyperhomo-
cysteinemia, defined as a concentration of Hcy above the 95th
percentile of controls, was diagnosed in 18/55 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<0.001.
The distribution 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: +/- =19.9+-9.7 +/-=11.5+-3.8
µmol/L; 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 MTH-
FR 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<0.05)
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<0.05/ controls: +/- =10.2+-5.3 +/-=8.2+-4.3
µmol/L; +/-=8.1+-2.5 µmol/L; p<0.05). 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
HOMOCYSITENE-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 ‘M ilian 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 antio-
xidants influence such early events in atherogenesis. We examined
the expression of intercellular adhesion molecule-1 (ICAM-1),
avascular 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
known inducers of endothelial 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 nega-
tive 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 homo-
cysteine-induced expression of adhesion molecules and, in par-
allel, reduced NF-κB activation. In conclusion, homocysteine, in
the concentration range achievable in hyperhomocysteinemia,
induces the expression of endothelial adhesion molecules pre-
sumably by a pro-oxidant mechanism as suggested by the neu-
tralizing effect of two structurally unrelated polyphenolic dietary
antioxidants. These results indicate a novel mechanism of induc-
tion of vascular disease by homocysteine and the possibility that
Mediterranean diets counterbalance some homocysteine vascu-
lar effects independent of folate levels.

P023
ELEVATED PLASMA HOMOCYSTEINE BUT NOT 677TT
METHYLETETRAHYDROFOLATE 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 tetrahydro-
folate 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 geno-
types 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. Results. The preva-
ience 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 geno-
type, mean plasma HC was significantly different across the four
groups, IS with 677TT MTHFR (8.7±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<0.001, Kruskal-Wallis). Dunn’s post hoc
analysis showed that mean plasma HC in IS patients with 677TT
MTHFR was significantly higher than in IS patients with 677TT
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. Ele-
vated plasma HC contribute to IS not only in patients with 677TT
MTHFR but also in patients heterozygous and non mutated. M
measurement of plasma HC may identify patients at a high risk
of IS requiring HC lowering.

haematologica vol. 87(suppl. to n 5):may 2002 77
ENDOTHELIAL MARKERS IN HYPERHOMOCYSTEINEMIC PATIENTS WITH VASCULAR DISEASES AT BASELINE AND AFTER VITAMIN SUPPLEMENTATION

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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 venous 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 venous vascular disease. Thirty-five matched non-hyperhomocysteinemic patients (group B), with clinical arterial and venous diseases, were taken as controls. A subgroup of 15 hyperhomocysteinemic patients (group B), with clinical arterial and venous diseases, were taken as controls. A subgroup of 15 hyperhomocysteinemic 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 are shown in Tables 1 and 2. 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.

<table>
<thead>
<tr>
<th></th>
<th>Group A (n. 35)</th>
<th>Group B (n. 35)</th>
<th>Difference mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy (µmol/L)</td>
<td>22.9±2.6</td>
<td>6.6±1.8</td>
<td>16.3 (12.3; 20.4)*</td>
</tr>
<tr>
<td>sTM (ng/mL)</td>
<td>13.5±5.7</td>
<td>7.0±4.6</td>
<td>6.5 (2.7; 9.3)*</td>
</tr>
<tr>
<td>sE-sel (ng/mL)</td>
<td>50.6±23.9</td>
<td>35.5±16.7</td>
<td>14.3 (4.9; 24.3)*</td>
</tr>
</tbody>
</table>

Table 2. Baseline (T0) and after 3 months of vitamin supplementation (T+).

<table>
<thead>
<tr>
<th></th>
<th>T0 (n. 15)</th>
<th>T+ (n. 15)</th>
<th>Difference mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy (µmol/L)</td>
<td>32.5±3.4</td>
<td>7.6±3.0</td>
<td>24.9 (17.4; 32.4)*</td>
</tr>
<tr>
<td>sTM (ng/mL)</td>
<td>18.7±3.3</td>
<td>10.3±2.7</td>
<td>8.4 (4.0; 12.7)*</td>
</tr>
<tr>
<td>sE-sel (ng/mL)</td>
<td>50.6±17.2</td>
<td>49.8±16.1</td>
<td>0.8 (2.0; 22.2)*</td>
</tr>
</tbody>
</table>

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.
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 (Immuliite 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.

Insulin Enhances Vascular Cell Adhesion Molecule-1 in Human Endothelial Cells in Culture

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:

- 0.01: 105 ± 7
- 0.1: 125 ± 10
- 1: 150 ± 15
- 10: 180 ± 20
- 100: 200 ± 25
- 1000: 225 ± 30

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.
MEDICINE DIET PHYTOCHEMICALS INHIBIT ENDOTHELIAL ACTIVATION THROUGH INTERFERENCE WITH REDOX-SENSITIVE TRANSCRIPTIONAL FACTORS

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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 Results. 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 cell incubation with bacterial lipopolysaccharide or cytokines to trigger adhesion molecule expression. Only oleuropein, hydroxytyrosol and resveratrol, possessing a marked antioxidant activity, reduced monocyte 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 or enhancer 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-Brzóska K., Manarini S., Martelli N., Evangelista V., Cerletti C.

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The pharmacokinetics and pharmacodynamic properties of two different doses (80 and 160 mg) of an enteric-coated formulation of aspirin (ASAFLow®) were evaluated in 16 normal subjects (34±5 years old, 9 females, 7 males) after two 3-day treatment periods. 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-PGF1α were measured. The kinetic parameters were calculated after two doses of aspirin in the absence or presence of an aspirin inhibitor. Plasma levels of ASA were about 10 times higher than those of aspirin and showed a tendency to accumulate at day 7 after the higher dose. As SA blunts the pharmacological effect of ASA, the latter finding is relevant in the long-term use of aspirin. Plasma levels of ASA and SA were remarkably delayed in respect to aspirin plasma. Plasma levels of ASA were about 30% lower 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 was significantly lower than on day 1. These results confirm and extend findings of previous studies (Circulation 1985; 72:1185).
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.

P031
A PARADOXICAL INCREASE IN THE SURFACE EXPRESSION OF VARIOUS CELL ADHESION MOLECULES IN HUMAN ENDOTHELIAL CELLS AFTER TREATMENT WITH STATINS

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 endothelial cells (HUVEC). Glucose endproducts (AGEs) for a further 12 h. After this time, the dose of 160 mg daily being pharmacologically more effective than the dose of 80 mg daily.

P032
WHICH STRATEGY TO CHOOSE FOR ANTI-THERMOBOTIC MANAGEMENT OF CORONARY ARTERY DISEASE IN HEMOPHILICAS UNDERGOING A PERCUTANEOUS INTERVENTION?

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 endothelial cells (HUVEC). Glucose endproducts (AGEs) for a further 12 h. After this time, the dose of 160 mg daily being pharmacologically more effective than the dose of 80 mg daily.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>VCAM-1 expression</th>
<th>Simvastatin 600 ng/mL</th>
<th>E-selectin expression</th>
<th>ICAM-1 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGEs 200 mg/mL</td>
<td>153±13</td>
<td>215±30</td>
<td>153±13</td>
<td>135±10</td>
</tr>
<tr>
<td>LPS 1 µg/mL</td>
<td>153±10</td>
<td>130±13</td>
<td>133±14</td>
<td>133±14</td>
</tr>
<tr>
<td>TNFα 10 ng/mL</td>
<td>153±13</td>
<td>150±23</td>
<td>150±23</td>
<td>133±10</td>
</tr>
</tbody>
</table>

SE stimulated expression.
compounds, belonging to different classes based on their mechanism of action: RGD-like peptides compete with fibrinogen to bind α6β3-illia, 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 RGD-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 (2T0) was considered for the analysis. The 2T0 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: 165-555 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 2T0 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


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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 normcholesterolemic 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 atorvas-tatin 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 (r=0.0013) and negatively with HDL (p=0.004). IL-6 also correlated with TNFα (p=0.003).

Table 1.

<table>
<thead>
<tr>
<th>Fibrinogen mg/dL</th>
<th>F1 + 2 mmol/L</th>
<th>IL-6 pg/mL</th>
<th>TNFα pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>(n=22)</td>
<td>(n=22)</td>
<td>(n=22)</td>
</tr>
<tr>
<td>P</td>
<td>(n=16)</td>
<td>(n=16)</td>
<td>(n=14)</td>
</tr>
<tr>
<td>P</td>
<td>(n=19)</td>
<td>(n=20)</td>
<td>(n=21)</td>
</tr>
</tbody>
</table>

1° (before) 385 (350-423) 1.8 (1.2-3.6) 2.6 (1.5-3.8) 2.6 (2.4-3.8)

2° (after) 345 (320-403) 0.9 (1.1-2.0) 1.0 (0.9-2.0) 1.0 (1.2-2.0)

ReoPro 2°/1° 0.9 0.8 0.8 0.9

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


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Recently, preliminary studies suggested that platelet glyco-protein 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 Reo-Pro 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 Reo-Pro to a receptor, cυβ3 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. Fibri nopeptide A (FPA), prothrombin fragment 1+2 (F1+2), thrombin/antithrombin III complex (TAT) and D-dimer were monitored by ELISA assays.
In all patients before PTCA β-TG (91.3±24.3 IU/mL vs 62±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 N.M/L vs 1.8±0.36 N.M/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±1.68 IU/mL 26.3±10.4 IU/mL, 18.8±7.7 IU/mL). FPA (6.89±1.3 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 (5.87±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 antiangiogenic in volunteers. 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**


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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 endarterectomy (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 endarterectomy 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**

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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. Antiplatelet autoantibodies anti- annexin V in 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 antiplatelet antibodies anti-β-2GPI (Radim, Pomezia, Italy), 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 against β-2GPI only are suffering from venous thrombosis. These results indicate that the evaluation of autoantibodies against 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.
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/3x10^9 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 2.69±0.57 vs 0.85±0.22, p<0.001). There was no correlation between blood pressure and platelet O2- production. After treatment 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
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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 determined by lucigenin assay. Results: Compared to healthy subjects, hypertensive patients had higher platelet production of O2- (2.5±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<0.001), that, however, was not correlated with blood pressure lowering. In vitro study showed that ibesartan (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
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The GP Ib/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 GP Ib/IIIa complex. In this study we assessed the effect of inhibition of the GP Ib/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 GP Ib/IIIa complex; ii) tirofiban (a nonpeptide) measuring the binding inhibition with PAC-1, MoAb that recognizes an epitope on the GP Ib/IIIa complex of activated platelets at or near the platelet fibrinogen receptor. We found that abciximab inhibited CD41 binding to GP Ib/IIIa in a concentration-dependent manner. PAC-1 binding to activated GP Ib/IIIa receptor was inhibited after exposure to tirofiban and eptifibatide in vitro and in vivo in a concentration-dependent manner. We measured the MoAb binding, expressed as antibody binding capacity, and we calculated the percentage of GP Ib/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.
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.

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.
SRC-DEPENDENT SIGNALING IS A KEY STEP IN THE PROCESS OF AUTOREGULATION OF MAC-1


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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 j2 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 j2-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 j2 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 j2 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.

HEMOLYTIC UREMIC SYNDROME/THROMBOTIC THROMBOCYTOPENIC PURPURA-LIKE SYNDROME AFTER SOLID ORGAN TRANSPLANTATION: A CASE SERIES

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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 multorgan 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 TIP protocol. After the second dose the patient improved (Plt: 214,000/m3) and she was discharged after the third administration, with stable platelet values.

GENOMIC RESTRICTION FRAGMENT LENGTH POLYMORPHISM TYPING OF FOUR HUMAN PLATELET-SPECIFIC ANTIGENS IN BLOOD DONORS FROM SOUTHERN ITALY

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The polymerase chain reactionrestriction-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+); 90.2%, 0.8% and 0% for HPA-4 (a−/b−), (a+/b−), (a−/b−); 75.8%, 18.8% and 0% 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 alloimmune thrombocytopenia (NAIT), post-transfusion purpura (PTP) and refractoriness to platelet transfusion therapy. So far we have successfully used our HPA genetic
A 26-year-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 mod-
nerential tests showed impaired sensitivity to ADP (>10 µmol/L) and collagen (>150,000/µl). Moreover, after cord section, i.v. desmopressin 0.3 µg/Kg for 2 weeks) treatment and her platelet count rose to 150,000/µl. Qualitatively and quantitatively similar data were found in platelets of GP Ia (CD49b, VLA-2) and GP Ic (CD49f, VLA-6) as monoclonal antibodies showed about 50% reduction on her coated with specific platelet GP). Cytofluorimetric analysis by IgG antibodies against glycoprotein (GP) Ia (commercial plates and ristocetin. Antiplatelet antibodies were also detected and interference of anti-GP Ia antibodies may be involved in this phenomenon 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.

**POST-TRAUMATIC BLEEDING AND EX VIVO FUNCTIONAL PLATELET ABNORMALITIES IN A YOUNG WOMAN WITH PARTIAL QUANTITATIVE ANALYSIS OF A YOUNG WOMAN WITH PARTIAL PLATELET FUNCTION ABNORMALITY.**

DEFECTS OF GP IA AND GP IC. POTENTIAL ROLE OF ANTI GPIA ANTIBODIES

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**SYSTEMIC MASTOCYTOSIS AND ESSENTIAL THROMBOCYTOSIS: A CASE REPORT AND AN UPDATE**

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**SPLENECTOMY IN PATIENTS WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA**

Iannacaro P, Santoro R, Muleo G

Haemophilia Centre, Azienda Ospedaliera “Pugliese- Ciaccio”, Catanzaro, Italy

**POST-TRAUMATIC BLEEDING AND EX VIVO FUNCTIONAL PLATELET ABNORMALITIES IN A YOUNG WOMAN WITH PARTIAL QUANTITATIVE ANALYSIS OF A YOUNG WOMAN WITH PARTIAL PLATELET FUNCTION ABNORMALITY.**

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, D’Inno G

Centro di Coordinamento Regionale per le Emocoagulopatie, Dipartimento di Medicina Clinica e Sperimentale and Dipartimento di Immunologia e Trasfusione, Università degli Studi di Napoli “Federico II”, and Servizio di Immunologia e Trasfusione, A.O.R.N. “A. Cardarelli”, Naples, Italy

**SYSTEMIC MASTOCYTOSIS AND ESSENTIAL THROMBOCYTOSIS: A CASE REPORT AND AN UPDATE**

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**SPLENECTOMY IN PATIENTS WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA**

Iannacaro P, Santoro R, Muleo G

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Splenectomy is the treatment of choice 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 17×10⁹/L (range 0-52×10⁹/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 µg/kg b.w. per day for five consecutive days, 5 received anti RhD immune globulin (HD Ig) 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 >50×10⁹/L or >100×10⁹/L respectively, poor if <50×10⁹/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 χ² 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**


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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 percent (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 thrombosis in 1 and hemorrhage in 2); none of which was fatal. Minor events (headache, erythromelalgia, paresthesias, minor bleeding) occurred 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 major 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).

**P049**

**LONG-POLYMERASE CHAIN REACTION IN FETAL TISSUE FROM PREGNANT WOMEN CARRYING THE FACTOR VIII GENE INVERSION**

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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 MgCl₂ 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 MgCl₂. In conclusion we want to underline that the results can vary according to DNA and MgCl₂ 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**

**WVF:RCo ASSAY: IMPLEMENTATION ON AN AUTOMATED COAGULOMETER**

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The WVF: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 WVF: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

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U/mL; L2 = 0.47 U/mL) were used to assess the test variability.

Methods. VFVF: 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 dilutions were similarly prepared for both methods. Results.

<table>
<thead>
<tr>
<th>WVF: RCo concentration U/mL (means and intervals)</th>
<th>AGM</th>
<th>ACL</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0.96 (0.42-1.57)</td>
<td>0.91 (0.39-1.93)</td>
</tr>
<tr>
<td>WVD1</td>
<td>0.30 (0.07-0.60)</td>
<td>0.22 (0.06-0.49)</td>
</tr>
</tbody>
</table>

The VWF: RCo values determined by AGM and ACL are very well correlated (r = 0.952, Passing-Bablok regression y = 1.024 × 0.99). 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.

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 WVF: RCo assay performed by ACL, suggest its routine application for WVD diagnosis.

P052
MONITORING HEPARIN TREATMENT IN DIFFERENT CLINICAL SETTINGS: COMPARISON OF TWO POINT-OF-CARE WHOLE BLOOD COAGULATION ANALYZERS
Paniccia R, Carbonetto F,* Bandinelli B, Palmari M F G,* Conti P,* Fedi S, Lari B, Lentù 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, Firenze, 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 Altmann analysis revealed a good performance for both systems (p < 0.001), although ACT values obtained by ISTAT 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; ACTII 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.

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. di *Centro Trombosi, Anestesiologia e Rianimazione 1, Centro Emodialisi, Azienda Ospedaliera Careggi, Firenze, Italy

Heparin therapy during cardiopulmonary bypass (CPB), interventional cardiology and hemodialysis, is traditionally monitored by a point-of-care clotting test, performed by a point-of-care instrument. In recent years, 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; ACTII 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.
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**

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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 pre-analytical rules. Hcy concentration was measured by AxSYM and IMx Hcy FPIA methods and by a rapid isotopic 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.

<table>
<thead>
<tr>
<th></th>
<th>AxSYM</th>
<th>HPLC</th>
<th>IMx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imprecision (CV%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intraassay</td>
<td>3.3</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>interassay</td>
<td>4.5</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>99.2</td>
<td>100</td>
<td>101</td>
</tr>
<tr>
<td>Sensitivity (µmol/L)</td>
<td>0.7</td>
<td>0.05</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Comparison data are reported in Table 2.

Table 2. Comparison data.

<table>
<thead>
<tr>
<th></th>
<th>Spearman’s correlation</th>
<th>Passing &amp; Bablok method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs statistic (p)</td>
<td>Intercept</td>
</tr>
<tr>
<td>AxSYM vs HPLC</td>
<td>0.83 (p &lt; 0.0001)</td>
<td>2.844</td>
</tr>
<tr>
<td>AxSYM vs IMx</td>
<td>0.97 (p &lt; 0.0001)</td>
<td>-0.367</td>
</tr>
<tr>
<td>IMx vs HPLC</td>
<td>0.83 (p &lt; 0.0001)</td>
<td>2.652</td>
</tr>
</tbody>
</table>

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 and IMx was +17% and +13%, respectively, whereas the median difference between AxSYM and HPLC was +11.7%. 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 STAACL-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. STAACL-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 STAACL-LA. The median (range) clotting time difference after hexagonal phospholipid was 38 (9-101) seconds. Thirty-nine of the 43 STAACL-LA-positive plasmas were also positive according to SCT (sensitivity relatively to STAACL-LA = 91%). The median (range) percentage correction for these 39 plasmas after phospholipid increase was 64% (23%-79%). Two of 19 plasmas that were STAACL-LAnegative were SCT-positive (specificity relatively to STAACL-LA = 89%). In conclusion, SCT performed at low and high phospholipid concentrations without normal plasma may be considered as reliable as STAACL-LA performed with hexagonal phospholipids and normal plasma to diagnose LA in patients on OAT. Advantages of SCT over STAACL-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 STAACL-LA AND SILICA CLOTTING TIME**

Chantarangkul V, Tripodi A, Clerici M, Bressi C, Mannucci PM

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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) clotting time difference 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 thawing non-filtered plasmas.

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 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.

PO56
USEFULNESS OF THE PLATELET FUNCTION ANALYZER PFA-100 IN MONITORING THE EFFICACY OF EPOPROSTENOL INFUSION

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 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.

PO57
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 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.
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 activat-
ed 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. 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 INR groups:

<table>
<thead>
<tr>
<th>INR</th>
<th>TGAt</th>
<th>PT</th>
<th>aPTT</th>
<th>Fib</th>
<th>TGCs</th>
<th>factII</th>
<th>AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.00</td>
<td>1.47</td>
<td>1.63</td>
<td>301</td>
<td>54</td>
<td>116</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00&lt;</td>
<td>2.13</td>
<td>2.61</td>
<td>378</td>
<td>29</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;3.50</td>
<td>2.41</td>
<td>4.20</td>
<td>428</td>
<td>14</td>
<td>123</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In detail, the TGAt mean value and the TGAt cut-off value during OAT are shown in the following Table:

<table>
<thead>
<tr>
<th>INR</th>
<th>ΔN</th>
<th>Mean</th>
<th>Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.00</td>
<td>1.40</td>
<td>1.30</td>
<td>0.70</td>
</tr>
<tr>
<td>2.00&lt;</td>
<td>2.10</td>
<td>1.49</td>
<td>0.93</td>
</tr>
<tr>
<td>&gt;3.50</td>
<td>2.64</td>
<td>1.12</td>
<td>0.70</td>
</tr>
</tbody>
</table>

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.

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>
<thead>
<tr>
<th>Week</th>
<th>TGAt</th>
<th>PT</th>
<th>aPTT</th>
<th>Fib</th>
<th>TGCs</th>
<th>factII</th>
<th>AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.60</td>
<td>0.96</td>
<td>1.00</td>
<td>371</td>
<td>69</td>
<td>301</td>
<td>99</td>
</tr>
<tr>
<td>5</td>
<td>0.62</td>
<td>0.93</td>
<td>1.00</td>
<td>434</td>
<td>53</td>
<td>110</td>
<td>97</td>
</tr>
<tr>
<td>10</td>
<td>0.75</td>
<td>0.90</td>
<td>0.97</td>
<td>485</td>
<td>46</td>
<td>115</td>
<td>100</td>
</tr>
</tbody>
</table>

Conclusions. The TGAt test is useful in recognizing clotting activation during physiological pregnancy.

Eosinophil cationic protein (ECP) is a highly cationic protein and a major compound of the human eosinophil cytoplasm granules. The biological function of ECP and the role of eosino-

phils 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 hyperesinophilia 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 hyperesinophilia so, in accord-

ing 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 hyperesinophilic syndrome of unknown origin. ECP plasma level was 75.1 ng/mL (ECP nor-
ACTIVATED PROTEIN C RESISTANCE AND FACTOR V LEIDEN: COMPETITION OR MUTUAL HELP?

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Introduction. APCR, acquired or congenital, causes an increased risk of venous thrombosis. Congenital APC 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 F 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 analysis was requested; in 162/195 DNA analysis (83%) resulted.

FEASIBILITY OF THE USE OF FILTERED PLASMA IN THE AUTOMATED SCREENING TEST FOR ACTIVATED PROTEIN C RESISTANCE

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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 available 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 work sessions 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™ APT™ 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.

MARKED CLOTTING ACTIVATION AND MONITORING OF HEPARIN TREATMENT DURING CARDIOPULMONARY BYPASS

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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,
Antiphospholipid Antibodies

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<0.001), 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 CHEMONEAIVE COLORECTAL CANCER PATIENTS
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Colorectal cancer is often associated with alteration of hemostasis. Thrombotic events, in fact, are more frequent than hemorrhagic 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 chemoneaive 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 ANTI COAGULANT AND/OR ANTICARDIOLIPIN-ANTIBODIES
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Internal Medicine I & Clinical Pathology Departments, Thrombosis Center, Azienda Ospedaliera Internal Medicine I and Clinical Pathology Departments, Thrombosis Center, Azienda Ospedaliera S.M aria 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 are not rarely 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 expressed 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 years, 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 = 0.026), followed by dRVVT and IgG - dRVVT association. The odds ratio for IgG and dRVVT 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.

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. L A activity was diagnosed using screening and confirmatory procedures based on criteria according to ISTH-recommendations. IgG and IgM ACA isotypes were assayed with the Autozyte 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%) 4 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.
Antiphospholipid Antibodies

P068
THE β2-GLYCOPROTEIN 1-DEPENDENT ANTICARDIOLIPIN ANTIBODIES: THEIR IMPORTANCE AS A RISK FACTOR FOR ISCHEMIC STROKE AND MYOCARDIAL INFARCTION


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 β2-glycoprotein 1 (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) in our series. 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 were also determined (data not shown). Results. One hundred and twelve out of 139 patients (80.5%, 95% CI: 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% CI: 18.11-35.47), 28 were aCL- a-β2GP1+ (25.00%, 95% CI: 16.51-33.49). The risk of thrombotic events was significantly increasing in aCL+ a-β2GP1+ compared with control group, (odds ratio 5.65, 95% CI: 2.11-15.13). The odds ratio of aCL+ a-β2GP1 was 0.85 (95% CI: 0.40-1.82); the odds ratio of aCL- a-β2GP1+ was 2.25 (95% CI: 0.82-6.19). Subsequently 93 patients had ischemic events; 40/93 were aCL- a-β2GP1+ patients, (43.01%, 95% CI: 33.31-52.71), 18/93 were aCL+ a-β2GP1- patients, (19.35%, 95% CI: 11.61-27.10), and 20/93 were aCL- a-β2GP1- patients, (21.51%, 95% CI: 13.45-29.56). The risk of thrombotic events in the follow-up also increased in aCL+ a-β2GP1+, (odds ratio 1.92, 95% CI: 0.89-1.10), 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


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Gaucher’s disease (GD) type I (β-glucocerebrosidase deficiency) is characterized by the storage of uncleaved β-glucocerebrosidase in the cells of the reticuloendothelial system leading to bone marrow infiltration, hepatosplenomegaly and skeletal lesions. Hematologic changes with anemia, thrombocytopenia and thrombocytopenia 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 brachiocephalic 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 methylene threhydrolfate reductase (MTHFR-C677T), prothrombin 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 APA were significantly increased (47.4 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 ANTI COAGULANT INHIBITOR DURING ORAL ANTICOAGULANT THERAPY

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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, especially 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 ± 0.29. The cut-off ratio for SCT50/50 and SCT 50/5 was, by our data, respectively 1.30 and 1.35.

Table 1.

<table>
<thead>
<tr>
<th>SCT ratio</th>
<th>SCT50/50</th>
<th>SCT50/5</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 2.00</td>
<td>18 (14)*</td>
<td>23 (15)*</td>
</tr>
<tr>
<td>cut off + 2.00</td>
<td>30 (6)*</td>
<td>38 (6)*</td>
</tr>
<tr>
<td>TOTAL</td>
<td>49 (21)*</td>
<td>61 (21)*</td>
</tr>
</tbody>
</table>

*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

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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 IgM 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 fatal losses (4). We carried out the original and the modified (plasma diluted 1:5 in FV defected plasma) APCR APTT based assays (Instrumentation Laboratory). We found that the prevalence of APCR original phenotype was significantly higher in patients with ACP (IgG) and anti β2GPI (+) (24/25) than in those patients ACP (IgG) and anti β2GPI (-) (21/25). The ACP (IgG) and anti β2GPI (+) group showed a mean APCR ratio significantly lower than the ACP (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 = .75) was found. However, when the analysis was limited to those patients with an abnormal APCR ratio, the correlation was increased (r = .86). We also found a linear correlation when plotting log anti β2GPI 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 ACP 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

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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-protein 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 (Beuty 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. Fifty 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 95th 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.
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.

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 (VWF) 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 the VWF native subunit probed by monoclonal antibodies. 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.

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.
LABORATORY DATA AND SONOCLOT ANALYSIS FOR COAGULATION ANALYSIS IN ORTHOTOPIC LIVER TRANSPLANT RECIPIENTS

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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 χ² tests as required (confidental 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 correlated 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.

GASTROESOPHAGEAL VARICES IN HEMOPHILIC PATIENTS WITH HEPATIS B OR C VIRUS INFECTION AND LIVER DISEASE PROGRESSION: THE NEXT LIFE-THREATENING CONCERN IN HEMOPHILIA?

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With regard to liver disease progression in the hemophilic 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 variceal, 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 hemophili 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 FVII, 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 laparotomy. Initially, we noticed 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 esophagus were seen together with abundant blood clots. The meticolous surgi-
Hemorrhagic complications of small bowel and a test for occult blood in the stool was started after 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, \(\alpha\)-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 needed in hemophiliacs who have had HBV and/or HCV infection. Periodic fiber optical endoscopy must also be performed to see possible esophageal-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 choice 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.

### P077

**EFFICACY OF VERY LOW DOSE RECOMBINANT FACTOR VIIA IN A BLEEDING PATIENT WITH FACTOR VIII INHIBITORS UNDERGOING SURGERY**

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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.
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 prothrombin 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 may favour the development of an abnormal lymphoid clone and in our case, probably, autoantibodies against FVIII.

Table 1. Coagulation profiles.

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<thead>
<tr>
<th></th>
<th>n.v.</th>
<th>At visit</th>
<th>1st wk</th>
<th>2nd wk</th>
<th>3rd wk</th>
<th>4th wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT  (sec)</td>
<td>34''</td>
<td>115''</td>
<td>82</td>
<td>63</td>
<td>45</td>
<td>33</td>
</tr>
<tr>
<td>FVIII (%)</td>
<td>60-150%</td>
<td>&lt; 1</td>
<td>4</td>
<td>41</td>
<td>53</td>
<td>69</td>
</tr>
<tr>
<td>FVIII inhibitor (BU/mL)</td>
<td>&lt; 0.01%</td>
<td>130</td>
<td>45</td>
<td>4</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

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.
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 enlargement and lanugosis 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 transferogin revealed hypoglycosylation. Analysis of lipid-linked-oligosaccharide (LLO) in the patient's fibroblasts suggested a diagnosis of CDG-1a. According to published data, a thrombotic tendency is frequent in CDG-1a, while an increased bleeding tendency is more frequent in CDG-1b. 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


We report on the evaluation of risks and complications concerning 12 knee prosthesis 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 thickening of the skin and subcutaneous tissue, limited joint function were considered. The choice of the prosthesis depended on the anatomic and pathologic 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 underestimated: 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 duration of replacement with coagulation factors concentrate and management of blood transfusions. Appraisal of infective risk and antifibrotic 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


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 anaemia due to macrohaematuria and recurrent subcutaneous bleeding. His past history was negative for bleeding. When admitted to our ward he had an extensive muscle haematoma 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

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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.

**P085**

**VITAMIN K RESCUE IN A PREGNANT WOMAN WITH PRE-TERM LABOR CAUSED BY A SUBCLINICAL CELIAC DISEASE**

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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 (45.7%). The ultrasound showed a small-for-age fetus confirmed by F.II: 5%; F.VII: 3%; F.X: 4%; F.IX: 18%; protein C: 23%; free protein S: 20%. 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.

**P084**

**FACTOR V SAN GIOVANNI ROTONDO: A NOVEL MISENSE MUTATION (ARG2074CYS) IN THE C2 DOMAIN OF FACTOR V ASSOCIATED WITH REDUCED COFACTOR ACTIVITY**


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 phosphatidylinerine-containing membranes.
Early treatment with steroids must be started as soon as the diagnosis is made to eradicate the inhibitor. Subcutaneous desmopressin (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, 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 F1, 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 transusions 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, Schla.vo, 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/kg/day1 with FVIII:C increase to 35% and inhibitor decrease to 0.91 BU mL-1. At week eleven, the inhibitor completely disappeared and FVIII:C was 88%; thus deflazacort therapy was stopped. The patient is at present (January 2002) completely disappeared and FVIII:C was 88%; thus deflazacort maintenance therapy was given for 3 weeks at doses ranging from 0.5 mg/Kg1 to 0.2 mg/kg/day1 with FVIII:C increase to 35% and inhibitor decrease to 0.91 BU mL-1. At week eleven, the inhibitor completely disappeared 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/kg/day1 for ten weeks with FVIII:C 20% while the inhibitor was 1.21 BU mL-1. No other disease or 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 occurs. 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.

P066
ACQUIRED HEMOPHILIA IN AN ELDERLY PATIENT WITH CHRONIC MYELOID LEUKEMIA
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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 (43.7×10^9/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 F1, IX, II, XI, XII or XII 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 cytometric and cytogentic evaluations and the PCR assay was employed to detect the presence of bcr-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. Intraavenous 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 disappearance of autoantibodies to FVIII was achieved 3 months after initiation of corticotherapy. No bleeds were recorded at recent follow-ups (October 2003). 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 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 Genar 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.
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 peptides 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 bleeding complications. At the age of 18 months, he also had a post-traumatic epidural hematoma without any fracture. He had no prior history of bleeding. The proband underwent dacyrstenosis 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.

**P087**

MOLECULAR AND GENETIC ANALYSIS OF A COMPOUND HETEROZYGOUS FOR DYSPROTHROMBINEMIA AND HYPOPROTHROMBINEMIA

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FOR DYSPROTHROMBINEMIA AND HYPOPROTHROMBINEMIA

MOLECULAR AND GENETIC ANALYSIS OF A COMPOUND HETEROZYGOUS

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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

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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 whose 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:

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<tr>
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<th>VWF:Ag</th>
<th>VWF-protease</th>
<th>VWF:CB</th>
<th>Plts x109/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>97±33</td>
<td>107±22</td>
<td>64±22</td>
<td>333±97</td>
</tr>
<tr>
<td>After</td>
<td>112±36</td>
<td>92±20</td>
<td>98±18</td>
<td>22±33</td>
</tr>
<tr>
<td>Before</td>
<td>156±25</td>
<td>76±24</td>
<td>166±19</td>
<td>171±85</td>
</tr>
<tr>
<td>After</td>
<td>156±24</td>
<td>75±22</td>
<td>171±7</td>
<td>49±18</td>
</tr>
</tbody>
</table>

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

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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-
possible 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 because of the low titer of inhibitor. On the 7th day the titre of inhibitor rose and replacement therapy with FVII became ineffective. rFVIIa was initiated by CI and continued for seven days with a total dose of 126 mg. The second case was femur osteotomy, treated successfully for 8 days. Two ileo-psoas hematomas, four lithotripsy sessions for renal calculus 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**

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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 KIU (21 µg/kg) that was repeated twice during the operation. CI began 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 KIU in days 11-12. A total of 0.93 µg/kg rFVIIa was used. FVII plasma 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 HEMORRHAX AND ILEOPSOAS HEMORRHAGE**

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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 hemorhax, 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 immunoglobulin (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 immunosuppressive treatment, the first-line use of HDIg enabled a fast control of FVIII inhibitor.

**P092**

**THROMBOPHILIC FAMILIES: A DIFFERENT GENOTYPE/PHENOTYPE CORRELATION**

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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 M L,* Capasso F,* Albinolo L,* Pudore L,* Torre S,* Russo V,* Iacca A,** Cipollino M,* D’Ambrosio A,* Pinto A,* Curcio M,* Sica G,* De Lucia D*

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Observational studies have suggested that hormone replacement therapy (HRT) may reduce the risk of arterial disease in healthy post-menopausal women. However an early increased risk of myocardial infarction was reported in women with coronary heart 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 be 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.

**P092b**

**MARKERS OF HYPERCOAGULABILITY AND PROC GLOBAL AS SCREENING TEST FOR THROMBOPHILIA**

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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 µmol/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 coagulumetric assay measuring the prolongation of an APTT induced by activation of protein C (PC) in the sample. The results are expressed as normalized 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 (coagulumetric 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 CYTOAFAERESIS EFFECTS ON HEMOSTASIS IN CD 34+ CELLS DONORS

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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, protein C, protein S, thrombin-antithrombin complex, prothrombin fragment 1+2, coagulant factor VIII, von Willebrand Factor, thrombomodulin, plasminogen and homocysteine 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 leukapheresis 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 fibrinolytic system as a consequence of either the extracorporeal circulation or a physiological 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.

**P094**

INHERITED FACTOR V LEIDEN MUTATION AND/OR PROTHROMBIN (G20210A) VARIANT CAN AFFECT THE CLINICAL OUTCOME OF BONE MARROW TRANSPLANT RECIPIENTS

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Background. Lower limb venous thrombosis (ULVT) is less frequent than iliac and lower limb venous thrombosis. It is often associated with anatomical abnormalities 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 (8%), in 3 cases associated with PE. Conclusions. One or more thrombophilic 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.
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 accompanied 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 allogeneic 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 amplification using the restriction enzyme Mnl I 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 APCR 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.

<table>
<thead>
<tr>
<th></th>
<th>Day -8</th>
<th>Day +7</th>
<th>Day +30</th>
<th>Controls N=15</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV:C</td>
<td>178±31</td>
<td>75±31*</td>
<td>89±25</td>
<td>95±13</td>
</tr>
<tr>
<td>FII:C</td>
<td>149±39*</td>
<td>111±22</td>
<td>127±19*</td>
<td>98±23</td>
</tr>
<tr>
<td>APCR ratio</td>
<td>2.8±0.9</td>
<td>2.1±1.2*</td>
<td>1.7±1.3*</td>
<td>3.1±0.9</td>
</tr>
<tr>
<td>FV Leiden mutation</td>
<td>3/19</td>
<td>0/19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothrombin (G20210A) variant</td>
<td>2/19</td>
<td>0/19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 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?

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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


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Background. Increased pre-operative levels of soluble fibrin polymers, as determined by an enzyme immunoassay (TePTM), correlate with the development of deep vein thrombosis after elective neurosurgery (Sonaglia, 1999). Aim of the study: To evalu-
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. 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**

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Few patients with septic thrombophlebitis, exclusively referred to Campylobacter fetus infection, are reported in the medical 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, cilastilin, and ciprofloxacin and calcium nadroparine 8000 U sc every 12th hour was started. Four days after, because of rapid aggravation of the clinical features and thrombosis progression associated with the appearance 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, Plt 132,000 m3, HYC 112 µM/L; normal values of APA, PANC, CA125, CA19-9, IP2; absence of LA and of FV G1691A and FII G20210A mutations. Bacteriological 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 (Ambín-GñFols) at doses of 400 U/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 was 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 the admission.
P099
DERMATAN SULPHATE IN A PATIENT WITH HEPARIN-INDUCED THROMBOCYTOPENIA AND PULMONARY EMBOLISM
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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, Italy) 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 QID). Ventilation-perfusion lung scan was diagnostic for acute pulmonary embolism (high probability) and the echocardiopplomer revealed a thrombus in the greater right saphenous vein. Hematocrit count showed severe thrombocytopenia (27,000/m3). 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/m3, 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
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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<0.003) and to controls (47.8 µg/L, p<0.001; 2421.3 µg/L, p<0.02, respectively). The levels of TAT and PAP remained high 2 hours after the end of reinfusion compared to controls (154.5 µL vs 37.6, p<0.1; 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.

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.
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 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.

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.
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 EUSA. 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 UHF (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, UHF 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 UHF may act differently in reducing the activation of blood coagulation triggered by tumor cell-derived products or by LPS in distinct EC types.

**P105**

**GENETIC THROMBOPHILIA: ASYMPTOMATIC AND SYMPTOMATIC CARRIERS AND CLINICS**

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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 LYMPHOBLASTIC DISEASE**

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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 7.2% (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.

The relative risk for thrombosis was 5.5 concerning the FV Leiden mutation and 0.83 for the FII G20210A mutation. The prevalence of FV 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, LPD appears to be an independent risk factor for venous thromboembolism in this category of patients.

**P107**

**COEXISTENCE OF THROMBOPHILIC GENE POLYMORPHISMS AMONG 623 UNRELATED CONSECUTIVE PATIENTS WITH A HISTORY OF THROMBOSIS**

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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 co-existence of at least two of these polymorphisms was found in 5 (2.2%) patients with VT, 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; χ² 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+MTHFR and 12 (3.5%) cases of MTHFR+AT combinations. 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+VT (0.3% for FV+MTHFR, 0.3% for FV+AT; p=0.02, 2.4% for FV+MTHFR; p=0.05, χ² 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+VT.

**P108**

**INHERITED PRO-THROMBOTIC CONDITIONS IN PATIENTS WITH A HISTORY OF VENOUS THROMBOEMBOLISM**

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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 G20210A 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±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; χ² test) and 40/291 (13.7%) vs 18/291 (6.2%) (OR 2.41, CI 1.3-4.5, p=0.004) for FII G20210A. 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, χ² 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**

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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**

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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 significantly 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 meteorological parameters that could influence the incidence of and mortality from PE.

**P111**

**HEPATIC VEIN THROMBOSIS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS**

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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), 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/10^9plt) 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-transplantation 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 DISORDER**

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Essential thrombocythemia (ET) and polycythemia vera (PV) are chronic myeloproliferative disorders (CM D) 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 x 10^9/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**

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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 sequestrated ascites of neoplastic nature due to peritoneal metastasis; CT scan examination confirmed the presence of peritoneal metastasis from pancreatic cancer 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±1.14), aPTT (1.01, ratio), fibrinogen (414mg/dl), protein C (77%), protein S antigen (81%), ATIII (72%), APC resistance (0.83, 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 vein thromboembolism or other thrombotic complications such as DIC in oncological patients, specially affected by pancreatic tumor. In particular, microthrombi are often found 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 (reparin 4200 UI/die) because of its selected inhibition of prothrombinase (f.Xa).

**References**


**P113**

**PROPHYLAXIS OF DEEP VENOUS THROMBOSIS AND PULMONARY EMBOLISM IN THE SURGICAL DEPARTMENT OF "A.S.L.4 CHIAVARESE" (LIGURIA REGION, ITALY)**

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Geerts et al. suggest that each hospital should write down an evaluation procedure of thromboembolic risk and prophylaxis especially in high risk patients. First, we have written and delivered to the directors of the surgical divisions (surgery, orthopedics, day-surgery, gynecology, otolaryngology, 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). Nowadays the schedule is used in each of our surgical divisions. Another important goal of this work is to validate our risk score.

**P114**

**THROMBOTIC PATHOLOGY: FROM THE LABORATORY TO CLINICAL ASSESSMENT OF THE RISK FACTORS. OUR EXPERIENCE**

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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 multiple 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:

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<th>Acquired factor</th>
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<td>Factor V Leiden</td>
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<td>+</td>
<td>4.1</td>
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<tr>
<td>Prothrombin G20210A</td>
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<td>11</td>
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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.

P116

PREVALENCE OF THROMBOPHILIC GENOTYPES IN PATIENTS WITH DEFICIENCY OF NATURAL ANTICOAGULANTS. A STUDY OF 34 CASES

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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 ischemic stroke) have had previous episodes of deep vein thrombosis (DVT) and/or pulmonary embolism. Results. Ten patients (29%) had TT677MTHFR genotype (1 AT, 5 PS and 4 PC deficiency) and 7 among 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.

A NET-SUPPORTED SCORING SYSTEM TO ESTIMATE THROMBOTIC RISK AND TO ADDRESS A TARGETED PREVENTION

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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 centered 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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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, hepatop-splenomegaly and visible cutaneous varices on the anterior abdomen wall. There was laboratory evidence of poliglycemia 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 antegrade 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).

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 venous 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 57.8±6.4, 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 smok-
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
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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 disobliterated with a Fogarty catheter under UF heparin continuous administration. Forty-eight hours later thrombotic recrudescence 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. 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 disobliterated 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-β2 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
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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 HyperHCY 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. Results. 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 HyperHCY 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.
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 THERMOLABLE METHYLENE TETRAHYDROFOLATE REDUCTASE VARIANT IN CANCER PATIENTS
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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 immunonassay (FPIA). C677T MTHFR polymorphism was determined by polymerase chain reaction and restriction analysis. Results. In cancer patients plasma homocysteine levels (µmol/L) were 11.1±3.72 in CC genotype (31.57%), 13.5±5.48 in CT genotype (48.21%) and 15±9.80 in TT genotype (30.35%). Plasma homocysteine levels were statistically different between CC and CT 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 homocysteinemia 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
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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:Ag) by the one-stage clotting assay and as antigen (TF:Ag) by ELISA. The results show that, in HMEC-1, the three heparins dose-dependently counteracted the IL-1β-induced expression of TF:Ag (% 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:Ag, 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.
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

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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-scarred 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 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 systemic lupus erythematosus was made and treatment with deflazacort (6 mg/daily), hydroxychloroquine (200 mg/daily) and thyroxin (0.05 mg/daily) was started. During the following 2 years she was in good health notwithstanding deflazacort tapering until 3 mg/daily. In 1995 and 1996 she had two pregnancies. As prophylaxis for pregnancies she received flucortolone (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 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: gender plays an important role in the time of appearance of the initial thromboembolic manifestation.
Venous Thromboembolism

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 < 0.05 years and 1 with relapsing CVRT) (11%) and 20 controls (4.7%, NS); TT677/MTHFR. Conclusions. A20210/FII genotype is associated with CVRT 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
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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 polymorphism. 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), homozygous FVL plus heterozygous FII/A20210 7% (1/15), homozygous MTHFR C677T with hyperhomocysteinemia 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 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 polymorphism. 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), homozygous FVL plus heterozygous FII/A20210 7% (1/15), homozygous MTHFR C677T with hyperhomocysteinemia 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 hyperhomocysteinemia 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
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A 43-year old woman with a controlled post-operative hypothyroidism–thyroidectomy in 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.

P131
CANDIDATE GENE POLYMORPHISMS IN THROMBOTIC DISEASE
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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 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.

<table>
<thead>
<tr>
<th>Marker</th>
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<td>Factor II G20210A</td>
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<tr>
<td>Factor V Arg506Gln</td>
<td>&lt; 0.001</td>
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<tr>
<td>ICAM gly214Arg</td>
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<td>Angiotensin Receptor 1 A1166C</td>
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**References**


**P132**

**RISK FACTORS FOR THROMBOSIS IN PATIENTS WITH THE NEPHROTIC SYNDROME**

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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 causative factors for the high thrombotic risk in these patients are not yet fully understood. The aim of the study was to investigate whether two common risk factors for thrombosis, resistance to activated protein C (RAPC) 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) in NS patients than in controls; 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 lower (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.

**P133**

**PREGNANCY IN INHERITED THROMBOPHILIA: FOLLOW-UP OF A SERIES OF 53 PATIENTS**

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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.

**P134**

**THROMBOEMBOLIC EVENTS IN CHILDREN ADMITTED TO “OSPEDALE INFANTILE REGINA MARGHERITA” OF TURIN BETWEEN SEPTEMBER 1998 AND DECEMBER 2001**

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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 1 day-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 prothrombosis 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**

THROMPHILIC SCREENING IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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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 methylenetetrahydrofolate reductase was evaluated. The same parameters were tested in 100 normal subjects (males = 50, females = 50; mean age 46 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). Anti-thrombin, 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 methyltetrahydrofolate reductase. Antiphospholipid antibodies were found in eight patients (61.5%) and in one (1%) normal control (OR=15.8, 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.

**P136**

CLINICAL SIGNIFICANCE OF ANTI-PROTHROMBIN ANTIBODIES IN PATIENTS WITH A HISTORY OF VENOUS AND/OR ARTERIAL THROMBOSIS

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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 immunosorbert assay (Orgentec kindly supplied by Bonty, 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 thrombosis than in controls (aPT IgG 21% vs 6%, p < 0.02; aPT IgM 22% vs 4%, p < 0.0001). In the group of subjects found negative for Lupus Anticoagulant activity (LA) and aCL and anti p2 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 (Cl) 2.9-25.3 and 1.3-13.2 respectively) and venous thrombosis (OR 9.9 and 6.7; 95% Cl 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.
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 be 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 in 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.

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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
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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.
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 aPTT prolongation. The direct thrombin inhibitors could be more effective than heparins in the prevention of 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 vitro thrombin generation.

PLASMA LEVELS OF ENDOTHELIAL PROTEIN C RECEPTOR RESPOND TO ANTICOAGULANT TREATMENT

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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 (184.5±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


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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.

Table 1.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mean age</th>
<th>Sex</th>
<th>% INR within range</th>
<th>% INR below range</th>
<th>% INR above range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age &lt; 75 years</td>
<td>102</td>
<td>67±7</td>
<td>65M/37F</td>
<td>73.2%</td>
<td>10.5%</td>
</tr>
<tr>
<td>(45%)</td>
<td>(37-74)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age &gt; 75 years</td>
<td>124</td>
<td>80±4</td>
<td>67M/57F</td>
<td>76.7%</td>
<td>12.2%</td>
</tr>
<tr>
<td>(55%)</td>
<td>(70-81)</td>
<td></td>
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</tr>
</tbody>
</table>

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 in an Anticoagulation Clinic.

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ORAL ANTICOAGULANT THERAPY AND THROMBOCYTOPENIA

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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 positivity to the hepatitis markers and autoantibodies. All patients at the time of bleeding complication were in a therapeutic range of anticoagulation.

P145

LOW-MOLECULAR-WEIGHT HEPARIN TREATMENT AND CANCER: POOLED ANALYSIS OF 908 PATIENTS TREATED FOR THREE MONTHS

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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-analysis 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
INTRACRANIAL BLEEDING IN REGGIO EMILIA: EPIDEMIOLOGY AND RELATIONSHIPS WITH ANTIARTHROMBOTIC TREATMENT

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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 χ² 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 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 1.1 in AP and 1.1 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 significantly 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.

LOW DOSE ORAL VITAMIN K REVERTS WARFARIN-INDUCED COAGULOPATHY MORE EFFECTIVELY THAN SUBCUTANEOUS VITAMIN K

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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.
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 pamaparin 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

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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 FVIIlc 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±60% in AF, 187±90% in PV, 206±72% in VTED, 90±12% in controls. Even though the mean value of FVIIlc levels detected in VTED patients was higher than that observed in other two groups the difference was not significant. The levels of FVIIlc 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 FVIIlc 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

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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, anthra-noides 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 absorption of the anticoagulant produced by a simultaneous intake of Senna derivatives. 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-CIRRHTIC PORTAL AND MENSETERIC THROMBOSIS

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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 5.19±7.16 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, T677 genotype of MTHFR, lupus anticoagulant and cardioliopin antibodies. Hematologic, hepatic and neoplastic diseases were inves-
Life-long anticoagulation. Relapse or extension of thrombosis seems to be exclusions. The following thrombophilic factors were found: myeloproliferative disease (22%), protein C deficiency (4%), FVL heterozygous (11%), MTHFR T677 (18%), PTHRA 20210 + MTHFR T677 (4%), PTHRA 20210 + FVL (4%), MTHFR T677 + 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) and 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.
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 nonvascular 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

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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 occurred 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 administrated 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, confirms that: a) women with ET have an increased 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 decrease 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.

**P154**

PREGNANCIES AT RISK: FACTOR XA ASSAY DURING LOW MOLECULAR WEIGHT HEPARIN TREATMENT

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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 pregnancy. 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 thrombotic 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 treatment 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.
ly. In patients with a target INR ≥ 3 was 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.

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.
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

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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 ELSA serological antibody detection test and platelet count. US scan revealed axial and subclavian venous thrombosis around the indwelling catheter. Serological screening tests for hereditary thrombophilia were negative. APA were not detected in serum.

LMW heparin administration was immediately stopped and der-


TREATMENT OF PULMONARY EMBOLISM SECONDARY TO ESSENTIAL THROMBOCYTOMIA: A CASE REPORT

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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). At 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/ml. A diagnosis of ET was formulated according to the Poly-
tiyemia 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 thromboendarterectomy (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 micro-


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DERMATAN SULPHATE AS A TREATMENT FOR HEPARIN-INDUCED THROMBOSIS AT SITE OF INTRAVENOUS CATHETER INSERTION

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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 ELSA serological antibody detection test and platelet count. US scan revealed axial and subclavian venous thrombosis around the indwelling catheter. Serological screening tests for hereditary thrombophilia were negative. APA were not detected in serum.

LMW heparin administration was immediately stopped and der-


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antithrombotic treatment. PTE, a surgical procedure indicated in selected patients with chronic thromboembolic pulmonary hypertension, did not have any advantage in this patient.

P160
PERIOPERATIVE MANAGEMENT WITH NADROPARIN OF PATIENTS ON LONG TERM ORAL ANTICOAGULANT THERAPY: A PROSPECTIVE STUDY
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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. Thirty-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: hemirotomy (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 heparination: 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 (hemorrhoids, hemoptysis). 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
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Prophylaxis with heparin and/or acetylsalicylic acid (ASA) improves pregnancy outcome and avoids thromboembolic complications in the antiphospholipid 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 gravidix. In puerperium Nadroparin in all the patients. Results. AT patients: 14 pregnancies; 6 treated with LM WH + 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 LM WH + 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.

P162
MONITORING OF PT-INR WITH THE HELP OF A PORTABLE COAGULOMETER
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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 prosthesis, 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.
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**

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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 thrombocytopeina-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**

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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.

(The project is totally supported by Emilia Romagna Regional Research Program, Azienda Ospedaliera and Azienda USL di Parma).
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 concomitant 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 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.

<table>
<thead>
<tr>
<th>All patients</th>
<th>Excluded pts. on heparin</th>
<th>Excluded pts. with symptoms &gt; 15 days</th>
<th>Patients with suspected GSV thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>81.5% 91.1% (69.1-93.9)</td>
<td>89.5% 93.8% (78.5-94.8)</td>
<td>87.1% 91.1% (81.3-100)</td>
</tr>
<tr>
<td>Specificity</td>
<td>78.4% 78.4% (70.8-86.6)</td>
<td>78.4% 78.4% (70.8-86.6)</td>
<td>78.4% 78.4% (79.5-98.8)</td>
</tr>
<tr>
<td>Positive PV*</td>
<td>60.7% 83.3% (47.3-74.1)</td>
<td>60.7% 83.3% (47.3-74.1)</td>
<td>60.7% 83.3% (68.1-98.8)</td>
</tr>
<tr>
<td>Negative PV*</td>
<td>91.2% 82.9% (85.6-94.4)</td>
<td>94.8% 82.9% (89.5-100)</td>
<td>90.8% 82.9% (91.3-100.4)</td>
</tr>
</tbody>
</table>

*Predictive value.

Interpretation and Conclusions. Our results show that previous or concomitant heparin administration (either at therapeutic or prophylactic doses), non-acute symptoms and thrombolysis localized to superficial veins reduces the clinical usefulness of the test due to the increase of the false negative result rate.
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 of 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 µg/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.

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 86.8±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 wave reflecting fibrin polymerization. Then, an inflection is produced as platelets are incorporated into the fibrin mesh and a secondary upstroke leads to a peak, which occurs at completion of fibrin formation. The subsequent downstroke 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: CTEPI remained prolonged in 9/10 patients who had baseline prolonged CTEPI. 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.
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**

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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 enrolled into the study. In both groups we recorded: prothrombin time (PT), fibrinogen (Fg), plasminogen (PLG), protein C (PC), protein S (PS), antithrombin III (ATIII), APC resistance test (APC-r), D-Dimer (D-D), tissue plasminogen activator (t-PA), plasminogen 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, F XII). Our results show that PAI-1 (M±SD) was 46.8±26.3 ng/mL in P and 100±55% 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 139.8±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**


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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 (2.3-14.0) in patients and 6.3 (0.0-9.8) 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.

**P169**

**ACTIVATION OF COAGULATION AND FIBRINOLYSIS DURING MYOCARDIAL REvascularization: ON-PUMP versus OFF-PUMP TECHNIQUES**

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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 (p = 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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