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Società Italiana per lo Studio dell'Emostasi e della Trombosi

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XIX Congress of the Società Italiana per lo Studio dell'Emostasi e della Trombosi

(Italian Society for Studies on Hemostasis and Thrombosis)

Milano, Italy, September 14-17, 2006

ABSTRACT BOOK

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(Italian Society for Studies on Hemostasis and Thrombosis)

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Scientific Reports

Oral Communications

Platelet abnormalities

C001

TPO AND IL-6 IN PATIENTS WITH ACUTE PHASE REACTION WITH OR WITHOUT THROMBOCYTOSIS

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Recent studies in mice indicated that during acute inflammation thrombopoietin synthesis is induced in the liver by IL-6. Moreover, it has been suggested that in selected human diseases TPO acts as an acute-phase protein. To verify the relationships between TPO, IL-6 and platelet counts (together with glycocalicin levels) during acute phase reaction, 53 consecutive patients with elevation of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) have been compared to 28 matched control patients with normal ESR and CRP. Serum TPO, serum IL-6 and plasma glycocalicin levels were determined using commercially available ELISA kits. Within the group of subjects with high ESR and CRP, 38 had normal platelet counts and 15 thromobocytosis. No control patient had thrombocytosis. Higher levels of serum IL-6 and serum TPO were found in patients with acute phase than in controls (Table). Patients with thrombocytosis had higher IL-6 values than patients with normal platelet counts (136.44 pg/mL vs 75.527 pg/mL, p=0.049). No statistical difference was found in TPO or IL-6 levels depending on the aetiology of the acute phase reaction. A positive correlation was found between ESR or CRP and TPO levels (r=0.459, p<0.0001 and r=0.485, p<0.0001 respectively) as well between ESR or CRP and IL-6 (r=0.318,p=0.0052 and r=0.410, p=0.0002 respectively), and between TPO and IL-6 (r=0.319, p=0.005). No significant correlation was instead found between TPO and platelet count or glycocalicin levels, while serum IL-6 correlated with platelet counts (r=0.390, p=0.0005). Conclusion: in humans with acute phase reaction TPO acts as an acute phase protein regardless of the causative illness, and both IL-6 and TPO contribute to thrombocytosis.

This work was supported by a grant from MIUR-FIRB (Fondo per gli Investimenti della Ricerca di Base), project number RBNE01T8C8-009.

Table.

	Matched Controls	*Patients with acute phase*	*p value*
PLT (×10⁹/L)			
m±SD	242±67	310±98	0.0016
median	222	295	
TPO (pg/mL)			
m±SD	62.04±83	189.55±139	<0.0001
median	38.54	161	
IL-6 (pg/mL)			
m±SD	7.25±14	90.79±103	0.0001
median	3.11	59.06	
GC (µg/mL)			
m±SD	2.035±0.9	2.854±2.1	NS (0.0558)
median	2.216	2.708	

C002

THE αIIBG236E MUTATION CAUSES GLANZMANN THROMBASTHENIA BY IMPAIRING ASSOCIATION WITH β3

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Glanzmann Thrombasthenia (GT) is a recessively inherited bleeding disorder caused by the deficiency of integrin αIIbβ3. The N-terminal domain of αIIb is folded in a complex structure sustained by a series of prolines. It had been postulated that the residue at position 236, spatially adjacent to proline 258, can only be a glycine for steric reasons. D'Andrea *et al.* (TH, 2002) found the αIIbG236E substitution in an Italian patient with type I GT. We decided to verify if the effect of the G236E substitution was to misfold αIIb and hence hamper αIIb-β3 association. pCDNA3.1αIIbG236E was generated by mutagenesis. Human embryonic kidney (HEK) cells were transfected either with normal or mutated αIIb in conjunction with β3. By flow cytometry analysis the percentage of HEK cells transfected with αIIbG236Eβ3 reacting with 10E5-FITC (antiαIIbβ3) was 7±1% while the percentage of cells transfected with αIIbβ3 reacting with 10E5-FITC was 37±13%. HEK cells transfected with either αIIbβ3 or αIIbG236Eβ3 were then lysed and analysed by SDS-PAGE electrophoresis and immunoblotting. Both lysates were reacting with antibodies directed against β3. In non-reducing conditions a band corresponding to αIIb was present in both lysates, although less intense in cells transfected with αIIbG236Eβ3. In reducing condition αIIb from cells expressing αIIbβ3 was nearly all mature, while in cells transfected with αIIbG236Eβ3 the ratio pro-αIIb:αIIb was 1:1 and the presence of degradation products was noted. Lysates were then immunoprecipitated with antibodies against αIIb and immunoblotted with an antibody reacting with β3. While in immunoblots from cells transfected with αIIb3 a band corresponding to β3 was strongly detectable, in immunoblots originating from cells transfected with αIIbG236Eβ3 no band at the same level of normal β3 was detected. In conclusion we demonstrated that αIIbG236E is a mutation that causes GT by impairing the association with β3 in the endoplasmic reticulum; the effect of the mutation is likely to create a misfold and the molecular modeling prediction of the need of a glycine at position 236 is confirmed.

C003

A CASE OF ACQUIRED GLANZMANN'S THROMBASTHENIA: COMPARATIVE EVALUATION OF DIFFERENT PLATELET FUNCTION TESTS

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Acquired Glanzmann' thrombasthenia (aGT) is a rare autoimmune disorder usually associated to lymphoproliferative or autoimmune conditions, caused by an autoantibody against platelet GPIIb/IIIa and characterized by bleeding symptoms similar to those of congenital Glanzmann' thrombasthenia. We characterized a new case of aGT and compared different platelet function tests for the detection of acquired GPIb/IIIa blocking autoantibodies. A young male patient (27 years) developed severe mucocutaneous bleeding with epistaxis, ecchymosis and hematemesis despite a normal platelet count. During diagnostic work-up a B-cell, non Hodgkin lymphoma was diagnosed. Platelet function was evaluated, on several occasions over a two years follow-up, by skin bleeding time (Simplate II), optical aggregometry, flow cytometry, PFA-100, β-TG and ATP content and release; anti-GPIIb/IIIa antibody was assessed by MAIPA and its functional effects by aggregometric and flow cytometric mixing tests. Bleeding time was >20 min; platelet count, aPTT, TT, fibrinogen, vWf:Ag, vWf:Rco and XDP were normal. Platelet aggregation showed complete absence of response to all platelet agonists and normal agglutination by ristocetin. Release reaction was slightly reduced with normal platelet content. Flow cytometry showed normal platelet GPIIb/IIIa expression with some antibodies (CD41 clone P2 and CD61 clone SZ21) but failure to bind other anti-GPIIb/IIIa antibodies

(CD41 clones SZ22 and A2A6/9). PAC-1 and fibrinogen binding to platelets induced by either TRAP 20 μM (normal value 93.1±3.4 and 47.7±5.1% of positive cells, respectively) or ADP 10 μM (normal value 95.6±2.1 and 61.2±7.2% of positive cells, respectively) were completely absent. PFA-100 (coll/epi and coll/ADP) were prolonged (>300 sec). Platelet specific antibodies against GPIIb/IIIa were identified in patient serum by MAIPA. Patient serum inhibited PAC-1 binding (-54%) and aggregation (-93%) of normal control platelets. We have applied several techniques to the diagnosis of a new case of aGT and shown that flow cytometry is a useful methodology for its sensitivity in the study of expression and activation of platelet GPIIb/IIIa and in the evaluation of the effect of circulating GPIIb/IIIa blocking antibody on platelet function.

C004

AN ASP217>VAL SUBSTITUTION IN β3 SUBUNIT IN A GLANZMANN THROMBASTHENIA VARIANT PATIENT, AFFECT THE FUNCTION OF αIIBβ3

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Glanzmann thrombasthenia (GT) is an inherited disorder characterized by lack of platelet aggregation in response to most physiological agonist, and associated with quantitative or qualitative abnormalities of αIIbβ3 integrin. We now report a functional variant Glanzmann thrombasthenia patient (GI) characterized by low platelet aggregation, normal αIIbβ3 integrin and mild bleeding severity. Genotyping of GI revealed a β3 homozygous mutation A-to-T leading to an Asp217 to Val. Asp217 residue is highly conserved among different species. In this study we have examined the effects of this mutation on the variant GT phenotype. We prepared two constructs, wild-type αIIbβ3 and β3 mutant in a pcDNA3.1 vector and transfected in Chinese hamster ovary (CHO) cells. Both CHO cell clones were analysed, by flow citometry to examine the αIIbβ3 integrin surface expression. Then, we tested the ligand binding function of the mutant β3 by the binding fibrinogen. To further examine the function of mutant construct, we performed cell adhesion and aggregation assay. For adhesion assay, CHO transfected clones were stained with crystal violet and quantized by absorbance at 550 nm. Aggregation assay was analysed by photographing random fields from wild-type and mutant sample. Surface expression of mutant receptors was approximately 60% of normal. As well the β3 mutant showed a binding ability for soluble fibrinogen, analysed by flow citometry, about 40% of wilde-type. CHO cells transfected with β3 mutant failed to form multicellular aggregates after stimulation with DTT and showed a more profonde defect binding for immobilized fibrinogen on the wells of microtitre plates. In conclusion this study demonstrates that, the Asp217Val mutation produce a Glanzmann thrombasthenia phenotype by qualitative abnormalities, suggesting that this amino acid is located at or close to the ligand-binding sites of β3 subunit.

C005

USEFULNESS OF A FLOW CYTOMETRIC ASSAY OF INTRAPLATELET VASP PHOSPHORYLATION FOR THE DETECTION OF PATIENTS WITH GENETIC DEFECTS OF THE PLATELET ADP RECEPTOR P2Y12

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Lack of inhibition of stimulated adenylyl cylase (which transforms ATP into cAMP) by ADP is the diagnostic hallmark of defects of the platelet ADP receptor P2Y12. The vasodilator-stimulated phosphoprotein (VASP), an intracellular actin regulatory protein, is phosphorylated by cAMP-dependent protein kinase A. The P2Y12-mediated Inhibition of adenylyl cyclase by ADP prevents VASP phosphorylation induced by agents that increase platelet cAMP, such as prostglandin E1 (PGE1). The aim of this study was to test whether a flow cytometric VASP phosphorylation assay is useful for diagnosing congenital defects of the platelet P2Y12 receptor. We studied 20 blood donors (median age 42y, range 20-66, 10 men and 10 women), 2 patients with severe P2Y12 deficiency due to frameshift mutations in the encoding gene (V.R. 68 year-old man, M.G. 56 year-old woman), 1 patient with incomplete defect of P2Y12 function due to compound heterozygosity of 2 missense mutations in the encoding gene (A.C, 63 year-old man), 2 patients with heterozygous P2Y12 defects (G.L, 23 year-old man, son of M.G., and F.C, 34 year-old man, son of A.C). The test was performed on citrated whole blood. ADP receptor reactivity ratio (ADP ratio) was calculated from the median fluorescence intensity (MFI), reflecting VASP phosphorylation, of samples incubated with PGE1 or PGE1 plus ADP, according to the formula: ADP ratio = [(MFI(PGE1) – MFI(PGE1+ADP))/MFI(PGE1)] x100. ADP ratios ranged between 76.7 and 95.3% in blood donors. Patients with severe P2Y12 defect had severely decreased ADP ratios (V.R., 0.7%; M.G., 0%); patient A.C. had moderately decreased ADP ratio (47.7%); patients with heterozygous P2Y12 defects had ADP ratios that were indistiguishable from those of healthy donors (G.L. 80.4%; F.C. 85.4%). Therefore, the flow cytometric assay of platelet VASP phosphorylation is useful for diagnosing patients with severe or moderate defects of P2Y12 receptor, but it is unable to detect milder defects.

C006*

MOLECULAR ANALYSIS OF THE PLATELET P2Y(12) RECEPTOR IN A FAMILY WITH CONGENITAL BLEEDING DIATHESIS

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Two related patients (sisters) with a congenital bleeding diathesis associated with a severe defect of the platelet ADP receptor coupled to adenylate cyclase, P2Y(12), have been described.(Cattaneo et al, ATVB 2000;20:883). Platelets from these patients are defective in ADP-dependent aggregation and in Gi-coupled ADP-mediated responses, but were normal in Gq-coupled ADP-mediated responses. Molecular analysis of the P2Y(12) gene of each sister revealed an identical single bp deletion (378delC) occurring just beyond the coding sequence for the third transmembrane domain in P2Y(12), causing a frame shift mutation (Thr126 frame shift X34) and premature truncation of the protein. In the present study we focused on the son, GL, of one of the two affected sisters He never underwent surgical operations and never suffered spontaneous bleedings; his bleeding time was moderately prolinged (13 min). Platelet aggregation and secretion studies demonstrated a moderate deficiency of platelet-binding sites for 2MeS-ADP and partial impairment of inhibition of adenylate cyclase by ADP. DNA sequence analysis of P2Y(12) gene did not reveal any DNA mutation. Therefore, for GL we hypothesized hemizygosity in P2Y(12) alleles. In order to verify our hypothesis, we analyzed the affected family by Bam HI DNA digestion, Southern Blotting and hybridization using a probe spanning the entire P2Y(12) gene. Patient GL carries the same DNA digestion pattern of his father but does not show the DNA fragment pattern present in his mother and affected aunt. This result supports our hypothesis for hemizygosity and suggests a null allele was inherited by GL from his mother. Real time Q PCR analysis of DNA will help in further verification of our hypothesis.

**PREMIO SISET 2006*

C007

CLINICAL, BIOLOGICAL AND GENETIC CHARACTERIZATION OF A COHORT OF PATIENTS AND THEIR RELATIVES WITH BERNARD-SOULIER SYNDROME IN SOUTH IRAN

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Bernard-Soulier syndrome (BSS) is a rare recessive congenital bleeding disorder caused by the deficiency of GpIb/IX/V, platelets von Willebrand factor receptor. In patients suffering from BSS platelet adhesion is typically impaired, while platelet aggregation is normal; macrothrombocytopenia is a common feature. In the present study we fully characterized a cohort of seven patients and their first and second degree relatives coming from three different families (A, B and C) from Southern Iran. In all the patients ristocetin-induced platelet agglutination was severely impaired and GpIb/IX/V platelet expression as detected by flow cytometry was less than 2% in 6 cases and 12% in the remaining one. Mean platelet count was 35000 platelets/microliter and iron deficient anemia was a common feature. Median age at first symptoms was 15

months and all the patients suffered from mucocutaneous bleedings at presentation. Mean follow up time was fifteen years and during this period mucocutaneous hemorrhages were frequent, often requiring platelet transfusions. All the patients were offspring of first or second degree consanguineous parents. Genetic analysis in families A and B demonstrated the presence of the GpIX Phe55Ser missense mutation; interestingly this mutation had already been reported in different individuals from very different ethnic backgrounds. In addition in family A we identified in two affected individuals out of three and in several relatives a heterozygous mutation of the splicing site of GpIbalpha at the beginning exon 2 (AGGT>AGGC). Heterozygous individuals in both family A and B did not exhibit the mild macrothrombocytopenia described in individuals carriers of other mutations responsible of BSS. In family C we identified a frameshift mutation in GpIbalpha gene never described before.

C008

MUTATIONS IN POSITION 702 OF NMMHC-IIA ARE ASSOCIATED WITH A SEVERE PHENOTYPE OF MYH9-RELATED DISEASE

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Background. MYH9-related disease (MYH9-RD) is an autosomal dominant disorder caused by mutations of MYH9, the gene encoding for the heavy chain of non-muscle myosin IIA (NMMHC-IIA). At birth all patients present macrothrombocytopenia and leukocyte aggregates of NMMHC-IIA, but many of them will subsequently develop sensorineural deafness, cataracts, and/or progressive glomerulonephritis leading to end-stage renal failure. Up to now mutations affecting 21 different positions of NMMHC-IIA have been associated with MYH9-RD. However, no genotype-phenotype relationships have been identified. *Patients.* We analyzed 91 consecutive patients from 47 unrelated families with MYH9-RD. *Results.* Genotype analysis of MYH9 identified mutations affecting 14 different positions of NMMHC-IIA. In 72% of families only 4 positions were involved: position 702, in the actin and ATP binding domain of NMMHC-IIA, and positions 1424, 1841, and 1933, all situated in the rod-tail domain responsible for NMMHC-IIA assembly. Patients with mutations in position 702 presented significantly lower platelet counts (median, 37×10^9/L) than patients with mutation in position 1424 (68×10^9/L), 1841 (65×10^9/L), or 1933 (88×10^9/L) (p=0.002, 0.04, and 0.02, respectively). The lower platelet counts caused a more severe bleeding diathesis in the 702 subgroup. Patients with 702 mutations presented a higher incidence of glomerulonephritis (77% of cases) with respect to patients with mutations in position 1424 (17%), 1841 (8%), or 1933 (0%) (p=0.001, 0.001 and 0.0002). Sensorineural deafness was significantly more frequent and had an earlier onset in the 702 mutations compared to each of the rod-tail mutations (100% vs. 61%, 37%, and 17%; p=0.002, 0.005 and 0.0004, respectively). Cataracts presented higher incidence in the 1424 subgroup (47%) compared to 702 (17%), 1841 (8%), or 1933 (0%) ones. *Conclusions.* Genotype is a major determinant of the phenotype of MYH9-RD. Substitutions involving position 702 in the catalytic domain of NMMHC-IIA are associated with the most severe disease phenotype.

Venous thromboembolism: epidemiology, risk factors and diagnosis

C009

RENAL TRANSPLANT RECIPIENTS ARE AT HIGH RISK FOR BOTH SYMPTOMATIC AND ASYMPTOMATIC DEEP VEIN THROMBOSIS

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Venous thromboembolism (VTE) is one of the thrombotic complications that occur in patients receiving renal transplantation (RT), however the prevalence of VTE in RT patients is undefined. In this study we evaluate the rate of a first episode of VTE in a series of 538 consecutive renal transplant (RT) recipients admitted to our Institution, the timing of occurrence of the thromboembolic events after transplantation and the rate of recurrence after thromboprophylaxis withdrawal. Risk factors for recurrence were also evaluated in particular in relation to the type of the first event (symptomatic or asymptomatic). During follow-up 47/518 patients (28 males, 19 females) (9.1%) developed a first episode of VTE at a median time of 17 (1-165) months after kidney transplantation. Cancer was associated with the occurrence of VTE with OR 4.8. Seventeen/43 patients (39.5%) with deep vein thrombosis were asymptomatic and the diagnosis was made during the routine ultrasound examination. Twenty two (46.8%) experienced a recurrence of VTE. A relevant rate of recurrence was documented among patients with a first episode both symptomatic (53%) and asymptomatic (23.5). The study confirms that RT patients are at high risk of symptomatic and asymptomatic VTE and this risk persists even after several years. Patients who experience VTE are at high risk of recurrence after thromboprophylaxis withdrawal.

C010

LIMITED VERSUS EXTENDED ULTRASONOGRAPHY FOR THE DIAGNOSIS OF CLINICALLY SUSPECTED DEEP-VEIN THROMBOSIS

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Compression ultrasonography (US) of the proximal vein system, repeating testing within one week in patients with abnormal D-Dimer (limited US) has been shown to be highly accurate and safe for the diagnosis of deep-vein thrombosis (DVT) of the lower extremities. Nevertheless, ultrasonography of the entire deep-vein system (extended US) is widely employed in clinical practice. In a prospective study, we randomized 2098 outpatients at their first episode of clinically symptomatic DVT to limited US, using the SimpliRED as D-Dimer test (1045), or extended US (1053). The aims of the study were to assess the prevalence and location of initial thrombosis, and the incidence of symptomatic venous thromboembolism (VTE) occurring in a 3-month follow-up period in patients with initial normal workup. In the limited US group, 217 patients had an abnormal baseline US; of the remaining 828, 256 (30.9%) had an abnormal D-Dimer, and US was found to be abnormal in 14 within one week. Thus, 231 of the 1045 patients (22.1%) allocated to the limited-US had a diagnosis of DVT, as compared to 278 of 1053 patients (26.4%) in the extended-US group (absolute difference 4.3%, 95% CI 0.5 to 8.1%, p=0.022). In the extended US group 65 (23.4%) had isolated (deep or muscular) calf vein thrombosis. Symptomatic VTE in the follow-up developed in 7 of 814 patients (0.9%; 95% CI, 0.3 to 1.8%) of the limited-US group with a normal workup, and in 9 of the 775 (1.2%; 95% CI, 0.5 to 2.2%) with baseline normal extended-US (p=0.62, two-tailed). We conclude that limited US leads to the diagnosis of a significantly lower initial rate of DVT in comparison to extended US, however the two strategies are equally safe.

C011

THE VALUE OF SPIRAL 4-SLICE COMPUTED TOMOGRAPHY FOR THE DIAGNOSIS OF PULMONARY EMBOLISM. A PROSPECTIVE COHORT STUDY

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Spiral computed tomography (CT) is increasingly being used as the first-line imaging procedure in the diagnostic work-up of patients with clinically suspected pulmonary embolism (PE). In contrast with extensive investigation available for the single-slice CT, virtually no study has investigated the value of the 4-slice detector CT for the diagnosis of PE. We performed a multicentre prospective cohort study aimed at assessing the clinical value of negative CT findings with the use of the 4-slice detector in a cohort of patients with clinically suspected PE free from leg(s) vein thrombosis. 702 consecutive patients (317 males; median age, 71 years) met the criteria for recruitment in this study. Of these, CT scan was interpreted as negative in 536 (76.3%), who received the determination of D-dimer according to local facilities. D-dimer was positive in 279, who had further diagnostic work-up, and negative in the remaining 257, who did not receive anticoagulant treatment, and had a 6-month follow-up. Of the former, PE was documented in 55 (19.7%). Of the latter, symptomatic thromboembolism in the follow-up period developed in 3 patients (1.17%; 95% CI, 0.24 to 3.38). We conclude that, when using the 4-slice detector the negative predictive value of spiral CT in patients with clinically suspected PE and positive D-dimer is unacceptably low. By contrast, it is safe to withhold anticoagulation from patients with negative findings and negative D-dimer.

C012

THE IMPACT OF AN AGING POPULATION ON DIAGNOSIS OF PULMONARY EMBOLISM: COMPARISON OF YOUNG AND ELDERLY PATIENTS

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The incidence of venous thromboembolic diseases such as deep vein thrombosis (DVT) and pulmonary embolism (PE) increases with age. PE may be difficult to diagnose in the elderly because of its different pattern of presentation, although the main causes of the age-related increase in thrombotic tendency are blood hyper-coagulability and vascular wall changes, and the higher incidence of PE in the elderly has also been attributed to the frequent presence of co-morbidities. We evaluated the effect of age on the prevalence of PE, and the sensitivity and specificity of diagnostic tests, as well as the role of co-morbidity as a risk factor, in 233 patients with a diagnosis of PE: 57 aged <65 years and 176 aged >65 years. The prevalence of PE was 31.6% in the patients aged <65 years and 40.2% in those aged >65 years. The presenting symptom was more likely to be chest pain in the former, and dyspnea and syncope in the latter. Statistical analysis showed that the ability of clinical scores to predict the probability of PE was not different in the two age groups. There was a significant association between PE and malignancies in the younger patients, but only a trend towards such an association among the elderly, who showed a significant association with recurrent thromboembolism and orthopedic surgery. There was no between-group difference in the overall specificity and sensitivity of Helical computed tomography and the lung perfusion scan test, revealed no observed differences between the two groups but, although the sensitivity of the D dimer evaluation was excellent in both groups, its specificity was age dependent. Analysis of variance (ANOVA) revealed a close association between the diagnosis of PE and age, and an increased Cumulative Illness Rating Scale (CIRS) score. PE is often under-diagnosed in the elderly, and the clinical laboratory findings are not specific. Only clinical suspicion can reduce the time to diagnosis and improve prognosis.

C013

PREVALENCE OF THE JAK2 V617F MUTATION IN PATIENTS WITH SPLANCHNIC OR CEREBRAL VENOUS THROMBOSIS AND WITHOUT SIGNS OF OVERT CHRONIC MYELOPROLIFERATIVE DISORDER

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Background. Thrombosis of splanchnic or cerebral veins can develop in patients with chronic myeloproliferative disoders (CMD) such as polycythemia vera (PV) and essential thrombocythemia (ET); CMD at very early stages not fulfilling diagnostic conventional criteria can account for a substantial proportion of splanchnic venous thrombosis. Recently a somatic mutation (V617F) of the Janus kinase 2 (JAK2) was reported in a high proportion of patients with overt CMD. No data are available on the presence of the JAK2 mutation in patients with thrombosis of splanchnic or cerebral veins not affected from overt CMD. *Aims.* To estimate the prevalence of the JAK2 V617F mutation in patients with thrombosis of hepatic/portal/mesenteric/splenic veins or cerebral veins in the absence of conventional criteria for diagnosis of CMD. *Methods.* We studied 111 adult patients (M/F 45/66, median age 40 years, range 18-79) with venous thrombosis of unusual sites: 12 with hepatic vein thrombosis (HVT), 60 with portal-mesenteric vein thrombosis (PMVT), and 39 with cerebral vein thrombosis (CVT). No patient fulfilled conventional criteria for diagnosis of PV or ET. For comparative purpose 19 patients (M/F 8/11, median age 33 years, range 21-80) with overt CMD (6 PV, 12 ET, and 1 idiopathic myelofibrosis IMF) were also investigated: 3 had HVT, 12 PMVT, and 4 CVT. All patients were screened for the presence of the JAK2 V617F mutation and thrombophilia (deficiency of antithrombin, protein C or S, factor V Leiden, prothrombin G20210A, mild hyperhomocysteinemia, lupus anticoagulant, anticardiolipin antibodies, anti-β2-glycoprotein antibodies). *Results.* The JAK2 mutation was found in 89.4% (95% CI 68.6-97.0) of the 19 patients with overt CMD. In the 111 patients without overt CMD a thrombophilic alteration was present in 36.9% (95% CI 28.5-46.2) of the cases. The JAK2 mutation was found in 18% (95% CI 10.8-28.4) of the 72 patients with HVT or PMVT and in 5.1% (95% CI 1.4-16.8) of the 39 patients with CVT. No difference was found in the prevalence of JAK2 mutation between patients with HVT and those with PMVT (p= 1.0). Overall, the JAK2 mutation was detected in 6 of 28 patients (21.4%, 95% CI 10.2-39.5) with otherwise unexplained thrombosis of splanchnic veins, in the absence of thrombophilia or any circumstantial risk factor (oral contraceptives, surgery, puerperium, trauma). *Conclusions.* The JAK2 mutation was detected in the large majority of the patients with overt CMD and splanchnic or cerebral venous thrombosis; in the absence of overt signs of CMD the mutation is still present also in a substantial proportion (18%) of patients with splanchnic venous thrombosis and in a minority (5%) of patients with cerebral venous thrombosis. Screening for the JAK2 mutation in such patients could identify CMDs at very early stages, having thrombosis as heralding symptom.

C014

THROMBOPHILIC ABNORMALITIES, ORAL CONTRACEPTIVES AND RISK OF CEREBRAL VEIN THROMBOSIS: A META-ANALYSIS

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Background. Recent studies suggest that thrombophilic abnormalities and the use of oral contraceptives (OC) are the leading causes of cerebral vein thrombosis (CVT). *Purpose.* To assess the association between CVT and thrombophilic states (factor V Leiden, prothrombin mutation G20210, hyperhomocysteinemia, antithrombin III, protein C, or protein S deficiency and antiphospholipid syndrome), OC and their interaction. *Data Sources.* The MEDLINE, EMBASE, Cochrane Library databases (January 1994 to March 2005), reference lists of retrieved articles and contact with content experts. *Study Selection.* Studies comparing the prevalence of OC use and the prevalence of prothrombotic abnormalities in patients with CVT compared with healthy controls. *Data Extraction.* Two reviewers independently selected studies and extracted study characteristics, quality and outcomes. Odds Ratios (Ors) on confidence intervals (CIs) were calculated for each trial and pooled using the Mantel-Haenszel method. *Data Synthesis.* Seventeen studies for a total of 6306 patients were included. There was an increased risk of CVT in patients using OC (OR, 5.59; 95% CI: 3.95 to 7.91; p<0.0001), and in patients with factor V Leiden (OR; 3.38; 95% CI: 2.27 to 5.05;

p<0.00001), with mutation G20210A of prothrombin (OR 9.27; 95% CI: 5.85 to 14.67; p<0.00001) and with hyperhomocysteinemia (OR, 4.07; 95% CI: 2.54 to 6.52; p<0.00001). Only few studies have evaluated the role of other prothrombotic risk factors. *Conclusion.* OC-users, and patients with factor V Leiden, the prothrombin G20120A mutation and hyperhomocysteinemia are at significantly increased risk of CVT.

C015

A COMMON PATHOGENETIC LINK BETWEEN VENOUS THROMBOSIS AND ATHEROSCLEROSIS: INCREASED PREVALENCE OF SMALL, DENSE LDL

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Background. Small, dense and highly low-density lipoproteins (sdLDL) are highly atherogenic and associated with endothelial dysfunction due to their increased susceptibility to oxidation. No information is available on the role of sdLDL in the pathogenesis of VTE. Patients and Methods. Fifty consecutive patients, aged 60±15 yrs, with a first episode of objectively documented VTE, with no previous history of symptomatic atherosclerosis were evaluated. Patients were classified as having secondary (cancer, estrogen use, trauma etc.) or spontaneous VTE. Patients with inherited thrombophilia, antiphospholipid antibodies, diabetes and those under lipid-lowering medications were excluded. Seventy healthy controls were also evaluated. Lipoprotein subclasses were isolated using density gradient ultracentrifugation and lipid measurements were performed by standardized enzymatic kits.Results. Twenty-one and 29 patients had secondary and spontaneous VTE, respectively. VTE patients had similar total, LDL, VLDL, IDL, HDL cholesterol and triglycerides as compared to controls. No significant differences in these parameters were found between spontaneous and secondary VTE patients. Despite similar LDL-C, patients with VTE had significantly higher cholesterol in the densest LDL subfractions (p>0.01) than controls. Interestingly, these subfractions carry only about 10-15% of the overall LDL-C. The increased cholesterol in the densest LDL subfractions was entirely due to the group with spontaneous VTE (p<0.01 vs. both controls and secondary VTE), while patients with secondary VTE had similar cholesterol distribution across the all lipoprotein density range. *Conclusions*. Patients with spontaneous VTE have significant increased cholesterol in the densest LDL, while no differences were found in the other lipoprotein subclasses. The increased susceptibility to oxidation and the pro-inflammatory potential of these denser LDL particles may contribute to the pathogenesis of VTE.

C016

VENOUS THROMBOEMBOLISM AND THE RISK OF SUBSEQUENT SYMPTOMATIC ATHEROSCLEROSIS

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Recently, we reported an association between asymptomatic carotid atherosclerosis and venous thromboembolism (VTE) of unknown origin. We hypothesized that patients with VTE of unknown origin would be at a higher risk of developing symptomatic atherosclerosis than patients with VTE induced by known risk factors. To examine this hypothesis, we studied 1,919 consecutive patients followed prospectively after their first VTE episode. Median follow-up was 48 months in the 1,063 patients with VTE of unknown origin, and 51 months in the 856 patients with secondary VTE. At least one symptomatic atherosclerotic complication was detected in 160 of the 1,063 patients (15.1%) with VTE of unknown origin and in 73 of the 856 (8.5%) with secondary VTE. After adjusting for age, risk factors for atherosclerosis, and thrombophilia, the hazard ratio (HR) for symptomatic atherosclerotic complications in patients with VTE of unknown origin compared to those with secondary VTE was 1.5 (95% CI: 1.1 to 1.9). When the analysis was restricted to patients without previous symptomatic atherosclerosis, the HR increased to 1.7 (95% CI: 1.1 to 2.4). Our findings challenge the common view that venous and arterial disorders are separate entities, suggesting instead that atherosclerosis may induce venous thrombosis or that the two conditions share common risk factors.

Inflammation and Thrombosis: experimental

C017

SRC FAMILY KINASES MEDIATE NEUTROPHIL ADHESION TO ADHERENT PLATELETS *IN VITRO*, UNDER PHYSIOLOGIC FLOW AND MEDIATE NEUTROPHIL ACCUMULATION AT THE SITE OF VASCULAR INJURY *IN VIVO*

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Platelet-PMN interactions at sites of endothelial damage contribute to vascular inflammation. We sought to determine the role of Src family kinases (SFKs) in PMN recruitment by adherent platelets under flow conditions *in vitro* and in an arterial injury model *in vivo*. Moreover, we investigated the role of the focal adhesion kinase Pyk-2 as down-stream effector of SKFs. Phase-contrast videomicroscopy was used to study platelet-PMN interactions under physiologic flow. As was observed with β-2-integrin blockade, SFKs inhibitors barely affected the number of interacting cells but dose-dependently reduced the percentage of firm adherent PMNs. Similarly, firm adhesion of PMNs from Hck/Fgr- or Hck/Fgr/Lyn-deficient mice was significantly reduced to 68±11 or 36±24% of control, respectively. Activation-dependent epitopes of β-2 integrins expressed at the sites of membrane-to-membrane contacts between PMN and platelets were abolished by SFKs inhibitors, confirming an active role of SFKs in sustaining activation-dependent conformational changes of the receptors. Phosphorylation of Pyk-2, a hallmark of β-2-integrin engagement, was abolished by SFK inhibitors. Pyk2 tyrosine phosphorylation was reduced in Hck/Fgr-/- PMNs and abolished in Hck/Fgr/Lyn-/- PMNs. PMN-treatment with the Pyk2 inhibitor tyrphostin A9 inhibited platelet-PMN adhesion, indicating that Pyk2 may be a main down-stream effector of SFKs. One hour after injury of the femoral artery, PMNs are recruited to the site of injury by adherent platelets in a β-2-integrin-dependent manner. Pretreatment of wild-type mice with SFK inhibitors PP1 or SU6656 prior to endothelial denudation significantly reduced the number of PMNs that accumulated along adherent platelets at the site of vascular injury by 85% and 60%, respectively. Furthermore, Hck/Fgr/Lyn-/- mice had significantly fewer PMNs that accumulated along the arteries at one hour after injury in comparison to matched controls (3.5±3 versus 20±10). This study unequivocally establishes a role for SFKs in PMN recruitment by activated platelets.

C018

ROSUVASTATIN EXERTS RENOPROTECTIVE EFFECTS IN STROKE-PRONE RATS MODULATING THE PLASMINOGEN/PLASMIN AND METALLOPROTEASE SYSTEMS

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Statin treatment improved renal and cardiovascular outcomes. Spontaneously hypertensive stroke-prone rats (SHR-SP), a model of brain and kidney disease, show a progressive increase of proteinuria and accumulation of acute-phase proteins (APP) in body fluids when subjected to salt loading. We have previously demonstrated that rosuvastatin (RSV) reduces APP accumulation and prolongs survival of SHR-SP. Objective. The aim of the present study was to evaluate the mechanisms responsible for the renoprotective effect exerted by RSV in SHR-SP fed a high-salt diet. RSV (10 mg/kg/day in drinking water; n=15) and vehicle-treated rats (n=15) were sacrificed when, in the latter group, 24-hour proteinuria exceeded 100 mg/day and MRI firstly detected brain abnormalities. For comparison, baseline kidneys from SHR-SP (n=6) were collected at the start of the high-salt diet. Results. Kidneys of the vehicle-treated rats presented massive inflammatory cell infiltration, accumulation of α-smooth muscle-positive myofibroblasts, collagen and fibrin deposition in comparison to baseline group. Moreover, by electron microscopy, we documented that vehicle-treated animals exhibited degenerative changes of podocytes, villous transformations, and focal detachment of cells from the glomerular basement membrane. These morphological changes were

reduced in rats given RSV. A greater expression of PAI-1, tPA, uPA, MMP-2 and plasmin activity, but less MMP-9 expression was documented in renal tissues of vehicle-treated rats. These features are reverted by RSV treatment. Conclusions. Our data indicate that RSV exerts a renoprotective effect in SHR-SP by preserving renal morphology, reducing inflammatory events, and modulating the imbalance in the plasminogen/plasmin and metalloprotease systems, without influencing plasma lipid levels.

C019

VCAM-1 EXPRESSION IN HUMAN ENDOTHELIAL CELLS EXPOSED TO HYPEROSMOLAR CONDITIONS: EFFECTS OF HYPERGLYCEMIA BEYOND SPECIFIC METABOLIC ACTIONS

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Background and Aim. High glucose is a major factor in the development of vascular complications once diabetic hyperglycemia has occurred. Exposure of endothelial cells to high glucose has at least three broad consequences, those attributed to the specific metabolic effects of increased levels of glucose (i.e. glucose-induced protein and lipid modifications - the advanced glycation endproducts or AGEs, as previously shown by us), those due to the hyperosmotic component of high glucose medium, and those due to the autocrine release of cytokines. Previous research has suggested that high glucose is per se sufficient in inducing endothelial activation and vascular cell adhesion molecole-1 (VCAM-1) expression. However whether such effects are attributable to the hyperosmolar component of high glucose or specific intracellular metabolic effects of glucose is unclear. We therefore assessed whether high glucose increases the endothelial expression of VCAM-1 and investigated the precise contribution of hyperosmolarity in this effect. *Methods.* Human umbilical vein endothelial cells (HUVEC) were exposed to 5.5 mmol/L glucose (control), high glucose (30 mmol/L) and hyperosmolar control (glucose 5.5 mmol/L plus mannitol 25 mmol/l). *Results.* Analysis of the time-course of VCAM-1 expression after short-term (0, 12, 24, 48, 72 h) and long-term incubation (1 and 2 weeks) was assessed by enzyme immunoassay and immunoblotting. Short-term exposure to high glucose or to the hyperosmolar condition did not induce VCAM-1 expression as assessed by both techniques. Conversely, a 2-week exposure to both high glucose (30 mmol/L) and hyperosmolar condition (glucose 5.5 mmol/L + mannitol 25 mmol/L), increased the total cellular content of VCAM-1 (protein expression at densitometric analysis of Western blots, expressed as % of control: 176±15 and 185±20, respectively, $p<0.05$ versus control, n=3), but did not exert any significant effect on VCAM-1 surface expression. *Conclusion.* High glucose resulted in a significant increase of total cellular content of VCAM-1 in endotelial cells through a hyperosmolar effect, but was not per se sufficient to activate any functional expression of VCAM-1 on the endothelial surface. These results indicate a low risk for direct, acute activation of endothelial cells by high glucose, and suggest that other mechanisms, such as modulation of VCAM-1 expression by AGEs, are prevalent in explaining long-term vascular consequences of hyperglycaemic states.

C020

INSULIN-LIKE GROWTH FACTOR-1 AXIS EXERTS PROTECTIVE EFFECTS AGAINST INFLAMMATORY ACTIVATION OF HUMAN VASCULAR ENDOTHELIAL CELLS

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Background and Aim. The insulin-like growth factor(IGF)-1/IGF-1 receptor (IGF-1R) axis has been identified as a pleiotropic regulator of vascular function, but its role in vascular inflammation and atherosclerosis is controversial. Increasing evidence indicates that individuals with traditional cardiovascular risk factors, but normal or elevated IGF-1 may be protected against disease, while individuals with reduced IGF-1 levels are more exposed to the detrimental effects of vascular risk factors through unopposed endothelial dysfunction and apoptosis. Furthermore, plasma concentrations of IGF-1 have been shown to be independently and positively associated with insulin sensitivity in subjects with different degrees of glucose tolerance and type 2 diabetes. We therefore investigated the effect of IGF-1 and its receptor on the expression of proatherogenic vascular cell adhesion molecole-1 (VCAM-1) in resting and cytokine-stimulated human endothelial cells. *Methods.* Human umbilical vein endothelial cells (HUVEC) were treated for 24 h with IGF-1 (1-100 ng/mL), ± lipopolysaccharide (LPS), tumor necrosis factor (TNF)-α or an IGF-1 receptor neutralizing antibody (0.1-10 ng/mL). Cell surface expression of VCAM-1 was measured by enzyme immunoassay (EIA). *Results.* At concentrations ranging from those measured in physiological and pathophysiological conditions such as type 2 diabetes (1-100 ng/mL), IGF-1 did not increase VCAM-1 expression, while inhibition of IGF-1 signaling pathway through the blocking of IGF-1R, concentration-dependently increased VCAM-1 expression. At 10 and 100 nmol/l, IGF-1 concentration-dependently reduced the surface expression of VCAM-1 in HUVEC stimulated with LPS and TNF (Table). *Conclusion.* IGF-1 reduces the expression of proatherogenic VCAM-1 and therefore may have antiinflammatory and antiatherosclerotic effects.

Table.

Treatment	*VCAM-1 at EIA* % of control
Untreated control	100±4
anti IGF-R1antibody 1 ng/mL	143±11*
anti IGF-R1antibody 10 ng/mL	246±57*
TNF-α 1 ng/mL	151±20*
LPS 50 ng/mL	207±6*
TNF-α + IGF-1 100 ng/mL	105±10**
LPS + IGF-1 100 ng/mL	150±6**

n=3, absorbance units expressed as percent of untreated control;
** p<0.05 vs untreated control; ** p<0.05 vs TNF or LPS*

C021

P38 MITOGEN ACTIVATED PROTEIN KINASE AND PROTEIN KINASE C ARE BOTH INVOLVED IN INSULIN-INDUCED VCAM-1 EXPRESSION IN HUMAN ENDOTHELIAL CELLS

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Backround and Aim. Insulin levels are a risk marker for cardiovascular events, but the link between hyperinsulinemia and atherosclerosis is poorly understood. We assessed the stimulatory effect of insulin on the proatherogenic vascular cell adhesion molecule (VCAM-1) expression in human endothelial cells, and investigated molecular mechanisms involved in this effect. *Methods and Results.* Human umbilical vein endothelial cells were incubated with insulin (0-24 h) +- inhibitors of signaling pathways potentially involved. At pathophysiological concentrations (10-9-10-7 mol/L), insulin selectively induced VCAM-1 expression (enzyme immunoassays, flow-cytometry, immunocytochemistry, immunoblotting and Northern analysis), and significantly increased U937 cell adhesion to endothelial cells (by a rotational adhesion assay). Incubation of endothelial cells with inhibitors of ERK1/2 failed to affect insulin-induced VCAM-1 expression. Conversely, the p38mitogen activated protein(MAP) kinase inhibitors SB203580 and SB202190, the protein kinase C(PKC)-β inhibitor LY379196 and, partially, the c-Jun NH2-terminal kinase (JNK) inhibitor SP600127, all tested at concentrations around their IC50 for inhibition of substrate phosphorylation, decreased insulin effect on VCAM-1. Gene silencing by small interfering RNA significantly reduced the expression of p38MAPK, and this was accompanied by suppression of insulin-stimulated VCAM-1 expression. Treatment with insulin also led to activation of NF-κB (Table). *Conclusions.* Pathophysiological insulin concentrations increase VCAM-1 expression and activate NF-κBB. This mostly occurs through stimulation of p38MAPK, with additional effects of PKC-beta.

Table.

Treatment	*VCAM-1 s fluorescence intensity unit (flow cytometry)*	*Cells/field (adhesion assay)*	*VCAM mRNA (arbitrary units)*	*NF-κB binding activity (arbitrary units)*
Control	5.11±1.53	2.2± 2	3±1	20±10
insulin 10-8 mol/L	15.6±3.2*	6± 2*	161±50*	118±41*
TNF-α 1 ng/mL	115.8±11*	20±5*	230±50*	179±22*

** p<0.05 vs control; ** p<0.05 vs TNF.*

C022

EXPRESSION AND REGULATION OF ENDOTHELIAL PROTEIN C RECEPTOR IN MONOCYTE-DERIVED DENDRITIC CELLS

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Background. Endothelial protein C receptor (EPCR) is a transmembrane protein, homologous to MHC class-1 molecules, that enhances the rate of protein C activation on endothelial cells. It is reported that EPCR mediates the anti-apoptotic activity of activated protein C on endothelial cells. EPCR was identified also in polymorphonuclear leukocytes and monocytes. We previously showed by immunohistochemistry that dendritic-like cells in the normal gut mucosa express EPCR. *Aims of the study.* 1. To characterize phenotypically the gut mucosa EPCR+ dendritic-like cell. 2. To study, in a model of dendritic cell generated *in vitro*, the expression of EPCR and its modulation. *Methods.* EPCR was identified by immunohistochemistry, immunofluorescence or flow cytometry. Dendritic cells *in vitro* were obtained from CD14+ peripheral blood leukocytes, cultured in the presence of interleukin-4 and GM-CSF (MoDCs). Specific messenger RNA (mRNA) was measured by RT-PCR. *Results.* We confirm that the gut mucosa dendritic-like cells have a phenotype characteristic of dendritic cells, namely they express CD80, CD83 and HLA-DR. We could not identify by immunohistochemistry EPCR+ dendritic cells in other tissues, such as lymph node, spleen, tonsil, liver, lung, and skin. EPCR surface expression on MoDCs was monitored by flow cytometry together with expression of the DC markers HLA-DR, CD1a, CD80 and CD83. After 7 days of culture, approximately 25% of immature DCs expressed EPCR on their surface. De novo expression of EPCR is not correlated with modulation of apoptosis or cell cycle. Lipopolysaccharide-induced terminal maturation of MoDCs down regulates the surface expression of EPCR by 40% while up regulating the expression of CD83. Incubation of cultured DCs with prostaglandin E2 upregulates EPCR mRNA and protein expression by about 3 fold at 50 hours. *Conclusions.* Contact with bacterial antigens modulates EPCR expression on MoDCs, suggesting that EPCR might be involved in antigen recognition or processing.

C023

IN VITRO INHIBITION OF MONOCYTE TISSUE FACTOR AND PAI-2 PRODUCTION BY OCHRATOXIN A

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The mycotoxin ochratoxin A (OTA) is a ubiquitous contaminant of human and animal food products. Apart from its known nephrotoxicity and carcinogenicity, OTA has been shown to variably affect several functions of mononuclear leukocytes. We have studied the effect of OTA on tissue factor (TF) and plasminogen activator inhibitor-2 (PAI-2) production by peripheral blood mononuclear cells (MNC) stimulated with endotoxin (1 μg/mL, 3 h and 18 h at 37°C for TF and PAI-2, respectively). TF was measured by functional (one-stage clotting time) and immunological (ELISA) assays, and by RT-PCR whereas PAI-2 was assessed by ELISA in conditioned media. OTA caused a dose-dependent reduction in TF activity and antigen (with more than 90% inhibition at the concentration of 1 μg/mL) and also reduced PAI-2 release (80% inhibition at 1 μg/mL). Inhibition of TF expression was also observed at mRNA level. The inhibitory effect disappeared if OTA was added to MNC suspensions 20-60 min after endotoxin. Moreover, OTA was much less efficient in reducing TF expression when MNC were suspended in medium containing 40 mg/mL human albumin. TF production was also impaired by OTA (1 μg/mL) when MNC were stimulated with 10-9 M PMA (99% inhibition), 10 ng/ml IL-1β (84%) or 100 ng/mL TNF-α (55%). Finally, we determined the effect of OTA on endotoxin-induced cytokine release by MNC and found that OTA inhibited IL-6, but not IL-8 or TNF-α production, thus ruling out an unspecific effect of the mycotoxin on protein synthesis. Because of the important role of blood clotting activation and fibrin deposition in cell-mediated immune responses, it is suggested that the inhibitory effect on cell TF and PAI-2 expression might represent one of the mechanisms whereby OTA exerts its immunomodulatory activities.

C024

HUMAN POLYMORPHONUCLEAR LEUKOCYTES PRODUCE AND EXPRESS FUNCTIONAL TISSUE FACTOR UPON STIMULATION

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Background. Blood-borne tissue factor (TF) plays a crucial role in thrombogenesis. *Aim.* To test whether polymorphonuclear leukocytes (PMN) are a source of TF. *Methods and Results.* Human PMN were carefully separated from other blood cells and stimulated for 3 min with purified P-selectin or the chemotactic peptide fMLP: they expressed both TF procoagulant activity, as identified by specific monoclonal antibody against TF and inactivated VIIa blockade; and TF:Ag (four to six times), as shown by flow cytometry and immunocytochemistry. About 40% of permeabilized PMN, both resting and stimulated, contained TF:Ag, indicating that stimulation only modifies the location of TF:Ag within PMN. By real time-polymerase chain reaction (RT-PCR), a very low amount of TF mRNA was detectable in resting PMN, but a three- to five- fold increase was observed after 1-h stimulation with P-selectin or fMLP, respectively. The effect of contaminating (0.02±0.02%) monocytes in PMN suspensions was ruled out by increasing concentrations of purified monocytes added to PMN samples: neither 0.02% nor 0.12% of contaminating monocytes increased the production of TF mRNA by PMN after stimulation. Similarly, the stimulation of purified monocyte populations with fMLP failed to produce any *de novo* synthesis of TF mRNA. *Conclusions.* These findings suggest that TF is not constitutively expressed in peripheral PMN, but can be up-regulated and produced upon stimulation and specific gene transcription, as for instance during contact with activated platelets or endothelium. The stored TF is rapidly expressed *in vitro* as a functional molecule on the surface of activated PMN. The availability of PMN TF supports the relevance of inflammatory cells and their interaction with platelets for fibrin deposition and thrombus formation.

Hemorrhagic diseases: diagnosis and molecular defects

C025

FACTOR XI DEFICIENCY: IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF SIX NOVEL MISSENSE MUTATIONS

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Factor XI (FXI) is the zymogen of a serine protease involved in the intrinsic pathway of blood coagulation by activating factor IX. It circulates as a homodimer and each chain, encoded by the 23-kb long F11 gene located on chromosome 4q35.2, is composed of four tandem apple domains (Ap1-4) and a serine protease (SP) domain. FXI deficiency (MIM*264900) is a coagulopathy generally inherited as an autosomal recessive trait and characterized by bleeding episodes associated with trauma or surgery and by both low FXI coagulant activity and low (type I) or normal (type II) FXI antigen level. This condition is rare (prevalence 1:106) in the general population except in Ashkenazi Jews. Currently, 89 disease-causing mutations have been reported in the F11 gene. We analyzed six FXI-deficient patients from Italy and Czech Republic with the aim to identify the genetic defect/s and to elucidate the pathogenetic mechanism/s underlying FXI deficiency. The mutational screening, performed by sequencing the whole coding region and intron-exon boundaries of F11, enabled the identification of a novel in-frame trinucleotide (GGA) deletion, which causes both the Lys8Asn substitution and the Asp9 deletion (localized in the Ap1 domain), and five novel single nucleotide variations causing the missense mutations Cys38Trp and Arg54Pro (in Ap1), Cys122Tyr (in Ap2), Arg184Gly (in Ap3), and Leu601Pro (in the SP domain). Computer-assisted analyses indicated that all substitutions are not conservative and are predicted to cause damaging structural effects. To confirm the pathogenic role of the identified missense mutations, expression of FXI mutants was accomplished in eukaryotic COS-1 cells. Preliminary results of quantitative analyses, based on ELISA and functional measurements of FXI levels in cell extracts and/or conditioned serum-free media, revealed secretion defects caused by all mutations except for the Arg184Gly substitution, which seems to only lower FXI activity.

C026

FACTOR VII-GFP CHIMERA AS A TOOL FOR THE ELUCIDATION OF MILD FORMS OF COAGULATION FACTOR VII DEFICIENCY

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Factor VII (FVII) is a plasma protease playing a crucial role in coagulation. FVII deficiency has been associated to a number of mutations responsible for reduced antigen levels. These mutations, peculiar models to investigate intracellular processing of coagulation factors, have been poorly characterized. As models we studied the prepeptide mutations (L-48P, L-42P) associated with mild FVII deficiency. The mutations were introduced into the native FVII, to evaluate consequences on secreted and intracellular protein levels. At steady-state, protein and activity levels of -42PFVII were remarkably decreased (~12% of WtFVII) in conditioned medium. The -48PFVII showed a markedly affected early secretion profile, but only mildly reduced (~60%) levels at steady-state, which did not mirror the reduction of FVII levels in patient's plasma. Upon sub-cellular fractionation, FVII protein was detected in organelles for WtFVII (10.3±3.7ng/mL) but not for prepeptide variants. To investigate at the intracellular level in living cells the effect of substitutions on the first biosynthetic steps, FVII was expressed as fusion protein with the Green Fluorescent Protein (GFP). Cells expressing WtFVII-GFP showed a bright and punctate fluorescence, excluded from the nucleus, which was maintained after plasma membrane permeabilization with digitonin, thus confirming correct targeting to endoplasmic reticulum (ER). As control, we expressed a cytosolic protein kinase C (PKCb)-GFP chimera, which showed a widespread fluorescence covering also the nucleus. Fluorescence of the –48PFVII-GFP and –42PFVII-GFP was diffuse and covered the nucleus, and declined upon digitonin treatment, thus clearly indicating impaired ER targeting and mislocalization in the cytosol. Noticeably, the residual fluorescence of â¢¢48PFVII-GFP (10%) and â¢¢42PFVII-GFP (20%) in organelles was fairly compatible with FVII levels in patient's plasma. This approach, exploitable to investigate altered intracellular trafficking in other coagulopathies, enabled us to elucidate the molecular bases of FVII deficiency and to explain the residual FVII levels responsible for a mild disease form.

C027

IDENTIFICATION OF SIX NOVEL MUTATIONS CAUSING COAGULATION FACTOR V DEFICIENCY

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Severe factor V (FV) deficiency (MIM 227400) is a rare coagulation disorder, characterized by very low or unmeasurable levels of functional and immunoreactive plasma FV, associated with a hemorrhagic phenotype of variable severity. This bleeding disorder has a prevalence of about 1 per million and is transmitted as an autosomal recessive trait. Among rare inherited coagulopathies, is one of the least characterized from the molecular point of view. The mutational screening of the FV gene (F5) in six FV-deficient patients disclosed eight mutations, six hitherto unknown (the missense mutations p.S83R, p.I1727M, and p.S1970I and the splicing mutations IVS21+1G>A, IVS24+1_+4delGTAG, and IVS8+6T>C), and two previously reported (p.R1002X and p.R2074H). To confirm the causal role of the newly identified splicing defects in the pathogenesis of the disease, transfections of appropriate FV minigene constructs (either wild type or mutant) were carried out in HeLa cells. RT-PCR analysis on mRNA extracted from transfected cells, demonstrated that the IVS8+6T>C transition causes the entire exon 8 to be skipped from the FV mRNA. Conversely, both IVS21+1G>A and IVS24+1_+4delGTAG cause the activation of cryptic donor splice sites, located in exons 21 and 24, respectively. In all cases, the splicing defect results in the generation of a premature stop codon in the FV protein (p.Lys346SerfsX17, p.Gly1952ValfsX2, p.Met2120IlefsX12). As far as missense mutations are concerned, their effect on FV expression is under investigation by transient transfection experiments. In conclusion, this study reports the molecular characterization of six hitherto unknown defects responsible for FV deficiency, further supporting the allelic heterogeneity of this disease.

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C028

CLINICAL AND MOLECULAR CHARACTERIZATION OF THREE ITALIAN PATIENTS WITH SEVERE FACTOR V DEFICIENCY AND BLEEDING DISORDER (PARAHAEMOPHILIA): REPORT OF A NOVEL HOMOZYGOUS MUTATION (FV: ASP524HIS)

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Background. Severe Factor V (FV) deficiency is a rare bleeding disorder, also called parahaemophilia, whose molecular basis is poorly characterized. We identified three parahaemophilic subjects (two were brothers) belonging to two unrelated families with bleeding tendency. Aim of the study. To identify the molecular basis of the severe FV deficiency found in the three parahaemophilic patients. *Methods.* Factor V levels were assessed in three patients with parahaemophilia (levels of FV activity of 1-3%) and in five family members, three of whom presented with FV activity levels of about 50%. The full length of exon 1 to exon 25 and the 5' untranslated sequence of FV genomic DNA was analyzed by polymerase chain reaction (PCR) and direct sequencing of the amplified fragments was performed in the three patients with severe FV deficiency. *Results*. A single base mutation was present at position 1744 (G->C) in exon 11 of FV gene, predicting an amino acid substitution from Asp to His at position 524 of FV molecule. This missense mutation was homozygous in the three parahaemophilic subjects. Family members with 50% FV activity were heterozygous for the mutation whereas those with normal FV in plasma presented only the wild type allele. Since the mutation creates a new restriction site for the enzyme RcaI, PCR and restriction analy-

sis was used to screen 200 healthy subjects from the same geographical area (the North East of Italy) of the probands. None of them presented with the mutated allele. Conclusions. We have described another novel homozygous mutation in FV gene associated with severe FV deficiency in three Italian patients. Due to the increasing number of different mutations found in FV deficient families, full screening of FV gene, even though time-consuming, remains the only approach for the molecular diagnosis of parahaemophilia.

C029

CONGENITAL AFIBRINOGENEMIA: TWO NOVEL FIBRINOGEN GENE MUTATIONS IDENTIFIED IN TWO PATIENTS FROM IRAN

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Congenital afibrinogenemia (CAF, OMIM #202400) is a rare autosomal recessive clotting disorder characterized by bleeding manifestations of variable entity and by the complete absence or extremely low levels of plasma fibrinogen. Mutational screening of the fibrinogen-chain genes in two unrelated Iranian patients affect by CAF led to the discovery of two novel homozygous mutations. A G>A homozygous transition involving the first nucleotide of the second intron of the fibrinogen γ-chain gene (FGG IVS2+1G>A) was detected in the first proband. A homozygous G>A transition coding for a nonsense mutation was identified in the fibrinogen Aα-chain gene (FGA W229X) of the other proband. The IVS2+1G>A mutation is predicted to cause either exon skipping/intron retention or the activation of a cryptic donor splice site, in all cases the most probable consequence being the introduction of a premature termination codon. Since fibrinogen expression is mainly confined to the liver and no liver biopsy specimens of the patient is available due to ethical constraints, to further elucidate the molecular basis of this mutation it would be necessary to conduct in-vitro transient expression and RT-PCR experiments using an appropriate minigene construct. In the light of previous data suggesting that transcripts carrying premature termination codons in fibrinogen Aα-chain gene escape nonsense-mediated mRNA decay, the W229X mutation is predicted to generate a truncated Aα polypeptide lacking the last 396 residues. Besides shedding light on the molecular mechanisms underlying CAF, these data will also be valuable for prenatal diagnosis purposes.

C030

CONGENITAL AFIBRINOGENEMIA: MOLECULAR CHARACTERIZATION OF THE FIRST MISSENSE MUTATION IN THE FIBRINOGEN Aα-CHAIN GENE

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Congenital afibrinogenemia (MIM#202400) is a rare autosomal recessive disease (prevalence 1:1,000,000) affecting coagulation, due to the decrease or absence of functional and immunoreactive fibrinogen in plasma and associated with a hemorrhagic phenotype of variable severity. Fibrinogen is composed of two sets of three chains: Aα, Bβ, and γ, encoded by the FGA, FGB, and FGG genes, respectively, clustered on chromosome 4q31.3-32.1. The six chains are assembled in a step-wise manner, with the last step being the Aα-Bβ-γ half-molecule dimerization. Among the 44 mutations described so far in the fibrinogen cluster, most are point mutations leading to severe truncations of the corresponding chain, while the only six missense mutations causing afibrinogenemia are located in FGB. Furthermore, also all the 14 missense mutations causing hypofibrinogenemia exclusively map to FGB (3) and FGG (11). We here report the identification and molecular characterization of the first missense mutation causing afibrinogenemia located in FGA in an Italian afibrinogenemic male proband. The identified mutation is a 1767T>G transversion in exon 3, leading to the substitution of a methionine (ATG) with an arginine (AGG) at codon 51 (numbering omitting the signal peptide). The mutation was identified in compound heterozigosity with a previously described frameshift mutation (1215delT) in the same gene. *In vitro* expression in COS-1 cells of the mutant fibrinogen showed the presence of small amounts of trimeric fibrinogen instead of the mature hexamer both in cell lysates and in conditioned media. The failure of the hexamer assembly could be explained considering that Met51 is located at the very beginning of the coiled-coil domain, in a region involved in trimer dimerization and hexamer stabilization. Western blot analysis confirmed the presence of the trimeric fibrinogen in the proband's plasma, further supporting the hypothesis that the mutation alters the final step of fibrinogen assembly.

C031

ENDOGENOUS THROMBIN POTENTIAL (ETP) IN SEVERE HEMOPHILIACS WITH MILD PHENOTYPE

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Some hemophiliacs classified as severe by FVIII/FIX:C (less than 1%), exhibit a mild bleeding tendency. This case-control study compares two groups of severe hemophiliacs A or B representing the extremes of the clinical spectrum, in order to assess whether ETP allows to distinguish a different coagulation profile. The role of PT G20210A and FV Leiden into the modulation of the hemophilia phenotype is evaluated. Patients: severe hemophiliacs 18 years old or more, without history of inhibitors and treated on demand. *Mild bleeders* (MB): no more than 2 spontaneous bleeding episodes/year, concentrate consumption smaller than 120U/kg/year, orthopedic joint score lower than 4 and radiologic score lower than 10. *Severe bleeders* (SB): at least 25 spontaneous bleeding episodes/year, concentrate consumption greater than 3000U/kg/year, orthopedic joint score higher than 45 and radiologic score higher than 40. Patients who did not fit the definition criteria of neither MB nor SB were defined as *intermediate* bleeders (IB). *Methods.* plasma samples were obtained after a minimum *washout* period of 5 days. FVIII levels were measured by chromogenic assay. ETP was measured in platelet-rich plasma after addition of tissue factor. *Results*. 22 MB, 22 SB and 28 IB were enrolled; age was comparable in the three groups (median: 32, 38 and 38 years). PT G20210A was detected in 5% MB and 4% IB; FV Leiden in 7% IB and 5% SB. ETP values were higher in MB (850nM) compared with both IB (478nM, p value less than 0.05) and SB (414nM; p value less than 0.05). P for trend was similarly significant (p value less than 0.05). This study shows that ETP allows to distinguish MB among severe hemophiliacs and suggests that ETP may be useful to select candidates for early preventive strategies.

C032

FETAL GENDER DETERMINATION WITH CELL-FREE FETAL DNA IN MATERNAL PLASMA IS A HELPFUL TOOL IN THE PRENATAL DIAGNOSIS OF HEMOPHILIA

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Fetal DNA identification in maternal circulation has provided a new approach for non-invasive prenatal diagnosis. However, fetal DNA can persist in maternal blood long after the delivery, severely hampering this possibility. We addressed the issue of fetal DNA persistence in maternal blood. Thus, we investigated cell-free fetal DNA as a potential approach in prenatal diagnosis of Hemophilia. Forty non pregnant women, who had had at least a male fetus, 29 control pregnant women, and 14 pregnant women, carriers of Hemophilia A or B. The assessment of Y-chromosomal sequences was obtained by analyzing SRY and Amelogenin genes using PCR-based techniques. A protocol consisting in a double centrifugation at full speed (13,000 g) followed by plasma filtration hampered the detection of Y chromosome-specific sequence in non pregnant women. In 29 control pregnant women, blinded determination of fetal sex confirmed the specificity and sensitivity of the method applied. In 14 pregnant carriers of Hemophilia, the investigation revealed a male fetus in 9 pregnancies. Excluding the 3 cases in which a spontaneous miscarriage occurred, the sensitivity and specificity of fetal sex prediction by SRY and Amelogenin gene analyses were both 100% as compared to the invasive approach and the fetal sex outcome at birth (6 males and 5 females). Because of its high correct prediction rate, fetal gender determination with cell-free fetal DNA in maternal plasma may be an useful tool in prenatal diagnosis of Hemophilia allowing for the avoidance of invasive procedures for female fetuses.

Anticoagulant drugs

C033

PERIOPERATIVE BRIDGING THERAPY WITH LOW MOLECULAR WEIGH HEPARIN IN PATIENTS REQUIRING INTERRUPTION OF LONG-TERM ORAL ANTICOAGULANT THERAPY

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Introduction The interruption of long-term oral anticoagulant (OAC) therapy for surgery or other invasive procedures puts patients at increased risk of thromboembolic events. Peri-operative "bridging" anticoagulant therapy may reduce this risk; however, the efficacy and safety has not been established. We conducted a study testing the hypothesis that low-molecular weight heparin is a safe and efficacious perioperative bridging therapy in patients on long-term OACs. *Methods.* This prospective, cohort study included patients requiring bridging therapy due to major surgery (defined as surgery lasting 1 hour), invasive procedures or minor surgery at increased risk for bleeding. Patients were considered to be at low or at high-risk for thrombosis. The first group was constituted by patients suffering of Atrial Fibrillation without previous Arterial Thromboembolism (AF-noAT), Venous ThromboEmbolism (VTE) lasted more than 3 months, or those with Prosthetic Aortic Valves (PAV). High-risk group was constituted by patients with Atrial Fibrillation with previous Arterial Thromboembolism (AF-AT), Prosthetic Mitralic Valves (PMV) or those with recent VTE. In all patients, Warfarin was discontinued 5 days prior to the procedure. In patients considered at low-risk for thrombosis, LMWH (at prophylactic dosage) was commenced the night before the procedure. In patients considered at high-risk for thrombosis LMWH (at therapeutic dosage) was started 3 days prior to, and continued until the night before the procedure. Warfarin was restarted the evening of the procedure and LMWH (at prophylactic or therapeutic dosage according to the patients' thrombotic risk) was reinitiated 12-24 hours post-procedure and continued until the INR was therapeutic. The primary efficacy endpoint was the incidence of thromboembolism from warfarin cessation to 28 days post-procedure. The primary safety endpoint was incidence of major haemorrhage from first dose of LMWH until 24 hours after the last dose. *Results.* Over a period of 4 years (2001-2005), a total of 228 patients (planned to major and minor surgery or invasive procedures) were included in the study. Conditions requiring long-term OAT were the following: 26 (11.4%) for VTE, 92 (40.3%) for AF-AT, 43 (18.8%) for AF-noAT, 4 (1.7%) for both, 53 (23.2%) for PAV/PMV and 10 (4.3%) for others (arterial hypertension, dilatative myocardiopathy, valvulopathy, myocardial infarction, coronary artery by-pass graft). All patients received LMWH (intention-to-treat group); among them, 132 (60%) belonged to low-risk group and 96 (40%) to high-risk group. In total, 43 (18.8%) underwent major surgery, 58 (25.4%) minor surgery and 127 (55.7%) invasive procedures. Thromboembolic events occurred in 4 patients (3 [3.1%] belonging to high-risk and 1 [0.75%] to low-risk group); 3 events (1 peripheral arterial thromboembolism and 2 transient ischemic attacks) occurred in AF-AT patients, 1 event (pulmonary embolism) occurred in VTE patient (Table 1). Major haemorrhages occurred in 5 patients belonging to high-risk (5.2%) and 1 (0.75%) to low-risk group (Table 1).

Table 1.

Patients characteristics	**LMWH* *Low-risk group*	***LMWH* *High-risk group*
Complications		
TE total n (%)	1 (0.75)	3 (3.1)
arterial	0	3 (3.1)
venous	1 (0.75)	0
Bleeding total n (%)	5 (3.8)	12 (12.5)
+major	1 (0.75)	5 (5.2)
minor	4 (3)	7 (7.3)

**Low Molecular Weight Heparin at prophylactic doses (accordingly to manufacturer); **Low Molecular Weight Heparin at therapeutic doses (accordingly to manufacturer); + All occurred during major surgery.*

All major haemorrhage occurred in patients undergoing major surgery; none of the haemorrhages were intracranial, retroperitoneal, or intraocular, were fatal, or required intervention (Table 2). *Conclusion.* Use of LMWH in accordance with the bridging regimen described is feasible and safe in patients undergoing minor surgery or an invasive procedure. Most major haemorrhages occurred in patients undergoing major surgery. Further studies are needed to optimize bridging therapy with LMWH in patients undergoing major surgery.

Table 2.

Events	*Major surgery (43)*	*Minor surgery (58)*	*Invasive procedure (127)*
Major hemorrhages (%)	6 (13.9%)	0	0
Type of procedure associated with major hemorrhage (n)	TKR (2), THR (2); Abdominal hernia repair (1), Aortofemoral construction (1)	-	-

TKR: Total Knee Replacement; THR Total Hip Replacement

C034*

IS COMBINED ASPIRIN-ORAL ANTICOAGULANT THERAPY JUSTIFIED IN PATIENTS WITH CHRONIC ATRIAL FIBRILLATION WHO HAVE CORONARY ARTERY DISEASE OR ARE AT HIGH RISK FOR STROKE?

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Background. Deciding whether to add aspirin to oral anticoagulant (OAC) therapy in patients with both atrial fibrillation (AF) and coronary artery disease (CAD) or high-risk patients with AF is a common clinical scenario with a no clear guidelines to aid practice. *Purpose.* To assess the therapeutic benefits and risks of combined aspirin-OAC versus OAC therapy alone, we performed a meta-analysis of randomized trials comparing these two treatment strategies. *Data Sources.* Randomized trials published to June 2005 in MEDLINE, EMBASE and Cochrane Library databases. *Study Selection.* Randomized trials with at least 3 months of follow-up that compared aspirin-OAC with OAC alone and reported arterial thromboembolism, all-cause mortality or major bleeding using objective methods. *Data Extraction.* Two reviewers independently extracted data on study characteristics and outcomes. Pooled odds ratios (ORs) and associated 95% confidence intervals (CIs) were calculated for each study outcome. *Data Synthesis.* Ten studies were included, totalling 4,180 patients. The risk for arterial thromboembolism was lower in patients receiving aspirin-OAC than OAC alone (OR 0.66; 95% CI: 0.52, 0.84; absolute risk reduction, 2.5%; number-needed-to-treat, 40). There was no difference in all-cause mortality with either treatment (OR 0.98; 95% CI: 0.77, 1.25). The risk for major bleeding was higher in patients receiving aspirin-OAC than OAC alone (OR 1.43; 95% CI: 1.00, 2.02; absolute risk increase, 1.0%; number-needed-to-harm, 100). Sub-group analyses confirmed these findings in studies of patients with a mechanical heart valve but not in studies of patients with AF or CAD. *Conclusions.* Compared to OAC therapy alone, combined aspirin-OAC therapy appears to reduce the risk for arterial thromboembolism and increase the risk for major bleeding and has no effect on all-cause mortality. Randomized trials are needed to assess the benefits and risks of these two treatment approaches in patients with both AF and CAD and high-risk patients with AF.

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C035

DOES CELECOXIB INFLUENCE THE INR STABILITY IN PATIENT ON ORAL ANTICOAGULANT TREATMENT? A RANDOMIZED, DOUBLE-BLIND, CONTROLLED TRIAL

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Background. The management of patients who are receiving warfarin therapy and have musculoskeletal problems that require treatment with a non-steroidal anti-inflammatory drug (NSAID) is problematic because NSAID use may increase the risk for bleeding. Cyclooxygenase-2 (COX-2) selective NSAIDs may be less likely to promote bleeding than non-

selective NSAIDs but there are concerns about potentiating the anticoagulation effect of warfarin. *Objective.* To determine if the concomitant use of celecoxib potentiates the anticoagulant effect of warfarin, as measured by the international normalized ratio (INR). *Design.* Randomized, double-blind, controlled, 2-phase cross-over trial. *Methods.* We studied the effect on INR of celecoxib and codeine in 15 patients who were receiving long-term warfarin therapy and who required an analgesic therapy for osteoarthritis. During the first phase of the study, patients were randomly allocated to receive celecoxib 200 mg/daily or codeine phosphate, 7 to 15 mg three- to four-times daily for five weeks. During the second phase of the study, patients stopped the first study medication and started the other study medication with no drug-free interval. Weekly INR testing was performed. *Results.* There was no significant difference in the mean INR values during each 5-week treatment period when patients received co-administered warfarin and celecoxib or co-administered warfarin and codeine. There was, therefore, insufficient evidence to reject the hypothesis that these two treatments had an equal effect on the INR ($F(1,9) <0.01$, $p=0.95$). This finding was confirmed after repeating the analysis using a generalized estimating equation(mean difference (95% CI); 0.10 (-0.04, 0.24), *p* value; 0.16). *Conclusions.* Our results are consistent with the hypothesis that treatment with celecoxib does not significantly influence INR values in patients who are receiving long-term warfarin therapy. Larger randomized trials powered to address clinical endpoints are warranted.

C036

SMALL INTESTINAL BACTERIAL OVERGROWTH INFLUENCES WARFARIN DOSE REQUIREMENT IN PATIENTS ON ANTITHROMBOTIC TREATMENT

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Background. The dose requirement of the vitamin K antagonist warfarin necessary to obtain the same therapeutic level of anticoagulation widely differs among patients and can sometimes undergo to dangerous abrupt changes for not ever identified reasons. Drug interactions and genetic factors can only partially explain these differences. Intestinal flora produces vitamin K, and experimental animals and patients with small intestinal bacterial overgrowth (SIBO) never develop hypothrombinemia. The aim of our prospective study was to investigate the correlation between SIBO occurrence and warfarin dose requirement in anticoagulated patients. Methods: We sampled 30 adult outpatients (15 females) on chronic oral anticoagulant therapy with stable INR within 2 and 3. Three groups were defined as follows: low dose <17.5 mg/wk of warfarin (LD); high dose within 35 and 70 mg/wk (HD); very high dose ≥70 mg/wk (VHD). The lactulose breath test was used to diagnose SIBO: after the ingestion of 10 mL of lactulose, breath samples were taken every 20 minutes for 4 hours using a two bag system. $\chi 2$ and logistic regression were used for analysis. *Results.* Symptomatic patients with > 2 SIBO symptoms were 1 in LD group, 4 in HD group and 5 in VHD group. Patients with an abnormal breath test were 50% in VHD, 10% in the HD and none in the LD group (c 2= 8.75; $p=0.013$). Known factors predisposing to SIBO showed an unbalanced distribution among the groups, while warfarin interfering variables did not. Logistic regression analysis showed a statistically significant OR to have an abnormal LBT for warfarin dose group (VHD vs L-HD, OR = 19; 95% CI 1.78 to 201.7) and previous surgery (surgery vs no surgery, OR = 22, 95% CI 2.36 to 204).*Conclusions.* This study suggests an additional explaining factor for the variability of warfarin dose requirement and its hard management.

C037

RISK OF BLEEDING IN VERY OLD ATRIAL FIBRILLATION PATIENTS ON WARFARIN: RELATIONSHIP WITH AGEING AND CHADS2 SCORE

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In atrial fibrillation (AF) patients, age≥75 years is one of the major risk factors for stroke. However, it is not clear if an upper limit for the indication to OAT exists. For this reason, we performed a prospective study on 290 AF patients on OAT aged ≥75 years (median age 82 years, total follow-up period 814 pt-yrs) followed by our Anticoagulation Clinic. Seventeen major bleeding events were recorded (rate 2.1×100 pt/yrs), 11 of which cerebral (1.35×100 pt/yrs). The occurrence of major bleedings was associated with history of previous TIA or stroke [OR 3.4 (1.1-12.5), $p=0.01$] and with diabetes [4.4 (1.3-14.7) $p=0.01$]. We found a trend to a progressive increase in the rate of bleeding risk with the increase of the CHADS2 score: patients with score 4-6 showed a rate of 3.4×100 pt/yrs with respect to 1.5×100 pt/yrs of patients with lower score. Number Needed to Harm (NNH) was calculated for the classes of CHADS2 score and age and compared with Number Needed to Treat (NNT) obtained from a metanalysis of clinical trials (Lip 2005). For CHADS2 score 1 and 2-3, NNHs were 51 and 68 without significant changes across the different classes of age (75-79, 80-84, >85 years). The corresponding NNTs were 58 and 32. For CHADS2 score 4-6, NNH was 29 (vs NNT of 16), but its values varied among the 3 groups of ages reaching a value of 10 in patients ï‚85 years. Our data suggest: 1) in patients with CHADS2=1 the indication of OAT is questionable, 2) in patients >85 years with CHADS2 4-6 both NNHs and NNTs are quite low and the use of OAT should be highly individualised. In all the other patients the balance is in favour of OAT.

C038

THE EFFECT OF INFLUENZA VACCINATION IN PATIENTS ON LONG-TERM ORAL ANTICOAGULANT THERAPY

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Background. Many patients on long-term oral anticoagulation therapy (OAT) need annual influenza vaccination (IV). Whether IV interferes with OAT is under debate. *Aim of the study.* To evaluate the interference between OAT and IV according to: INR and warfarin mean weekly dosage variations; thrombotic and bleeding events. *Study design*. Prospective randomized, multicentre, single blinded, cross-over study. Materials and Methods: the influenza virus vaccine and placebo were provided by CHIRON (Fluad, Chiron, Italy, batch release 2004-2005). The recruitment was conducted in the peripheral units of the National Health Care system encharged of IV and point-of-care (POC) determination of INR. The venous PT-INR was also checked at predefined sampling times. Sample size calculation was based on the population of the OAT centre and was set to find out a systematic deviation of 0.5 INR units in 10% of the patients. ANOVA model for repeated measure was used. Results: Between October and November 2004, 104 patients (57 males, 47 females, mean age 71.3±9.2, mean BMI 27±3.8) were entered in the study, followed up for 10 weeks and tested at baseline and on week 1, 2, 4, 7, 8, 10; 4 patients dropped out because of no compliance. POC-INR and PT-INR showed a statistically significant correlation ($R2 = 0.88$ $p<$ 0.001). We observed no statistically difference in the weekly average variation of INR and weekly dosage of OAT. We recorded one episode of recurrent TEV in a cancer patient. No major bleeding event was found. Eleven minor hemorrhagic events with the following distribution occurred: 9 in patients after IV, and 2 in patients after placebo. Ten patients were within the therapeutic range. We suppose a platelets disorder caused by IV as reported in the literature. Conclusions: in patients with stable baseline INR, no statistically relevant interaction was found between IV and OAT.

C039

A TWO DIMENSIONAL-ENANTIOSELECTIVE LIQUID CHROMATOGRAPHY/ELECTROSPAY TANDEM MASS SPECTROMETRY (2D/LC-MS/MS) METHOD FOR THE RAPID MULTIPLE QUANTIFICATION IN HUMAN PLASMA OF THE ENANTIOMERS OF WARFARIN, ACENOCOUMAROL, AND PHENPROCOUMON

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Oral administration of anticoagulant drugs is one of the most employed therapies in clinical practice for prevention and treatment of patients with arterial and venous thromboembolic disorders. Oral anticoagulants are clinically administered as a racemic mixture concentration of enantiomers that shows in blood marked difference in pharmaco-

kinetics and pharmaco-dynamics because of stereoselective metabolism. Few analytical methods for quantification in human plasma of racemic Warfarin, Acenocoumarol, and Phenprocoumon have been reported so far positive mass spectra and product ion spectra of warfarin, acenocoumarol, and phenprocoumon were obtained. Resulting measurement specificity was assessed by using different blank donor plasma samples where no interfering reagent peak appeared at the retention time (RT) of the targeted analytes. Measurement linearity of the assay where over the range exceeding 625 ng/mL was linear for both R and S warfarin, R and S acenocoumarol, R and S phenprocoumon. Typical linear regression coefficients have been 0.9983 for R Warfarin, 0.9935 for S Warfarin.0.9915 for R Acenocoumarol and 0.9973 for S Acenocoumarol. The recoveries ranged from 98 to 118%, for warfarin and acenocoumarol. Determinations in 10 normal healthy individuals, revealed a high reproducibility of RTs,. This renders the method suitable for large population studies. We describe, for the first time to our knowledge, a fully validated enantioselective LC-MS/MS method for stereospecific quantitation of both the racemic forms of either Warfarin, Acenocoumarol, and Phenprocoumon in human plasma. The proposed methodology is an accurate, rapid, sensitive, and reliable tool to independently resolve and quantify Warfarin and Acenocoumarol enantiomers with a minimal sample preparation step. Since the proposed internal standard is Phenprocoumon the method can be rotated in the way that Phenprocoumon can be quantified as well as an analyte, and by using one of the other two pharmaceutical drugs as internal standard.

C040

COAGULATION PROFILES IN PRIMATE RECIPIENTS OF PORCINE KIDNEYS EXPOSED TO LONG-TERM TREATMENT WITH RECOMBINANT HUMAN ACTIVATED PROTEIN C

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Clotting cascade activation still remains an unresolved problem to the long term survival of porcine xenograft and is central to the rejection of pig organs transplanted into primates. Here we have studied the efficacy of recombinant human Activated Protein C (rhAPC) on the prevention of consumptive coagulopathy and rejection in xenotransplanted primates. *Methods.* Six nephrectomized cynomolgus monkeys received a hDAF transgenic pig kidney and received our standard immunosuppression. Extracorporeal perfusion (2 hrs) was performed using the controlateral donor kidney prior to transplantation. rhAPC (Xigris, provided by Eli Lilly) was administered by iv infusion. Group A (n=3) received 24 μg/kg/h rhAPC from days 2-7. In group B (n=2), rhAPC was administered on day 2-7 at 24 μg/kg/h and, from day 8 onwards, at 8 μ/kg/h. In both groups episodes of rejection were treated with 4 days of rhAPC at 24 μg/kg/h. In group C rhAPC was initiated at 24 μg/kg/h and subsequently at 48 μg/kg/h throughout the post-operative period. Prothrombin time (PT), activated partial thromboplastin time (aPTT), antithrombin antigen (AT), protein C, free and total protein S antigen, TAT, F1+2, D-dimer, platelets and fibrinogen were monitored. *Results.* Mean survival time was 22.3±10.9 days (median 24, range 10-37). There were no cases of post-operative disseminated intravascular coagulation or overt consumptive coagulopathy. Similarly, we did not observe any bleeding. When compared to the pre-transplant values, we did not observe significant variations of PT, aPTT, AT, PC, fibrinogen or platelets except in the pre-euthanasia phase in some animals. As expected D-dimer levels increased rapidly immediately after transplantation and remained elevated throughout the follow-up period. Importantly, no significant fibrin deposition was observed in biopsies. However, humoral rejection was observed in all explanted xenografts. *Conclusions.* At least in the first 2 postoperative weeks, regardless of the regimen used, rhAPC prevents fibrin deposition in porcine xenografts transplanted into primates. Furthermore, in all cases rhAPC prevents the onset of overt consumptive coagulopathy. rhAPC appears to be a safe agent for long-term use in pig-to-primate xenotransplantation.

Genetic factors and thrombosis

C041*

NATURAL MUTATIONS IN THE EGF4 DOMAIN OF PROTEIN S PRODUCE EXTENDED CONFORMATIONAL CHANGES AND ALTER PHOSPHOLIPID AND C4BBP INTERACTIONS

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Protein S (PS), through its anticoagulant activities, plays a crucial role in the control of coagulation. Rare natural PS mutations responsible for dysfunctional molecules in plasma (type II deficiency) represent unique models to relate plasma phenotype with recombinant PS (rPS) expression, and to elucidate the functional role of PS domains. We characterized the first type II PS deficiency affecting the EGF4 domain, a module with a poorly defined functional role. The proband suffered from recurrent deep vein thrombosis and showed reduced PS anticoagulant activity (31%), and total, free PS antigen and C4bBP levels in the normal range. The patient was heterozygous for the IVSg-2A/T mutation that, by activating a cryptic splice site, causes the deletion of codons I203-D204. Recombinant rPSwt and rPSDelI203D204 variants were expressed in BHK21 cells and immunopurified from conditioned medium. The rPSDelI203D204 showed reduced anticoagulant (<10% of rPSwt) and APC-independent activities (38% of rPSwt). Binding of the variant to phospholipid vesicles was significantly reduced (Kd=235.7±30.8 nM, rPSwt; Kd=15.2±0.9 nM) and the Kd for C4bBP was increased (Kd=107.7±14.0 nM; rPSwt, Kd=8.2±1.0 nM). The rPSDelI203D204 showed markedly reduced binding to conformation-specific monoclonal antibodies for GLA and EGF1 domains, whereas the affinity of the mutant rPS for the SHBG recognizing antibody was only slightly different from that of rPSwt. A more conservative alteration (N217S) in the EGF4, previously found, was characterized. The rPS217S showed functional (10% and 41% of anticoagulant and APC-independent activities, respectively) and binding (phospholipid vesicles, Kd=175.5±53.1; C4bBP, Kd=47.2±3.7) features indistinguishable from those exhibited by the micro-deleted PS. These data contribute to characterize the functional role of EGF4 domain in the anticoagulant activities of PS and to define the thrombophilic nature of type II PS deficiency. Moreover, they provide examples of extended conformational changes transmitted from the EGF4 module to the GLA and SHBG domains through intra-molecular communication.

**PREMIO SISET 2006*

C042*

SOLUBLE ENDOTHELIAL PROTEIN C RECEPTOR (SEPCR) LEVELS, A3 HAPLOTYPE OF EPCR GENE AND THE RISK OF THROMBOSIS IN CARRIERS OF ANTITHROMBIN (AT), PROTEIN C (PC) AND PROTEIN S (PS) DEFECTS

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Note: M.O. and W.B. are employed by Diagnostica Stago and Stago R&D whose product was studied in the present work. The plasma levels of sEPCR have been assessed in 406 individuals (probands excluded) belonging to families with inherited clotting inhibitors defects. DNA analysis for the A3 haplotype was performed in all subjects. Among 169 individuals belonging to PC deficient families 10 presented with double heterozygous PC defect and A3 haplotype, 74 with PC defect alone, 19 with A3 haplotype alone and 66 were non-carriers of either defect. VTE had occurred in 20%, 8.1%, 0% and 1.5% of family members, respectively. In addition, by considering the upper quartile of sEPCR levels, individuals with PC defects presented a risk 3-fold higher to develop VTE as compared to those without PC defects. In contrast, in the lower quartile of sEPCR levels, the risk for VTE was similar regardless of the presence of PC defects. Among 134 individuals from PS deficient fami-

lies, 14 presented with double heterozygous PS defect and A3 haplotype, 52 with PS defect alone, 17 with A3 haplotype alone and 51 were non-carriers of either defect. VTE had occurred in 7.1%, 23%, 5.9% and 3.9% of family members, respectively. In the upper quartile of sEPCR levels, individuals with PS defects presented a risk 3-fold higher to develop VTE as compared to those without PS defects. In the lower quartile of sEPCR levels, the risk for VTE was about 2-fold higher in carriers as compared to non-carriers of PS defects. Among 103 individuals from AT deficient families, 8 presented with double heterozygous AT defects and A3 haplotype, 43 with AT defects alone, 9 with A3 haplotype alone and 43 with no defect. VTE had occurred in 25%, 33%, 5.5% and 1.2% of family members, respectively. The risk of VTE in carriers of AT was 5-fold higher as compared to non-carriers both in the upper and in the lower quartile of sEPCR levels. In conclusion, the presence of A3 haplotype and sEPCR plasma levels might modulate the clinical expression of thrombophilia in clotting inhibitor deficient patients. Prospective studies are needed to confirm these findings.

**PREMIO SISET 2006*

C043

EFFECT OF APC RESISTANCE ASSOCIATED WITH FACTOR V MUTATIONS ON PLASMA FIBRINOLYSIS

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Factor V is endowed with both procoagulant and anticoagulant properties. APC resistance caused by homozygous FV Leiden mutation has been shown to inhibit fibrinolysis through the enhancement of thrombin-mediated activation of TAFI (thrombin activatable fibrinolysis inhibitor). It is unclear, however, whether heterozygous carriers of Leiden mutation or carriers of other FV genotypes associated with APC resistance have a defective fibrinolytic activity. We studied 107 subjects with different FV genotypes (table). Sixty-seven were unrelated asymptomatic subjects with or without Leiden mutation while 40 belonged to 6 families with combined FV mutations. Plasma fibrinolytic capacity was studied by evaluating the lysis time of tissue factor-induced clots exposed to 25 ng/ml exogenous t-PA. The assay was performed in the absence and in the presence of APC (1 microg/ml) and the fibrinolytic response was calculated by the ratio of the two lysis times (APC lysis ratio). Heterozygous Leiden mutation was associated with a significantly reduced APC lysis ratio both in unrelated subjects and in members of thrombophilic families. Moreover, the combination of Leiden mutation with type I FV deficiency (pseudohomozygous APC resistance) made the plasma totally unresponsive to the fibrinolytic effect of APC, mimicking the homozygous Leiden mutation. Neither the HR2 haplotype nor type I FV deficiency, when present in heterozygous form, influenced the response to APC. Interestingly, the two patients with Leiden plus HR2 and the patient with HR2 plus type I deficiency were also refractory to the fibrinolytic activity of APC. These data indicate that heterozygous carriers of the Leiden mutation are resistant to the fibrinolytic effect of APC. This resistance, however, is attenuated by the presence of normal factor V as indicated by strong refractoriness to APC of the combinations of Leiden with either type I deficiency or HR2 haplotype.

Table 1. APC lysis ratio in patients with different factor V genotypes

Unrelated subjects		*Members of thrombophilic families*						
Normal	Leiden Het	Normal	Leiden Het	HR2 Het	Type I def Het	Leiden+ Type I def	Leiden+ HR2	HR2+ Type I def
n=33	n=34	n=13	n=10	n=2	n=5	n=7	n=2	n=1
+1.75 ± 0.26	1.57* ± 0.26	1.57 ± 0.29	1.21* ± 0.22	1.44 ± 0.24	1.54 ± 0.30	1.10*† ± 0.09	1.08 ± 0.02	1.11

** p<0.05 as compared to normal; † p<0.05 as compared to Leiden Het.*

C044

TISSUE FACTOR A-603G GENOTYPE ASSOCIATES WITH CAROTID INTIMA-MEDIA THICKNESS IN SUBJECTS UNDERGOING CARDIOVASCULAR RISK PREVENTION

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Tissue factor (TF), key initiator of coagulation, is also ascribed a non-haemostatic function in inflammation, cell migration and proliferation, suggesting a role of TF not only in thrombosis but also in atherosclerosis development. Polymorphisms in the TF gene promoter have been shown to modulate the expression of TF, and thereby potentially also its involvement in atherosclerosis and individual predisposition to atherosclerotic disease. Hence, this study was aimed at investigating associations between TF promoter polymorphisms and carotid intima-media thickness (IMT), a well-established surrogate marker of atherosclerotic disease. To this end, the TF A-603G polymorphism was analysed in 316 subjects enrolled in a primary and secondary cardiovascular risk prevention programme, with measurements of carotid IMT by B-mode ultrasound. Also, the TF Ins-1208Del polymorphism was investigated in a limited number of subjects, which confirmed the previously reported complete concordance between the -603A and -1208Del alleles. The subjects were aged 60.2±8.4 years, 80% were male, and 78% were undergoing secondary prevention with a history of coronary, cerebrovascular, or peripheral atherosclerotic disease. Both mean and maximum carotid IMT (measured at the common carotid artery, carotid bifurcation, and internal carotid artery) differed significantly according to A-603G genotype, being highest in -603A/A (n=93), intermediate in A/G (n=161) and lowest in G/G (n=62) (mean IMT: A/A 1.31±0.36 mm, A/G 1.27±0.33 mm, G/G 1.19±0.32 mm; max IMT: A/A 2.36±0.88 mm, A/G 2.26±0.85 mm, 2.05±0.88 mm; both $p<0.05$; adjusted for age, gender, and statin treatment). In summary, a significant association between TF promoter genotype and carotid IMT was observed, perhaps mediated via alterations of TF expression levels in the circulation or within the carotid vessel wall. These findings support the hypothesis that TF plays a role in the atherosclerotic process, beyond its well-known role in haemostasis and thrombosis, thus further implicating TF not only in thrombotic complications of atherosclerotic disease, but also in plaque progression.

C045

HIGH-THROUGHPUT MULTIPLEX SINGLE-NUCLEOTIDE POLYMORPHISM (SNP) ANALYSIS IN GENES INVOLVED IN METHIONINE METABOLISM

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Hyperhomocysteinemia is a well known independent risk factor for atherothrombotic diseases. Mild hyperhomocysteinemia may result from both acquired and genetic influences. Several polymorphisms are suspected to be associated with hyperhomocysteinemia, but often date are limited and inconsistent due to the lack of robust studied populations and/or of data on the possible interaction among SNPs in genes coding molecules influencing methionine metabolism. High-throughput genotyping technologies, such as that provided by the GenomeLab SNPStream from Beckman Coulter (4,600-890,000 genotypes/day) are now available and an appropriate definition of SNPs to be analysed could represent a strong resource in order to quickly and definitively define the role of genetic risk factors on diseases. We developed a multiplex PCR-oligonucledotide extension assay with the GenomeLab SNPStream platform to detect SNPs in genes coding molecules involved in the methionine metabolism. We selected SNPs based on their putative function and frequency in candidate genes extracted from PubMed resources. The annotation of each SNP and its frequency in Caucasian populations was assessed in several databases. We selected 72 SNPs: 7 in MTHFD1, 5 in NNMT, 3 in PON1, 2 in PON2, 5 in TCNII, 4 in AHCY, 7 in MTRR, 5 in BHMT, 3 in GCP2, 5 in MTHFR, 6 in MTR, 3 in TYMS, 3 in cSHMT1, 5 in RFC1, 7 in CBS, 2 in BHMT2 gene. They were analyzed in 6 panels of 12 SNPs each according to their nucleotide substitution (1 AT, 1 CA, 1 GC, 1 GA and 2 CT panels). Among the 6 panels analyzed, in 3 panels 12/12 and in 3 panels 11/12 designed SNPs worked: therefore, we could analyze 69 out of 72 (96%) SNPs. As concerns 3 SNPs, C677T and A1298C in MTHFR gene and A2756G in MTR gene, we compared data obtained with an electronic microchip technology (Nanogen) in 288 subjects. We showed a 98.5% concordance with the two technologies. The

developed approach could represent a useful tool to investigate the genotype-phenotype correlation and the association of these genes with hyperhomocysteinemia and correlated diseases.

C046

NEW VARIATIONS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS-γ (PPARG) PROMOTERS IN ACUTE CORONARY SYNDROME PATIENTS AND CONTROLS

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Objective. PPARg is a nuclear transcription factor involved in the control of energy, lipid and glucose omeostasis. The gene is located on chromosome 3 (3p25) and produces 4 different PPARg mRNAs by alternative splicing and promoter usage. The 4 different promoters are thought to allow a more fine tuning in the control of gene expression. PPARg may act directly on local vasculature in several critical aspects of atherothrombosis (lipid metabolism, foam cell responses, inflammation), suggesting that it may be an important determinant of gene expression during atherogenesis and it is a potential candidate gene for acute coronary syndrome (ACS). Few studies on PPARg promoters (Pr) polymorphisms have been performed, but no data on the role of these polymorphisms in cardiovascular diseases are available. *Aim of the study.* 1) to look for new genetic variations in PPARg Pr, 2) to investigate PPARg Pr polymorphisms association with ACS. *Methods.* We studied 202 patients affected by ACS, (median age 66 ys,145 males) compared with age- and sex-matched control group (n=295). The genetic variants were evaluated using heteroduplex analysis on dHPLC and direct sequencing or RFLP analysis. *Results.* We identified in PPARg promoters 3 new variants: T65597C, C25924T, T93640C and 4 already published polymorphisms: C25819G, T26233A, T93673C, C93695T. the C93695T (Pr 4) mutation showed a significantly different distribution between control and ACS populations (genotype distribution and allele frequency: $p<0.001$). The rare allele T of Pr4 (C93695T) conferred a significant protection against ACS at both univariate (O.R.:0.45,95%C.I.:0.29-0.69, $p<0.001$) and multivariate analysis adjusted for sex, age and traditional cardiovascular risk factors (O.R.:0.44,95%C.I.:0.25-0.76, $p<0.005$). *Conclusion.* These results suggest a new role for PPARg polymorphism C93695T in ACS. Further investigations in order to understand the role of PPARg promoters polymorphisms in the regulation of atherosclerosis process are needed.

C047

INTERLEUKIN GENES TAG-SNP AND RISK OF MYOCARDIAL INFARCTION: THE WESTERN NEW YORK STUDY

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Introduction. Polymorphisms in interleukin (IL) genes were associated with both levels of ILs and risk of myocardial infarction (MI). We investigated whether IL-1b and IL6 variations were associated with MI in the frame of the Western New York Study, by using a Tag-SNP approach. *Methods.* The study is a population based case-control evaluation of 953 MI cases and 1381 healthy controls (without cardiovascular disease or cancer), all residents of the Erie and Niagara counties in NY State, (35-69 yr). 637 white MI male patients were individually matched with 637 controls and 225 white MI females with 450 controls by age (± 5 years), smoking habits, menopausal status and years since menopause (± 2 years). SNPs were identified in the SeattleSNPs variation discovery panel http://pga.mbt.washington.edu/. Linkage disequilibrium was used to select SNPs that tagged common genetic variation. SNPs within an BIN were selected on the bases of allele frequency (≤10%), position in the gene, type of sequence and already known associations with quantitative or disease phenotypes. *Results.* The following SNPs were finally selected: 794C/T; 3298G/A; 2766A/T; 5277C/T for IL 1-β and 1510G/C; 205del/CT; 4638G/C; 7592G/A for IL6. The SNP 3298G/A of IL 1b gene was significantly associated with the risk of MI in the whole population. Homozygosity for the A allele reduced by 30% the risk of MI (OR:0.70; 95% CI: o.52-0.73). The SNP 1510C/G and 205 -/CT of IL 6 gene were positively associated with the risk of MI (OR GG vs GC+CC= 1.28 (1.03-1.60) and OR -- vs -CT+CTCT= 1.30 (1.05-1.61), respectively in a dominant inheritance model). After stratification by gender, all the SNPs remained significantly associated with MI in males, but not in females. *Conclusions.* Inflammatory genes are associated with the risk of Mi. Our results support a primary role of inflammation in the pathogenesis of myocardial infarction.

C048

HEMORHEOLOGICAL PROFILE IN SYSTEMIC SCLEROSIS: ROLE OF ENOS -786T>C AND 894G>T POLYMORPHISMS IN MODULATING BOTH THE HEMORHEOLOGICAL PARAMETERS AND THE SUSCEPTIBILITY TO THE DISEASE

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Objective. Microvascular disorders are relevant in systemic sclerosis. Hyperviscosity, due to alterations of blood cells and plasma components, may play a role in the pathogenesis of microcirculatory disorders. An impairing nitric oxide availability, related to eNOS gene polymorphisms, might influence erythrocyte deformability. Aims of this study were to investigate 1) the hemorheological profile in systemic sclerosis; 2) the role of eNOS polymorphisms in modulating the hemorheological status of systemic sclerosis patients. Methods: We studied 113 consecutive systemic sclerosis patients (75 with limited and 38 with diffuse subset), and 113 healthy controls. Hemorheological profile was performed by assessing whole blood (WBV 0.512s-1 and WBV 94.5s-1) and plasma (PLV) viscosity, and erythrocyte deformability index (DI); eNOS polymorphisms by molecular analysis. *Results.* A marked alteration of hemorheological parameters in both limited and diffuse systemic sclerosis patients was found ($p<0.0001$). At the multivariate analysis rheological variables were significantly associated to the disease (WBV 94.5s-1 OR: 5.4 95%CI 1.4-19.9 $p=0.01$; PLV OR: 2.8 95%CI 1.2-6.5 $p=0.01$; DI OR: 3.9 95%CI 1.4-10.8 $p=0.007$), and eNOS -786C and 894T alleles significantly affected the deformability index (OR -786C 2.3 95%CI 1.01-5.4, $p=0.04$, OR 894T 2.2 95%CI 1.01-4.8, $p=0.04$, respectively). The contemporary presence of -786C and 894T alleles represented a susceptibility factor for systemic sclerosis (OR 2.84 95%CI 1.40-5.74, $p=0.004$). *Conclusion.* Our findings document an altered rheological profile in systemic sclerosis, and demonstrate a relationship between this alteration and eNOS polymorphisms, so shedding light on a potential novel influencing the microcirculation in this disease.

Antiplatelet drugs

C049

INTERINDIVIDUAL VARIABILITY OF PLATELET SENSITIVITY TO ASPIRIN IS DUE TO IMPAIRED PLATELET COX-1/ASPIRIN INTERACTION

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We have recently demonstrated that an interindividual variability of platelet sensitivity to aspirin is due to the persistent platelet production of thromboxane A2 (TxA2). Aspirin efficacy may be dependent on variable plasma concentration of the drug, pharmacodynamic interindividual variability, or on TxA2 derived from sources different from platelet COX-1. In order to address this controversial matter we studied plasma salicylate concentration, serum TxA2 and collagen-induced TxA2 production in 100 patients under chronic treatment with aspirin. Our results demonstrated that the observed interindividual variability of the response to aspirin is not dependent on the rate or the extent of the absorption, in that both serum TXA2 levels and collagen-induced TxA2 values were not higher in patients with salicylate values below the median respect to values above the median. Thus, in order to evaluate whether TxA2 derives from sources different from platelets, we studied: a) Collagen-induced TxA2 production in the absence and in the presence of aspirin, alone or in combination with the COX-2 inhibitor, NS-398; b) immunoblot analysis of COX-1 and COX-2 platelet expression.

Our results demonstrated that COX-2 activation has a marginal role in TxA2 production. In fact, despite the slight reduction of TxA2 levels achieved by the combined use of NS-398 plus aspirin, only in a limited number of patients we found COX-2-positive platelets. It is worth noting that the amount of COX-2 is markedly lower than COX-1. Moreover, those patients whose platelets were COX-2 negative had both serum and collagen-induced TxA2 levels similar to those found in COX-2 positive platelets. In conclusion, our data demonstrate that the interindividual variability of platelet sensitivity to aspirin is a phenomenon that is due to a reduced efficacy of aspirin on platelet COX-1 despite normal plasma salicyate concentration.

C050

REDUCED PLATELET COX-1 SENSITIVITY TO ASPIRIN IN TYPE 2 DIABETES

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Type-2 diabetes mellitus represents an important risk factor for coronary artery disease. Although benefit of aspirin in primary prevention in diabetic patients has been documented, the PPP trial suggests that aspirin might be less effective in diabetics than in nondiabetics. *Aim.* to investigate platelet function in a diabetic population to understand the mechanisms of the reduced platelet sensitivity to aspirin. To this purpose 50 patients with type-2 diabetes under aspirin treatment were recruited and compared with 50 nondiabetic patients taking aspirin for primary prevention. Plasma concentrations of salicylate were evaluated by HPLC to assess patients' compliance. Platelet function was studied with: a) platelet aggregation in response to collagen and ADP; b) collagen-induced TxA2 production in the absence and in the presence of aspirin, alone or plus the COX-2 inhibitor, NS-398; c) serum TxA2 concentration; d) immunoblot analysis of COX-1 and COX-2 platelet expression. *Results.* platelet aggregation was higher in diabetics compared with nondiabetics (57.3±26.3% vs. 41.7±26.2% for collagen; 40.9±10.6% vs. 31.3±16.7% for ADP). Collagen-induced TXA2 levels were significantly higher in diabetic subjects (896.4±232.3 pg/108cells; median 212; min 1; max 7344), compared to nondiabetics (130.5±31.5 pg/108cells; median 44; min 2; max 250). *In vitro* aspirin treatment decreased TXA2 production (51±12 vs 896.4 +/-232.3 pg/108cells) that was further reduced by combining aspirin with NS-398 (31±10 vs 51±12 pg/108cells). A positive correlation between collagen-induced TxA2 formation and serum TxA2 levels (1716.9±286.5 pg/mL, R=0.778) was found. Platelet COX-2 expression (although in a lower amount than COX-1) was observed in all diabetics, but only in 8/20 nondiabetics. *Conclusion.* this study provides evidence that *in vivo* aspirin intake in diabetic patients is less effective in inhibiting platelet COX-1. This phenomenon might account for the higher incidence of cardiovascular events during a chronic treatment with aspirin in diabetic, than in nondiabetic patients.

C051

GALLIC ACID, A DIETARY POLYPHENOLIC COMPONENT, BLUNTS THE INHIBITION OF PLATELET FUNCTION BY ASPIRIN: AN EXAMPLE OF NUTRIENT-DRUG INTERACTION

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Gallic acid, a polyphenol mainly present in fruits, nuts, tea and red wine, was recently shown to interact with platelet and endothelial P-selectin. The chemical structure of gallic acid is similar to that of salicylic acid, the main aspirin metabolite, another natural polyphenol. The aim of this study was to test whether gallic acid, similarly to salicylic acid, would prevent the platelet inhibitory effect of aspirin through an interaction with the COX-1 binding site. Citrated platelet-rich plasma (PRP) was obtained from healthy subjects, platelet aggregation was induced by arachidonic acid (AA, 0.8-1 mM) and percent aggregation recorded at 5 minutes. The amount of TxB2 released by AA-stimulated platelets was measured by EIA. Aspirin (10 μM, preincubated for 10 min at 37°C) completely blocked AA-induced platelet aggregation and reduced TxB2 production to less than 5% of control values (2.4±1.1 μ/mL). Gallic acid (0.4 -1 mM) was unable to inhibit AA-induced platelet aggregation, but when preincubated with PRP at 37°C for 10 min before aspirin, completely blunted aspirin inhibition of aggregation and restored TxB2 production to 51.2±3.6% and 60.1±1.0% of control, at 0.4 and 1 mM, respectively. These effects were comparable to that of 1 mM salicylic acid, that blunted aspirin inhibition of AA-platelet aggregation and restored TxB2 production to 71.4±28.6% of control. ETYA, a fatty acid structural analogue of AA, completely inhibited, at 200 microM, AA-induced platelet aggregation by competition with AA at the COX-1 active site. Inhibition by ETYA was not prevented by either gallic acid (1 or 4 mM) or salicylic acid (1 mM). In conclusion, gallic acid -like salicylate and other non-steroidal anti-inflammatory compounds - interferes with aspirin at the platelet COX-1 binding site level. This novel nutrient-drug interaction may be a possible mechanism, so far underestimated, underlying the variability of aspirin's effects on platelets.

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C052

ASSOCIATION OF THE POLYMORPHIC GENETIC VARIANTS OF GLYCOPROTEIN IA WITH ASPIRIN RESISTANCE IN ACUTE CORONARY SYNDROME PATIENTS

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Variability in response to antiplatelet treatment has been described and the widespread use of aspirin requires clarification of the aspirin resistance phenomenon. Various polymorphisms of the glycoprotein Ia/IIa receptor (GpIa/IIa) have been investigated, but the influence on platelet reactivity in acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI) patients is not still elucidated. The aim of this study was to assess whether, in ACS patients undergoing PCI on antiplatelet treatment, the platelet reactivity profiles may be influenced by the C807T, G873A and C837T polymorphisms of the GpIa/IIa gene. 519 patients on dual anti-platelet therapy were genotyped by an electronic microchip technology. Reactivity of platelets was assessed by PFA-100 on whole blood obtained after PCI by measuring the closure times (CT) for collagen/epinephrine. Aspirin resistance was diagnosed in the presence of CT/EPI ≥ 203 sec. The C807T and G873A polymorphisms resulted in complete linkage disequilibrium. The distribution of C837T genotypes was similar in aspirin resistant (CC 81.7%, CT 17.4%, TT 0.9%) and responders patients (CC 77.2%, CT 22.5%, TT 0.3%), whereas a significant difference ($p<0.05$) in genotype distribution of C807T/G873A polymorphisms between aspirin resistant (CC/GG 25.6%, CT/GA 59.0%, TT/AA 15.4%) and responder patients (CC/GG 40.2%, CT/GA 47.9%, TT/AA 11.9%) was observed. We observed 94 patients with two rare alleles of GpIa/IIa polymorphisms (A), 240 patients with one rare allele (B) and 107 patients without rare alleles (C). The genotype combination distribution was significantly different ($p<0.05$) between aspirin resistant patients (A: 25.4%, B 58.8%, C 15.8%) and responder patients (A: 19.9%, B 52.9%, C 27.2%). The presence of one as well as

of two rare alleles of GpIa/IIa polymorphisms was a significant (p<0.05) predictor of aspirin resistance (B vs C: OR= 1.9, 95% CI 1.1-3.4, p<0.05; A vs C: OR= 2.2, 95% CI 1.1-4.3, p<0.05). Our results indicate that the 807TT/873AA genotypes are significant predictors of aspirin resistance in patients with ACS.

C053

POST-TREATMENT PLATELET FUNCTION IS AN INDEPENDENT PREDICTOR OF MAJOR ADVERSE CARDIAC EVENTS IN PATIENTS WITH MI UNDERGOING PRIMARY PCI

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Purpose. Antiplatelet therapy is a cornerstone of cardiovascular medicine. Recently, a great interest has been focused on the phenomenon of aspirin resistance which may be defined as laboratory resistance - defined as the failure of aspirin to inhibit platelet thromboxane A2 production or inhibit tests of platelet function- or clinical resistance. No data are available on the possible clinical implications of aspirin resistance in the setting of acute coronary syndromes. Aim of our study was to evaluate the role of aspirin resistance in the occurrence of major adverse coronary events in patients with acute myocardial infarction. *Methods.* We prospectively evaluated 146 [115 M/31 F; age: 65(30-84)yrs] patients with MI undergoing primary PCI. Exclusion criteria were the use of GpIIb/IIIa inhibitors, hematocrit <50% and platelet count <100,000/mm^3. PFA-100 closure times by collagen-epinephrine (CT/EPI) (reference values: 90±24.6 sec) and collagen-ADP (CT/ADP) (reference-values: 146.3±56.7 sec) were used for measuring platelet function, on venous blood samples obtained 12-15 hrs after the primary PCI. Aspirin resistance was diagnosed in the presence of CT/EPI <203 sec (95° percentile of control distribution). Results: After a median follow up of 12 months, MACE were recorded in 46/146 (31.5%) patients: 16 restenosis, 14 cardiovascular death and 16 new ischemic events. A significantly higher percentage of patients with MACE had aspirin resistance (39.1% vs 23.2%; p<0.05). In particular, among the 16 restenosis, 11/16 patients (68.7%) had aspirin resistance with respect to 30/129 (23.2%) without a subsequent restenosis (p<0.0001). At the Kaplan-Meier survival curve, the overall risk of MACE was significantly higher among patients with aspirin resistance (p=0.02). At Cox regression analysis – adjusted for age, gender, traditional cardiovascular risk factors, renal function, the number of stenosed vessels and history of previous MI, PCI or CABG – aspirin resistance was a significant and independent risk factor for the future occurrence of MACE [HR=3.8 (95%CI 1.1-15.6), p<0.05]. *Conclusion.* Our data demonstrate that post-PCI aspirin resistance is a significant and independent predictor of MACE in patients with MI undergoing primary PCI.

C054

PERSISTENT PLATELET ACTIVATION IN DIABETIC PATIENTS TREATED WITH LOW DOSES OF ASPIRIN

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The percentage of individuals with type 2 diabetes (DM2) who do not benefit from the protective effect of low dose aspirin is larger than in other populations at cardiovascular risk. We explored the hypothesis that, in diabetes, aspirin fails to adequately suppress the biochemical activity of its primary target, cycloxygenase(COX)-1 and, as a consequence, fails to adequately reduce platelet activation. Following a case-control design, the study enrolled 82 patients with DM2 (cases) and 39 non diabetic subjects (controls), with or without previous cardiovascular events, treated with low dose aspirin (minimum 75 mg/d). Urinary 11-dehydro-Tx-B2, and plasma CD40L, were measured as indexes of COX-1 activity and of functional platelet activation, respectively. 11-deidro-TxB2, was significantly higher in cases than in controls (38.9 [27.8-63.3] vs. 28.5 [22.5-43.9] ng/mmol of creatinine, p=0.02; values are median and interquartile range). After adjusting for age, sex, and smoking, the risk to have 11-deidro-TxB2 within the upper quartile was over 3 times higher (OR=3.6 IC 95% 1.01-12.6) in cases than in controls. When compared to non-smokers non-diabetic patients, the risk to have 11-deidro-TxB2 within the upper quartile was increased by 3 times (OR=3.1 IC 95% 0.9-10.8) in non-smokers diabetic patients, and 8 times (OR=7.8 IC 95% 1.2-52.3) in the presence of both, diabetes and smoking. Circulating plasma levels of CD40L were significantly higher in cases than in controls (1.06 [0.42-3.06] vs. 0.35 [0.22-0.95] ng/mL; p=0.0001); 34.6% of patients with DM2 had CD40L values within the higher quartile as compared with 5.1% in non-diabetic subjects (p<0.001). In DM2 patients treated with low doses of aspirin, markers of COX-1 and platelet activity remain elevated in a percentage of subjects significantly higher than that of non-diabetic patients. The persistent high TxA2 production in these patients suggests a reduced pharmacological efficacy of aspirin on its specific pharmacological target COX-1.

C055

NCX-4016, BUT NOT ASPIRIN, PREVENTS THE ACUTE HYPERGLYCEMIA-INDUCED ENHANCEMENT OF SHEAR STRESS-INDUCED PLATELET ACTIVATION IN TYPE 2 DIABETES MELLITUS (TD2M)

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T2DM is associated with a 2 to 4-fold increased risk of ischemic cardiovascular disease and platelet hyperreactivity has been suggested as a potential mechanism of enhanced arterial thrombosis. We have previously shown that acute, short-term, hyperglycemia enhances shear stress-induced platelet activation in T2DM (Gresele et al., J Am Coll Cardiol 2003, 41: 1013). High shear stress-induced platelet activation is known to be resistant to aspirin inhibition. NCX-4016 (NCX), a nitric oxide-donating aspirin, is a novel antiplatelet and vascular anti-inflammatory agent with wider activity than aspirin. We have evaluated comparatively the effects of aspirin and NCX-4016 on the acute hyperglycemia-induced platelet activation in patients with TD2M. In a prospective randomized double-blind parallel group study, 40 diabetic patients underwent 4hrs of acute hyperglycemia (13.9 mmol/l, 250 mg/dL) after a 14-day treatment with placebo or NCX (800 mg bid) or aspirin (100 mg od) or the combination of the two. Primary endpoint was the enhanced shear stress-induced platelet activation (O'Brien filter test) provoked by a hyperglycemic clamp (4hrs, blood glucose 250 mg/dL) Platelet adhesion to collagen under high-shear conditions (3000 sec-1), expression of P-selectin on platelets recovered in the blood from a sking wound (bleeding time-blood) and circulating platelet/leukocytes aggregates were also measured. NCX was significantly better than aspirin (p=0.043) and than placebo (p=0.039) in preventing hyperglycemia-induced enhancement of shear stress-induced platelet activation (post minus pre-clamp filter closure time: placebo -13.5±21.9 sec, aspirin -12.7±22.9, NCX +10.6±24.9, NCX+ASA +12.0±34.0). Secondary endpoints were consistent, showing a significant inhibitory effect of NCX vs placebo, and not of aspirin, for adhesion under high shear conditions (placebo +7.1±18.8 plts/μ m^2, NCX -26.8±30.2; p=0.008), and for the formation of circulating platelet/leukocyte aggregates (placebo +1.2±2.4%, NCX -0.5±1.2%; p<0.05). The increased expression of P-selectin on platelets in bleeding-time blood induced by hyperglycemia was significantly reduced by both NCX and aspirin. All active treatments significantly inhibited serum TxB2 and urinary 11-dh-TxB2. These results suggest that NCX-4016 possesses antiplatelet effects additional to those exerted by aspirin in diabetics, and therefore deserves further clinical testing for cardiovascular prevention in T2DM.

C056

NITRO-ATORVASTATIN (NCX-6560) INHIBITS CHOLESTEROL BIOSYNTHESIS AND SHOWS ANTI-THROMBOTIC PROPERTIES SUPERIOR TO THOSE OF ATORVASTATIN

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Recently, a new class of compounds, incorporating a statin and a nitric oxide (NO)-releasing moiety, have been reported to display enhanced antiproliferative, antinflammatory and antithrombotic activities. Aim of the present study was to evaluate a novel Nitro-atorvastatin deriva-

tive (NCX6560), in comparison with its parent compound atrovastatin (Ato) for its effectson cholesterol levels and on platelet dependent thrombosis. *In vitro*, NCX6560 and Ato induced comparable inhibition of cholesterol biosynthesis by rat smooth muscle cells (IC50 = 1.9+0.4 and 3.9+1.0 μM, respectively). However, a 5-week daily oral treatment with NCX6560 (46.8 mg/kg/day) was more effective than an equivalent Ato dose (40 mg/kg/day) at lowering serum cholesterol in CD1 mice on a hyperlipidemic diet (NCX6560: -21% vs controls, $p<0.05$; Ato: -14% vs control, p=NS). *In vitro*, NCX6560-induced vasodilation of rabbit aortic rings (EC50 = 53.5+8.3 μM)while Ato was ineffective. The vasorelaxant effect was confirmed *in vivo* by the reduction of blood pressure of NCX6560, but not of Ato, in NOS-3 knockout mice (NCX=119.2±2.9*, Ato=134.9Ã,±3.6, vehicle=140±2.1 mmHg, n=12, $p<0.001$* vs vehicle); the blood pressure lowering effect was not observed in normal wild type mice. Platelet pulmonary thromboembolism in mice, induced by the i.v. injection of U46619 or collagen plus epinephrine, was reduced by a single oral dosing of NCX6560 (46.8 mg/kg) (mortality -44% and -56% vs vehicle, respectively; $p<0.05$), but not by Ato (40 mg/kg). Similarly, NCX6560, but not Ato, inhibited mortality induced by mechanical pulmonary microembolism by exerting a vasodilatory effect on the pulmonary microcirculation. Finally, administration of NCX6560 (46.8 mg/kg, po) to CD1 mice on a high fat diet, but not of equimolar Ato, significantly reduced ex vivo platelet adhesion to collagen at high shear rate (C=56±1.6% surface coverage; Ato=5,±1.6%; NCX6560= 40±1.25%, $p<0.001$ vs control and Ato). In conclusion NCX6560 exerts greater lipid lowering, anti-thrombotic and anti-inflammatory effects than Ato both *in vitro* and *in vivo*.

Venous thromboembolism: epidemiology, risk factors, recurrences

C057

PULMONARY EMBOLISM IN THE MULTICENTRE ADVANCED STUDY FOR A THROMBOEMBOLISM REGISTRY (MASTER): STUDY DESIGN AND PATIENTS RECRUITED

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Background. The outcome of patients affected by Pulmonary Embolism (PE) has been described only in the short term period. *Aims of the study.* The MASTER registry aims at describing the outcome of patients recruited for a non-fatal episode of PE and the incidence and risk factors for PE in patients recruited for an episode of Deep Venous Thrombosis (DVT) in a 2 years follow-up. *Patient and methods.*MASTER (Multicenter Advanced Study for a ThromboEmbolism Registry) was an multicenter registry of consecutively patients with symptomatic, objectively confirmed, acute VTE. *Results.* From January 2002 to October 2004, 2119 patients (1056 males), aged 59.2+18.4 years, were included in the registry in 29 centres; 1541 patients were affected by DVT, 372 by DVT and PE, and 206 by PE only. The frequency of PE was significantly higher in females (OR=1.44, 95% C.L. 1.18-1.76) and in the 955 subjects with a temporary risk factor (TRF), such as surgery, immobility, recent trauma, and other (OR=1.57, 95% C.L. 1.29-1.91). The risk of PE was higher in females also after adjustment for the presence of TRF, pregnancy, and use of oral contraceptives. The risk of PE was not increased in the 424 subjects affected by malignancies or in those treated with chemo or hormone therapy. The location of DVT in the caval or in the lower right limb veins was associated with an increased risk for PE. *Conclusions.* The preliminary analysis of our cohort suggests that the survivors an acute episode of PE are not at risk of a premature death in the long term period when compared with subjects affected by an episode of DVT only. The complete follow-up of the MASTER cohort could confirm these results and allow for an assessment of many long term outcomes of the thromboembolic disease.

C058

RATES OF CLINICALLY APPARENT VENOUS THROMBOEMBOLISM AND DEATH RESULTING FROM PULMONARY EMBOLISM AMONG UNSELECTED HOSPITALIZED ACUTELY ILL MEDICAL PATIENTS VERSUS RATES PREDICTED FROM CLINICAL STUDIES.

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Background. Without evidence from autopsies, the majority of deaths resulting from pulmonary emboli (PE) are indistinguishable from deaths due to other cardiovascular diseases. This has led to a gap in perceptions between the benefits and risks of providing venous thromboembolism (VTE) prophylaxis. In this study, we estimated the incidence of clinically apparent VTE in hospitalized acutely ill medical patients in The International Medical Prevention Registry on Venous Thromboembolism (IMPROVE), and compared this with the expected incidence derived from clinical studies that used autopsy or prospective venographic confirmation of clinically important VTE. *Methods.* Beginning in July 2002, a consecutive, unselected sample of patients who were aged ≥18 years and hospitalized for ≥3 days with an acute medical illness, were enrolled in this observational cohort from 49 hospitals in 12 countries. Up to 31 March 2005, 6946 patients were enrolled. *Results.* Based on autopsy series of all-cause in-hospital deaths reported in the literature, PE is associated with 10% of deaths, is the primary cause in 5%, and is clinically recognized as the primary cause in 1.5% of deaths. A review of clinical stud-

ies with mandatory venography resulted in predicted rates of 10% for all VTE and 1% for clinically recognized (confirmed) VTE. In IMPROVE, there were 4/291 (1.4%) deaths due to clinically recognized PE (vs. 4 predicted). There were 79 (1.1%) treated VTE events (vs. 69 predicted). *Conclusions.* Observed rates of death due to PE and clinically recognized VTE in a real-world setting are consistent with predictions from clinical study data. Physicians should be aware of the significant gap that exists between clinically important and clinically evident VTE events. Reliance on the low rates of clinically recognized events to assess the seriousness of this disease can lead to a significant under-estimation of its impact on public health and a consequent failure to realize the proven benefits of VTE prophylaxis in hospitalized acutely ill medical patients.

C059

INCIDENCE, CLINICAL ASPECTS AND TREATMENT OF ISOLATED CALF (IC) DEEP VEIN THROMBOSIS (DVT) IN THE MASTER MULTICENTER ITALIAN REGISTRY

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Background. The incidence, risk factors, clinical importance, need for diagnosis and best treatment of IC-DVT are still controversial. *Methods.* MASTER is a multicenter registry aimed to prospectively collect information on acute venous thromboembolic events (VTE) in a large cohort of patients. *Results.* IC-DVT was diagnosed in 240 (13.5%; 121 males) out of the 1772 patients with acute leg DVT. No significant differences were recorded as regards sex, age, left or right leg; 5.4% of IC-DVTs were bilateral. Diagnosis, obtained by ultrasonography in all cases, was more delayed (at least 10 days from onset of symptoms) in IC than in proximal DVT (15.0% and 5.5%, respectively; $p<0.0001$). At least one temporary risk factor (49.6% vs 41.1%; $p= 0.016$) was more frequent in IC than in proximal DVT, but cancer was less frequent (10.8% vs 20.4%; $p<0.001$). A pulmonary embolism was equally present in patients with IC (24.6%) or proximal DVT (19.9%). The majority of patients with IC-DVT were treated with heparin (or derivatives) followed by oral anticoagulation (67.5%), while 30.4% received heparin or derivatives only. *Conclusions.* These data show that: a) most Italian centers (at least among those participating to the MASTER registry) look for IC-DVT by a complete ultrasonographic examination of the deep vein system in symptomatic patients; b) the incidence of IC-DVT was, as expected, about 14%; c) its diagnosis is often more delayed than in proximal DVT; d) transient risk factors are more frequent in calf than in proximal DVT; e) unexpectedly, patients with IC are at the same risk of pulmonary embolism than those with proximal DVT, and f) most centers treat these patients in the same way as those with proximal DVT. Some of these findings are unexpected and highlight the clinical importance of IC-DVT. Further clinical studies are necessary on this issue.

C060

CLINICAL PRESENTATION AND RISK FACTORS OF UPPER LIMB DEEP VEIN THROMBOSIS (DVT): FINDINGS FROM THE MASTER REGISTRY

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Background. The clinical presentation and risk factors associated with upper limb-deep vein thrombosis (DVT) is not completely elucidated. *Aim of the study.* To prospectively evaluate the epidemiology, clinical presentation and risk factors associated with upper limb-DVT, in comparison to lower limb-DVT. *Patients and Methods.* MASTER was a multicenter registry of consecutively recruited patients with symptomatic, objectively confirmed, acute VTE. Information about clinical presentation, diagnostic methods, temporary and permanent risk factors and prophylaxis were captured by an electronic data network at the time of the index event. *Results.* From January 2002 to October 2004,1789 patients with lower limb-DVT and 124 with upper limb-DVT were included in the registry. Upper limb-DVT affected more frequently the male gender than lower limb-DVT (60.5% vs 51.5%, $p=0.041$).The mean patient age was significantly lower in patients with upper limb-DVT than in patients with lower limb-DVT (52.58 vs 59.81 years, $p<0.001$). Edema was more frequently associated with upper limb-DVT than lower limb-DVT (88.7% vs 79.2%, $p=0.01$). Confirmatory tests were performed significantly earlier in patients with upper limb-DVT than in patients with lower limb-DVT ($p<0.001$). The incidence of pulmonary embolism was significantly lower in patients with upper limb-DVT than in patients with lower limb-DVT (3.2% vs 20.6%, $p<0.001$). The incidence of known cancer at the time of the index event was 32.3% in patients with upper limb-DVT and 17.2% of patients with lower limb-DVT ($p<0.001$). No difference between patients with upper limb- and lower limb-DVT was found regarding the incidence of newly diagnosed cancer (1.6% and 2.5%, $p=0.40$). CVC was a risk factor for upper limb-DVT but not for lower limb-DVT (3.2% vs 0.1%, $p<0.001$). *Conclusions.* The clinical presentation and risk factors of upper limb-DVT are only partially similar to than of lower limb-DVT. Most of cases are associated with known cancer and use of CVC.

C061

THE RISK OF RECURRENT VENOUS THROMBOEMBOLISM IN PREGNANCY AND PUERPERIUM WITH NO ANTITHROMBOTIC PROPHYLAXIS

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Background. Wheter or not pregnant women with a previous venous thromboembolism (VTE) should receive antithrombotic prophylaxis is a matter of debate. *Aims.* To estimate the probability of recurrent VTE during pregnancy and puerperium. *Methods.* We studied a retrospective cohort of 1401 women with a first VTE occurred before 40 years of age. Inherited thrombophilia was defined as the presence of deficiency of antithrombin, protein C, and protein S, factor V Leiden (FVL), prothrombin G20210A. Women with antiphospholipid antibodies were preliminarly excluded. After the first VTE 197 women were pregnant at least once. Further criteria of exclusion were a history of recurrence between the first VTE and pregnancy (n=11) or antithrombotic prophylaxis during all the pregnancies after the first VTE (n=38); thus 148 women (52 with thrombophilia) after a single VTE were pregnant at least once with no antithrombotic prophylaxis. *Results.* Overall, 259 pregnancies were recorded and 193 ended with a live birth; post-partum periods (defined as the 6 weeks period after delivery >16 weeks of pregnancy) with no prophylaxis were 197. We recorded 15 antepartum and 22 postpartum first recurrent VTE (10 and 17 recurrences, respectively, were related to the first pregnancy after VTE). The probability of recurrence during pregnancy was analysed according to the Kaplan-Meier method; in order to ensure the independence of the observations, we selected for each woman only the first pregnancy after VTE. The cumulative probability of recurrence during pregnancy was 7.8% (95% CI 3.1-12.6). The probability of postpartum recurrent VTE related to the first pregnancy after VTE was 11.5% (95% CI 7.3-17.6). In women with a pregnancy-related first VTE the probability of recurrence in pregnancy was 9.9% (95% CI 2.4-17.5) and in puerperium 20.3% (95% CI 12.3-31.7). Women with a pregnancy-related first VTE had a 3.5-fold (95% CI 1.4-8.4) risk of recurrence in puerperium than women with a first VTE occurred in other circumstances. Carriers of FVL had a probability of recurrence in puerperium 2.8 times (95% CI 1.3-6.1) higher than women without thrombophilia. In women with both FVL and a pregnancy-related first VTE the risk of recurrence in puerperium was 8.8 times (95% CI 2.6-30.4) higher than in non-carriers with other circumstances of first VTE. *Conclusions.* We observed an increased risk of puerperium-related VTE recurrence in women with a pregnancy-related first VTE (3.5-fold) and in carriers of FVL (2.8-fold). Other than during puerperium, antithrombotic prophylaxis may be extended in pregnancy of women with a pregnancy-related first VTE.

C062

LONG-TERM OUTCOMES OF POST-SURGICAL DEEP-VEIN THROMBOSIS IN ASIA: THE AIDA-EXTENSION STUDY

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Background. We have recently demonstrated in a large multinational, multiethnic study that the incidence of venographic deep-vein thrombosis (DVT) in Asian patients undergoing major orthopedic surgery of the lower limbs and not receiving thromboprophylaxis was similar to that observed in orthopedic patients in Western countries. Overall, 407 patients (20-99 years) undergoing THR (n=175), TKR (n=136) or HFS (n= 96) were recruited in 19 centres across Asia (Indonesia, South Korea, Malaysia, Philippines, Taiwan and Thailand). 72.5% of the enrolled patients had adequate venograms. Total DVT was diagnosed in 121 of 295 evaluable patients (41.0%, [95% confidence interval: 35.4-46.7]). Proximal DVT was found in 30 patients (10.2% [7.0-14.2]). However the long-term outcomes following deep vein thrombosis such as recurrence of DVT, incidence of pulmonary embolism (PE), and post-thrombotic syndrome (PTS) remain unknown. PTS has received little attention in the literature, and particularly in Asia. *Objectives.* To assess the long-term complication rates (at 1 and 2 years) of patients with objectively assessed DVT compared to those observed in patients without post-operative DVT. *Methods.* Annual visits were performed to record the occurrence of DVT/PE, incidence of PTS, cumulative incidence rates and related predictive factors. PTS scoring were assessed using the Villalta scale (signs and symptoms). In addition in a subset of 18 out of 27 (66%) patients from South Korea with centrally adjudicated venographic DVT, a second venogram was performed at 1 year in order to verify possible spontaneous modifications of the thrombus (none of these patients had undergone any anticoagulant treatment). Results: From the 332 patients having completed the AIDA study in the 19 centres involved in the extension study, 236 patients (71.1%) agreed to participate in the extension phase and were assessed at year 1. Year 2 follow up visits were completed in May 2005. Complete data analysis of the final study results are ongoing and should be completed by September 2005. Of the 18 patients who underwent a second follow-up bilateral or unilateral ascending venography at 1 year±3 months post-operatively, 11 (61%) patients demonstrated resolution of the thrombus, with 3 (16%) patients showing incomplete resolution or persistence of the venous thrombosis. The venograms of the remaining four (23%) patients presented with development of collateral circulation confirming the progressive state of PTS. The majority of patients with centrally adjudicated venographic DVT demonstrated positive resolution following venographic assessment at year 1. The high incidence of thrombus resolution may have been due to the fact that most patients were diagnosed with distal DVT, which would suggest that a high percentage of DVT of the distal veins may resolve spontaneously compared to other types of DVT.

C063

D-DIMER AND FACTOR VIII ARE INDEPENDENT RISK FACTORS FOR RECURRENCE AFTER ANTICOAGULATION WITHDRAWAL FOR A FIRST IDIOPATHIC DEEP VEIN THROMBOSIS

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Background. D-dimer (D-d) and Factor VIII (FVIII) have been separately shown to be risk factors for recurrent venous thromboembolism (VTE) after oral anticoagulant therapy (OAT) suspension. Objective: to assess the predictive value of D-d and FVIII in combination for recurrent VTE after OAT withdrawal. *Patients and Methods.* Consecutive outpatients with a first episode of idiopathic proximal deep vein thrombosis of the lower limbs were enrolled on the day of OAT suspension. After 30+ 10 days, D-d (cut-off value: 500 ng/mL), chromogenic FVIII activity and thrombophilia were determined. Follow-up was 2 years. *Results.* Recurrences occurred in 55 of the 336 patients enrolled (16.4%; 95% CI:13 - 21%), in 8.9% (14/157 ; 95% CI: 5-15%) and in 22.2% (39/176; 95% CI:16-29%) of subjects with normal and abnormal D-d, respectively. Recurrences occurred in 12% (30/251; 95% CI:8 -17%) and in 27.7% (23/83; 95% CI:18-39%) of subjects with FVIII below and above the 75th percentile (2.42U/mL), respectively. The multivariate hazard ratio (HR) for recurrence was 2.45 (95% CI: 1.24-4.99) for abnormal D-d and 2.76 (95% CI:1.57-4.85) for abnormal FVIII after adjustment for age,sex, thrombophilia, OAT duration and residual venous obstruction. When compared with normal D-d and FVIII, the multivariate HR was 4.5 (95% CI: 1.7-12.2) for normal D-d with FVIII >75th percentile and 2.7 (95% CI: 1.2-6.6) and 7.1 (95% CI :2.8-17.6) for abnormal D-d with FVIII, respectively, below and above 2.42 U/mL. *Conclusions.* D-d and FVIII at 30 + 10 days after OAT withdrawal are indipendent risk factors for recurrent VTE.

C064

THE NATURAL HISTORY OF D-DIMER LEVELS IN SUBJECTS WITH NEGATIVE D-DIMER AFTER SUSPENSION OF ORAL ANTICOAGULATION FOR A FIRST EPISODE OF VENOUS THROMBOEMBOLISM: A PRELIMINARY REPORT ON THE PROSPECTIVE OBSERVATIONAL PROLONG II STUDY

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on behalf of the Italian Federation of Anticoagulation Clinics (FCSA)

Background. The PROLONG randomized clinical trial has shown that patients with unprovoked venous thromboembolism (VTE) who have an abnormal D-dimer (D-d) result at one month after oral anticoagulation suspension have a statistically significant higher risk for recurrent VTE as compared with those with normal D-d. Continued anticoagulation in patients with elevated D-d significantly reduced the risk of recurrent VTE. Patients with normal D-d at 1 month after anticoagulation suspension had a low risk of VTE recurrence, however it is unknown whether D-d levels change in the subsequent follow-up. These results prompted us to design the prospective observational PROLONG II study with the aim to assess the natural history of D-d in those patients who have a negative D-d after treatment suspension. Experimental design: This is a prospective multi-center (19 centres) study. After at least 4-6 months of vitamin K antagonist (VKA) therapy for a first episode of symptomatic unprovoked VTE, D-d is measured during treatment with the Clearview Simplify D-dimer assay (Agen Biomedical Limited, Brisbane, Australia; kindly provided by Instrumentation Laboratory, Milan), a qualitative, fast, whole blood method. If D-d is abnormal, treatment is prolonged for 4-6 months, while if D-d is normal anticoagulation is stopped and D-d reassessed after one month. At one month after VKA cessation, patients with abnormal D-d resume VKA for 3-6 months while patients with normal D-d do not resume VKA and D-d measurement is repeated every 2 months for a year. The primary outcome measure is the composite of recurrent venous thromboembolism and major bleeding. Results: 50 patients have been enrolled so far (M/F: 30/18; mean age: 63,2; range: 21-81) with a total follow-up of 22 pt-y. *Conclusions*. these are preliminary results and a larger number of patients is expected to be enrolled.

Inflammation and thrombosis: clinical and epidemiologic studies

C065

THROMBOXANE-DEPENDENT CD40 LIGAND RELEASE IN TYPE 2 DIABETES MELLITUS

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Objectives. Aims of the study were to characterize the platelet contribution to soluble CD40 ligand (sCD40L), to correlate its formation with the extent of oxidative stress and platelet activation and to investigate the effects of improved metabolic control and low-dose aspirin on these processes. *Background.* Inflammation, oxidative stress and platelet activation are involved in the pathogenesis of type 2 diabetes (T2DM) and its complications. CD40-CD40L interactions result in inflammatory and prothrombotic responses. *Methods.* Urinary 8-iso-prostaglandin (PG)F2α and 11-dehydro-thromboxane (TX)B2, *in vivo* markers of oxidative stress and platelet activation, respectively, plasma CD40L and C-reactive protein (CRP) were measured in 114 T2DM patients and 114 controls. A randomized, parallel group, 17-day study of aspirin (30, 100 or 325 mg/day) was performed in 18 T2DM patients. A similar study was performed in 6 healthy volunteers (aspirin 100 mg/day). Twenty poorly controlled T2DM patients were studied before and after improved metabolic control. *Results.* Compared with controls, diabetic patients showed significantly higher levels of 8-iso-PGF2α, 11-dehydro-TXB2, sCD40L and CRP. On multiple regression analysis, 11-dehydro-TXB2 and 8-iso-PGF2α excretion rates predicted sCD40L levels. sCD40L linearly correlated with 11-dehydro-TXB2 (Rho 0.67, $p<0.0001$) and both were reduced after 1-week aspirin ($p<0.0026$), with slow recovery over 10 days after aspirin withdrawal. Improved metabolic control was associated with a reduction in sCD40L, 8-iso-PGF2α and 11-dehydro-TXB2. *Conclusions.* This study provides several lines of evidence for the dependence of sCD40L release on TXA2-dependent platelet activation in T2DM and provides novel mechanistic insight into the amplification loops of persistent platelet activation in this setting.

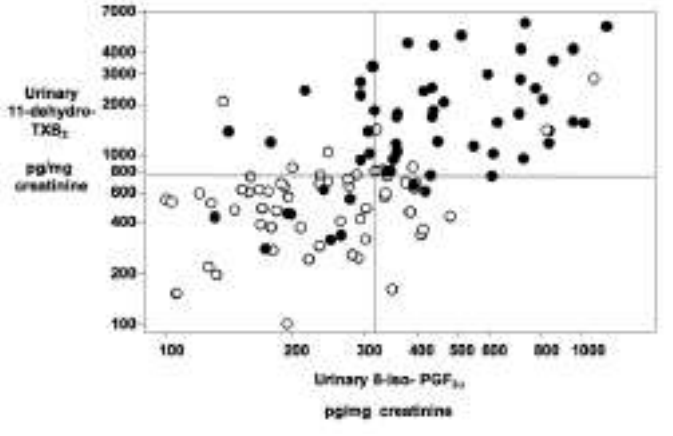

Figure 1.

C066

HCV LOCALIZES INTO CAROTID PLAQUES AND FACILITATES PLAQUE PROGRESSION

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The present study was aimed at evaluating whether HCV infection could facilitate the establishment of carotid atherosclerotic lesions in a group of high risk European patients affected by chronic ischemic heart disease consecutively admitted to our Institution. The presence of HCV RNA sequences was investigated in plaque tissues. In 31 HCV seropositive (HCV+) patients (17 m, 14 f, aged 72+8 yrs) and in 120 age and sex-matched HCV- patients the intima-media thickness (IMT) in carotid bifurcation and the prevalence and severity of plaques in internal carotid artery was studied by ultrasound. The prevalence of hypertension, diabetes, dyslipidemia, family history of premature coronary artery disease (CHD, smoking habits, markers of inflammation and main liver function tests were also studied. In 3 HCV+ patients who underwent PTCA, HCV RNA sequences were investigated by RT-PCR in plaque tissues, surrounding endothelium and serum. The prevalence of an IMT >1 mm ($p<.001$) and of prevalence (P>.005), but not severity, of internal carotid plaques was higher in HCV+ than in HCV- patients. None of the risk factors for atherosclerosis showed a higher prevalence in the HCV+ than in HCV- group. At univariate analysis HCV infection was significantly associated with >1 mm IMT ($p<.01$) and at multivariate regression analysis remained as an independent risk factor. Main liver function tests and markers of inflammation did not differ between the 2 groups. In 2 patients HCV RNA sequences were shown within carotid plaque tissues but not in the surrounding normal endothelium or in serum. In one patient HCV RNA was found in plaque tissues, in normal endothelium and in serum. The novel finding of genomic HCV RNA sequences within plaque tissues suggests a local action inside the plaque.

Our data strongly support that HCV infection does facilitate the occurrence of carotid atherosclerotic lesions in chronic CHD patients, independently of other risk factors.

C067

EXPRESSION OF TF AND ALTERNATIVE SPLICED TF MRNA ISOFORMES IN LINFOMONOCYTES AND PLATELETS OF PATIENTS WITH ACUTE CORONARY SYNDROMES

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Tissue Factor (TF) is involved in the pathogenesis of cardiovascular diseases, being responsible for the thrombogenicity of the atherosclerotic plaque. Studies on patients with acute coronary syndrome (ACS) showed that TF plasma levels, monocyte- and platelet-associated TF are higher than in stable angina (SA) patients. Recently, an alternative spliced form of TF (asTF) has been discovered, which is soluble, circulates in the blood and exhibits procoagulant activity. *Aim.* To examine TF and asTF mRNA expression in linfomonocytes and platelets of patients with ACS, SA and in control subjects. *Methods.* We studied 14 patients with ACS 10 patients with SA and 12 healthy subjects. Total RNA was extracted from peripheral linfomonocytes and from washed platelets free of leukocyte contamination and full length TF as well as asTF mRNA levels were assessed by RT-PCR and real time PCR. *Results.* TF mRNA expression in resting linfomonocytes was barely detectable in all subjects. Conversely, a consistent expression of asTF mRNA levels was observed in ACS (r.e.: 1.9 [1.4-2.5]) and in SA patients (r.e.: 1.7 [1.4-2]) compared to controls (r.e.: 1.0 [0.8-1.2]). *In vitro* lipopolysaccharide stimulation of linfomonocytes upregulated TF mRNA expression in all samples with the highest induction observed in ACS patients (TF r.e. vs unstimulated sample: 70 [64.5-75.9] in ACS, 36.6 [33.7-39.8] in SA and 6.8 [6.4-7.2] in con-

trol subjects). By contrast, the asTF induction by lipopolysaccharide was highest in control subjects, being double than that observed in ACS and SA patients. Platelet associated TF mRNA levels were significantly higher in SA patients (r.e.: 13.3 [10.4-17]) compared to ACS (r.e.: 8.2 [6.9-10.1]) and control subjects (r.e.: 1 [0.8-1.3]). No asTF mRNA was detectable in any platelet sample. *Conclusions.* These data provide evidence for the first time that different TF mRNA isoforms present in linfomonocytes and platelets of ACS patients can contribute to the hypercoagulability associated with the disease.

C068

SOLUBLE E-SELECTIN AND TISSUE FACTOR PATHWAY INHIBITOR, BUT NOT C-REACTIVE PROTEIN, CORRELATE WITH CAROTID INTIMA-MEDIA THICKNESS (C-IMT) IN STABLE CORONARY PATIENTS: THE BASELINE DATA OF THE MIAMI STUDY

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Inflammation plays an essential role in the initiation and progression of atherosclerosis.Several inflammatory markers have been shown to be correlated with cardiovascular risk. C-IMT can be used as a marker of atherosclerosis in other vascular districts. Only few data have so far addressed the relationships between inflammatory markers and progression of atherosclerosis. The MIAMI study investigates whether changes in C-IMT induced by atorvastatin treatment can be at least partially explored by concomitant changes of circulating markers of inflammation, thrombosis and endothelial dysfunction. We report here the cross-sectional relationships between the same variables measured at baseline visit. *Methods.* The MIAMI study is a prospective, open-label, multicenter clinical trial. The study enrolled 86 patients with stable coronary disease to receive moderate dosage of atorvastatin (20 mg/daily) for two years. At time of enrollment, soluble vascular cell adhesion molecules-1, soluble intercellular adhesion molecules-1, soluble E-selectin, interleukin-6, -8, -10, -18, tumor necrosis factor-alfa, high-sensitivity C-reactive protein (hs-CRP), matrix metalloproteinase-9, tissue factor, tissue factor pathway inhibitor (TFPI), soluble CD40-ligand, von Willebrand factor, fibrinogen, total cholesterol, high-density and low-density lipoprotein cholesterol, and tryglicerides were measured, in parallel with C-IMT assessment. *Results.* Univariate and multivariate analysis showed that E-selectin and TFPI were correlated with C-IMT at different sites (bifurcation-IMTmean, ICA-IMTmean, IMTmax) and with aggregate measurements (IMTmean), while hs-CRP was feebly associated with the common C-IMT mean only. *Conclusions.* The baseline results of the MIAMI study indicate that E-selectin and TFPI, but not hs-CRP, are linked with atherosclerotic burden. The longitudinal results will provide further information on the effect of statins on changes of proinflammatory/prothrombotic markers and changes of C-IMT.

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C069

ANTIBODIES TO TISSUE-TYPE PLASMINOGEN ACTIVATOR IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Background. Patients with inflammatory bowel disease (IBD) have an increased prevalence of thromboembolic events whose pathogenetic mechanisms include reduced fibrinolysis, which may be caused by antibodies to tissue-type plasminogen activator (tPA). *Objectives.* To evaluate anti-tPA antibodies in patients with inflammatory bowel disease; considerations will be made concerning clinical correlations, biochemical and functional characteristics. *Patients and methods.* We immunoenzymatically measured anti-tPA antibodies in plasma from 97 consecutive IBD patients and 97 age- and sex-matched healthy controls. Moreover we assessed the antibody interactions with different epitopes of tPA, the antibody inhibition on tPA activity and the correlations with clinical features and other serum antibodies. *Results.* IBD patients had higher median anti-tPA antibody levels (5.4 U/mL vs 4.0 U/mL; $p<0.0001$): 18 were above the 95th percentile of the controls (OR 5.3; 95% C.I. 1.7-16.3; $p<0.003$), and the six with a history of thrombosis tended to have high levels (6.9 U/mL). Anti-tPA antibody levels did not correlate with IBD type, activity, location or treatment, or with age, sex, acute phase reactants or other antibodies, and were frequently IgG1 and bound tPA in fluid phase; they recognised the catalytic domain in 10 patients and the kringle-2 domain in six. The IgG fraction from the three patients with the highest anti-tPA levels slightly reduced tPA activity *in vitro*. *Conclusions*. The prevalence of anti-tPA antibodies is high in IBD patients. By binding the catalytic or kringle-2 domains of tPA, they could lead to hypofibrinolysis and contribute to the prothrombotic state of IBD.

C070

DO HIGH SENSITIVITY C-REACTIVE PROTEIN AND CYSTATIN-C SERUM LEVELS PREDICT TOTAL MORTALITY IN HEART FAILURE PATIENTS?

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Arterial wall remodelling and atherogenesis are favoured by overexpression of elastolytic and collagenolytic cathepsins and by the reduction of their inhibitor Cystain C (Cys-C). In atherosclerotic lesions the elastolytic and collagenolytic cathepsins are over-expressed, whereas their endogenous inhibitor Cys-C resulted to be reduced. Heart failure (HF) resulted to be associated with both inflammation and Cys-C, a novel endogenous marker of glomerular filtration rate. Aim of this study was to evaluate hsC-reactive protein (hsCRP) and Cys-C levels in patients with HF and the role of Cys-C and inflammation in affecting the clinical outcome of HF. We studied 131 patients with HF (102 M/29 F), median age 74 (43-95 yrs) and 131 healthy subjects, comparable for age and sex. Cys-C and hs-CRP serum levels were assayed by nephelometric methods. After adjusting for age, sex, BMI and creatinine, Cys-C, as well as hsCRP serum concentrations were significantly higher in HF patients than in controls [Cys-C: 1.39 (1.33-1.46) mg/L vs 0.99 (0.95-1.04) mg/L, $p<0.001$; hsCRP: 5.6 (4.6-6.9) mg/L vs 2.6 (2.2-3.2) mg/L; for HF patients and controls respectively ($p<0.0001$)]. HF patients were stratified into two groups based on their serum creatinine values [group A: patients with normal creatinine values(<1.3 mg/dL for male and < 1.1 mg/dL for females); group B: patients with elevated creatinine values]. In group A, Cys-C levels, but not hsCRP levels, were significantly higher in HF patients who died with respect to survivors patients [Cys-C: 1.52 (1.13-2.055) mg/L vs 1.15 (1.08-1.25) mg/L, $p<0.05$; hsCRP: 2.26 (1.96-2.61) mg/L vs 2.29 (1.71-3.04) mg/L n.s.]. In group B, no significant difference in Cys-C and hsCRP levels between survivors and non-survivors HF patients was observed. In both group A and group B, Cys-C and hsCRP serum levels were not different in relation to re-hospitalization. In conclusion, our data suggest that elevated Cys-C values provide prognostic information for clinical outcome in HF with normal creatinine values.

C071

MARKERS OF HYPERCOAGULABILITY AND INFLAMMATION PREDICT MORTALITY IN PATIENTS WITH HEART FAILURE

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Purpose. Plasma levels of inflammatory markers are increased in chronic heart failure (HF) and are also subclinical indicators of future HF. Inflammation is strictly correlated with clotting activation, but the association between inflammation, hypercoagulability and prognosis in HF has not been previously reported. *Methods and Results.* Markers of inflammation (IL6, CRP) and hypercoagulability (DD, TAT) were prospectively assessed in 214 subjects with NYHA functional class II-IV HF. During a median follow-up of 8.5 months, 32 patients had an event: 13 died and 19 were hospitalized due to worsening of HF. Both IL6, DD and TAT levels were significantly associated with increased risk of death after adjustment for other known HF prognostic factors (age, gender, traditional cardiovascular risk factors, NYHA class, systolic left ventricular function, renal failure, haemoglobin, serum sodium) in a Cox multivariate

proportional hazard model (p=0.003,p=0.01 and p=0.02, respectively). When these markers were added simultaneously to the known prognostic factors in a new Cox multivariate model, only DD levels were significant predictors of mortality (HR (95%CI): 11 (2.7-45.1), p=0.001). The Kaplan Meier curve revealed a significantly better outcome in patients with DD below 450 ng/mL. NT-pro-BNP was the only significant predictor of rehospitalization (HR(95%CI): 5.3 (2.0-13.8), p<0.001). *Conclusion.* Hypercoagulability and inflammation, as assessed by DD, TAT and IL6 levels, are associated with an increased mortality risk in HF.

C072

ASSOCIATION BETWEEN METHYLENETETRAHYDROFOLATE REDUCTASE C677T POLYMORPHISM AND INFLAMMATORY MARKERS IN THE INCHIANTI STUDY

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Homocysteine levels (Hcy) have been shown to depend on both environmental and genetic factors, and to be associated with atherosclerosis. Prospective studies have identified many inflammatory markers as predictors of cardiovascular events. A link between Hcy levels and a pro-inflammatory state has been documented in several studies.

Aim of this study was to evaluate the association between C-reactive protein (CRP) or interleukin-6 (IL-6) levels and genetic polymorphisms both of methionine metabolism enzymes [5, 10-methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C, methionine synthase (MTR) A2756G gene polymorphisms] and of inflammatory genes [CRP 1059 G/C and IL-6 -174 G/C] in the InCHIANTI Study, a prospective population-based Italian study of risk factors for disability in late life. We studied 586 men (78% aged ≥65 years) and 784 women (81% aged ≥65 years) randomly selected from people living near Florence. In the InChianti participants, serum CRP and IL-6 were 2.38 (0.2-147.0) mg/L and 1.27 (0.0-90.0) pg/mL, respectively. After adjustment for age, sex, creatinine, folate, vitamin B6, vitamin B12 serum levels and dietary vitamin intakes, MTHFR C677T polymorphism was significantly associated with Hcy levels [CC genotype:13.7 (13.3-14.1) µmol/L; CT: 13.9 (13.45-14.1) µmol/L; TT: 16.2 (15.7-16.7) µmol/L].

A significant genotype-phenotype association (p<0.01) between CRP or IL-6 levels and C677T MTHFR polymorphisms was observed; no significant association with A1298C MTHFR or A2756G MTR polymorphisms was found. Adjusting for age, sex, BMI, smoking habit, physical activity and creatinine levels, CRP serum levels were significantly (p<0.001) higher in 677TT compared to 677CC and 677CT genotypes [CC: 2.29 (2.03-2.58) mg/L; CT: 2.24 (2.07-2.42) mg/L; TT: 2.92 (2.58-3.30) mg /L].IL-6 serum levels were significantly (p<0.001) higher in subjects carrying 677TT genotype than in subjects with 677CT and 677CC genotypes [CC: 1.19 (1.08-1.31) pg/mL; CT: 1.21 (1.13-1.29) pg/mL; TT: 1.44 (1.31-1.59) pg /mL]. No significant effect of the MTHFR A1298C and MTR A2756G polymorphisms on CRP and IL-6 serum levels was found. In the InChianti participants we did not observe a significant association between CRP 1059 G/C polymorphism, IL-6 -174 G/C polymorphism and CRP, IL-6 and Hcy levels. In conclusion, our results indicate that homocysteine-related polymorphisms may be implicated in mechanisms that modulate the inflammatory response.

Hemorrhagic diseases: clinical

C073

UN UPDATE OF THE ITALIAN AICE-GENETIC HEMOPHILIA A DATABASE

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The Italian Association of Hemophilia Centres (AICE) planned to characterize the FVIII gene mutations to elucidate molecular basis of haemophilia A in Italy and to provide insight into protein structure-function relationships. An additional aim was to obtain the mutation in an index person from each affected family to allow for the construction of a confidential national database of mutations for the optimization of genetic service. To identify mutations responsible for Hemophilia A, first the presence of an inversion of the intron-22 or intron-1 was investigated. Then, all coding regions of the FVIII gene and intron/exon boundaries were analyzed. Since now, a total of 1482 patients with Hemophilia A were enrolled. Of them, 1167 had a severe, 189 a moderate, and 126 a mild form of Hemophilia. A complete genetic characterization was obtained in 885 (76%) of patients suffering from a severe, 121 (64%) a moderate, and 99 (79%) a mild phenotype. The high mutational heterogeneity within the FVIII gene locus was confirmed, the inversion of the intron-22 accounting for about one half of gene mutations identified in severe Hemophiliacs. Other classes ranged from missense mutations (15%) to large deletions (2%). In moderate and mild phenotypes, most of the patients characterized (85%) carried missense mutations. The information about the absence/presence of allontibodies against FVIII was obtained in 750 patients suffering from a severe phenotype, in which the molecular genetic characterization was available. The large number of possible causative mutation found and the fact that a series of patients bear the same mutation offers the opportunity to investigate the existence of a genotype/phenotype correlation. Our intention is to improve, and update the database to provide useful information to elucidate molecular basis of haemophilia A and to support genetic counselling and service.

C074

THE HEMOPHILIA REGISTRY OF THE ITALIAN ASSOCIATION OF HEMOPHILIA CENTRES

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Reason for the study. To build up a National Registry of congenital coagulation diseases. The program was started in 2003 by the Italian Association of Hemophilia Centres (AICE). *Methods.* The AICE identified an expert panel to steer the registry program. A computer software to assist patient care management was developed, and all the AICE Hemophilia Centres were prompted to adopt it. Twice a year a predefined set of anonymized data was centralized and merged into a national database. Duplicated entries are managed through a confidentiality sparing mechanism. Draft of local and national reports are submitted to a validation step by all Hemophilia Centres Directors through a web-based procedure. The Registry covers sociodemographic, clinical, laboratoristic and treatment data. A subset of data is shared with the ISS. *Findings and Results.* Overall, data were collected six times by 45/51 Hemophilia Centres; 34 centres updated their patients' records up to December 2005.

The database contains 6183 records (347 duplicates), 380 of which relating to dead patients. Database growth and missing data clearance showed a constantly positive trend over time. The database collects records of the following alive patients: Hemophilia A: 1368 severe (mean age = 31.25, range 0.75-83.08), 385 moderate and 810 mild; Hemophilia B: 207 severe (mean age = 28.08 range 1.83-74.83), 128 moderate and 169 mild; vWD: 1023 type 1, 315 type 2 and 92 type 3; Factor VII deficiency187, other rare deficiencies 531. Median age at diagnosis was 2 year for severe and 16 year for mild hemophilia patients. Inhibitor patients were 213 (107 high responder, of which 34 undergoing immunotolerance treatment and 48 low responder, of which 40 transient). *Conclusions.* The AICE runs a National Registry intended to become a powerful tool for policy making and epidemiological research.

C075

SYSTEMATIC REVIEW OF RANDOMIZED CONTROLLED TRIALS OF PROPHYLACTIC CLOTTING FACTOR CONCENTRATE IN HEMOPHILIA

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Introduction. The best considered approach to managing persons with hemophilia entails the use of prophylactic treatment with clotting factor concentrates (CFC) to prevent bleeds and bleeding related complications. Objective: To conduct a systematic review/meta-analysis (SR/MA) regarding the effectiveness of clotting factor concentrate prophylaxis in the management of people with hemophilia A or B. *Methods*. We conducted a SR of all randomized controlled trials (RCTs) that studied the effect of prophylactic CFCs in people with hemophilia A or B including a control group. We searched all major electronic databases (MEDLINE, LILACS, EMBASE, and the Cochrane Cystic Fibrosis and Genetic Disorders Group's Controlled Trials Register). *Results.* We identified 29 studies, of which 4 (including 37 participants) were eligible for inclusion. Three studies evaluated hemophilia A; one showed a decrease in frequency of joint bleeds with prophylaxis compared to placebo (non-physiological dose), with a rate difference (RD) -10.80 (95% confidence interval (CI) -16.33 to -5.27) bleeds per year. The remaining 2 studies evaluating hemophilia A compared two prophylaxis regimens, one study showed no difference in joint bleed frequency, RD -5.04 (95%CI -17.02 to 6.94) bleeds per year and another failed to demonstrate an advantage of factor VIII dosing based on individual pharmacokinetic data over the standard prophylaxis regimen with RD -0.14 (95% CI -1.34 to 1.05) bleeds per year. The fourth study evaluated hemophilia B and showed fewer joint bleeds with weekly (15 IU/kg) versus bi-weekly (7.5 IU/kg) prophylaxis, RD -3.30 (95% CI -5.50 to - 1.10) bleeds per year. *Conclusion*. Basing solely on RCTs, there is insufficient evidence to determine whether prophylactic CFC decrease bleeding and bleeding-related complications in hemophilia A or B, compared to placebo, on-demand treatment, or prophylaxis based on pharmacokinetic data from individuals. The results of two well-designed unpublished RCTs are waited to strengthen the evidence about prophylaxis.

C076*

EARLY VIROLOGICAL RESPONSE AND VIRAL KINETICS IN HEMOPHILIACS WITH HIV INFECTION AND CHRONIC HEPATITIS C TREATED WITH PEGILATED INTERFERON (PEG-IFN) A-2A PLUS RIBAVIRIN

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Early virological response (EVR; decrease in HCV viremia of at least 2 Log within the twelfth week of therapy) represents the main predictor of sustained virological response (SVR) during combined therapy in patients with chronic hepatitis C. Recent studies show that the virological response obtained after the first 4 weeks of therapy is consistent with the long-term outcome. The aim of this study was to evaluate the early viral kinetics in hemophiliacs coinfected with HCV and HIV, treated with Peg-IFN α-2a plus ribavirin. Main inclusion criteria were: a stable HIV infection (HIV-RNA greater than 100000 copies/mL, CD4+ cell count of at least 200 cells/mm^3), ALT levels more than 1.5 folds the upper limit of the normal range and compensated liver disease. Treatment: Peg-IFN α-2a 180mcg subcutaneously once weekly plus oral ribavirin 1000-1200 mg/day (according to body weight) for 48 weeks. Post-treatment follow-up: 12 months. Patients who did not achieve EVR were considered non-responders and stopped treatment. Twenty-five patients were included (median age: 38 years, range 22-59). HCV genotype was 1 in 76%, 2 in 4% and 3 in 20%. At study entry the median CD4+ cell count was 580 cells/mm^3 (range: 310-1290) and HIV-RNA was undetectable (sensitivity threshold of 50 copies/mL) in 81% of cases. EVR was obtained in 15 of 17 valuable patients (88%). The kinetics of HCV-RNA reduction within the first 12 weeks of therapy was evaluated in 13 patients: it was of 2 Log at week 4 in 8 (61%) and at week 8 in 3 (23%). Our results show that combined therapy produces an early linear decline of HCV viremia in more than 60% of coinfected hemophiliacs, indicating great chances of SVR.

**PREMIO SISET 2006*

C077

EFFICACY OF RITUXIMAB TREATMENT IN POST-PARTUM ACQUIRED HEMOPHILIA

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Background. Selective B cell depletion following Rituximab treatment, has been shown to be an effective strategy for the therapy of immune disorders. *Aims.* To evaluate the efficacy of Rituximab in patients with high titer post-partum acquired inhibitor against factor VIII resistant to several therapy lines. *Patients' history and results.* A 25 years old woman, with a previous diagnosis of post-partum acquired hemophilia A, came to our Institution in December 2002 because she was not responsive to prednisone (1 mg/kg/day). The aPTT ratio was 2.59; FVIII:C <1%; inhibitor titer 621 BU/mL. The patient was subsequently treated with dexamethasone (40 mg/day for 4 days), immunoglobulins (2 g/kg/ for 4 cycles), oral cyclophosphamide (100 mg/day for two months) without any response. Intercurrent hemorrhagic events were treated with rFVIIa. In July 2004, a sudden abdominal blood shedding was diagnosed (hemorrhagic corpus luteum) and the patient was treated with rFVIIa (90 µg/kg every 3 hours) and red blood cell transfusions. In August 2004, she began therapy with Rituximab (375 mg/m^2/once a week for four doses). Inhibitor titers were as follows: pre-Rituximab, 206 BU/mL; first week after therapy 75 BU/mL; first month 50 BU/mL; third month 9.7 BU/mL; fifth month 2.1 BU/mL; seventh month 0.71 BU/mL; ninth month 0 BU/mL; twelfth month 0 BU/mL. FVIII:C values: third month 1.2%; fifth month 11%; seventh month 20%; ninth month 40%; twelfth month 46%. CD19+ B cell values: pre-Rituximab, 205×10^6/L; first week after therapy, 0; fifth month, 0; ninth month, 56×10^6/L; twelfth month, 212×10^6/L. Since the start of Rituximab, the patient experienced no hemorrhagic events. *Conclusions.* Rituximab therapy is effective in the treatment of acquired hemophilia A with high titer inhibitor resistant to other immunesuppressive therapy lines.

C078

AMINOGLYCOSIDE ANTIBIOTICS AS ALTERNATIVE THERAPY IN SEVERE COAGULATION FACTOR VII DEFICIENCY

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Factor VII (FVII) is the plasma protease triggering coagulation, and its absence is lethal. Life-threatening hemorrhagic symptoms in severe FVII deficiency are prevented by frequent administration of fresh frozen plasma or recombinant FVII. Recent studies in animal and cellular models of human diseases showed that aminoglycoside antibiotics partially suppress nonsense mutations. Nonsense mutations in clotting factors, a relatively frequent cause of severe bleeding, represent ideal models to test this strategy, because tiny increases in functional full-length protein levels in patients significantly ameliorate hemorrhagic phenotypes. We explored the aminoglycoside-mediated rescue of FVII function impaired by nonsense changes (K316X,W364X) found in infants with intracranial bleeding. A FVII-GFP chimera, resulting in a fluorescent model of FVII expression in living cells, was exploited. Appreciable fluorescence in cells transfected with nonsense FVII-GFP mutants was detected only upon

geneticin and gentamicin treatment, thus demonstrating suppression of premature termination of translation. The newly devised fluorescence approach, extendable to other disease proteins, represents a tool for screening in living cells of drugs able to restore protein synthesis. To investigate restoration of FVII function, nonsense variants of native FVII without GFP (p316X-FVII and p364X-FVII) were transfected and found to secrete low amounts of FVII (~1% of Wt-FVII activity), thus suggesting a spontaneous stop codon readthrough. Geneticin treatment of cells resulted in a significant and dose-dependent increase of secreted FVII molecules (p316X-FVII, 24±12 ng/mL, 3.6±0.8% of Wt-FVII activity; p364X-FVII, 26±10 ng/mL, 3.7±0.6%) characterized by reduced specific activity, thus indicating the synthesis of dysfunctional proteins. Similar results were observed with gentamicin, a commonly used aminoglycoside of potential interest for patient treatment. Our approach, extendable to other coagulation factors, represents an effective tool for a systematic study of effects of aminoglycosides and neighboring sequences on nonsense codon readthrough. These results provide the rationale for a mutation-specific therapeutic approach in FVII deficiency.

C079

PROSPECTIVE EVALUATION OF BIOLOGICAL RESPONSIVENESS TO DESMOPRESSIN IN PATIENTS WITH CONGENITAL DISORDERS OF PRIMARY HAEMOSTASIS

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The synthetic analogue of antidiuretic hormone desmopressin (DDAVP) has been successfully used since the 80's for prophylaxis and treatment of bleeding in most patients with type 1 von Willebrand Disease (VWD) and a variable percentage of patients with type 2 VWD and platelet abnormalities. Thus, a test dose of DDAVP (0.3 μg/Kg) is recommended in each patient for defining its therapeutic indication. Although many studies evaluated DDAVP responsiveness in this setting, only few prospective data are available. Between January 2002 and January 2006, we performed a test dose of DDAVP (using the concentrated preparation administered subcutaneously, Emosint, Kedrion) in all patients receiving diagnosis of congenital disorder of primary haemostasis at our Centre (clinical features and laboratory abnormalities including prolonged aPTT and/or bleeding time [BT], moderate reduction -->60,000/μL- of platelet count, abnormal *in vitro* platelet aggregation). After excluding patients with acquired conditions, consanguineous and patients with type 3 or 2B VWD, 52/137 patients (38%) were studied (34 women, 18 males, mean age 40±22 yrs, range 8-75). DDAVP responsiveness (at 2 hrs: reduction of BT below 12 min or >20% than baseline; FVIII:C and VWF:RCo increase at least 3-fold than baseline and at least 30% in VWD) was found in 35/52 (67%) patients, being 88% (15/17) in type 1 VWD, 57% (4/7) in type 2 VWD, 43% (9/21) in platelet function abnormalities and 29% (2/7) in isolated abnormality of BT. Due to the number of women with VWD, higher responsiveness was detected in women (76%) than in men (50%). Patients with BT below the median value (600 sec) had significantly higher responsiveness than those with BT>600 sec (25/26, 92%, vs 10/26, 39%, p=0.003), irrespective of FVIII and VWF:RCo levels. Our data show that DDAVP is an useful and well tolerated therapeutic agent especially for type 1 VWD and other mild-moderate abnormalities of primary haemostasis.

C080

TAFI IS A STRONG PREDICTOR OF SURVIVAL IN PATIENTS WITH LIVER CIRRHOSIS

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TAFI (thrombin activatable fibrinolysis inhibitor) is a potent antifibrinolytic and antinflammatory factor of liver origin and is markedly reduced in liver diseases. We evaluated the prognostic value of TAFI assay in predicting bleeding and mortality in patients with cirrhosis. Sixty-five consecutive patients (53 males, age 29-70 y) of different Child-Pugh class (11 A; 17 B; 37 C) were studied. TAFI antigen was assayed by ELISA (American Diagnostica) and plasma fibrinolytic capacity (PFC) by a turbidimetric method on samples collected at hospitalization. During 3-year follow-up 32 patients had gastrointestinal bleeding and 25 died because of terminal liver disease. No difference in TAFI level and PFC was observed between patients with and without bleeding. Moreover, neither TAFI nor PFC was associated with bleeding by logistic regression analysis. On the contrary, TAFI, but not PFC, was significantly lower in non survivors than in survivors (24.8±10.9% vs 38.1 ±10.3%, p=0.0001). In univariate Cox regression analysis TAFI level (continuous variable), but not PFC, was strongly associated with survival (b= -0.0671, p<0.0001). In a stepwise multivariate analysis that included age, sex, Child-Pugh class, albumin, bilirubin, factor VII, INR, fibrinogen and platelets, only TAFI and Child-Pugh class were identified as independent risk factors. Using a TAFI cut-off level of 37,3% (derived from ROC curve analysis) TAFI antigen assay achieved a sensitivity of 92% (95% CI: 74-99), a specificity of 55% (CI: 39-71), and a negative predictive value of 91.7%. By logrank test the risk of fatal outcome in patients with TAFI below 37.3% was 9.2 times higher (CI: 2.0-9.9) than in patients with TAFI above this cut-off level (p=0.0002). These data suggest that TAFI is a strong predictor of survival in patients with cirrhosis and may be useful to select candidates for liver transplantation.

Atherothrombosis and Cardioembolism

C081

MODERATE BUT NOT INTENSE REGULAR PHYSICAL ACTIVITY IMPROVES LIPID AND VITAMIN PROFILES AMONG A CLINICALLY HEALTHY ITALIAN POPULATION

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Introduction. Physical activity is an independent protective factor for cardiovascular diseases (CVD). The amount and frequency of physical activity to be regularly done for obtaining this protection, however, are not completely known. Some studies indicate that an intense regular physical activity, accounts for a lesser favourable health status in terms of thrombophilic risk factors. Aim of this study was to evaluate the possible influence of a different amount of regular physical activity and circulating levels of several biomarkers associated with an increased risk of CVD [(lipid parameters: total cholesterol, LDL-cholesterol, HDL-cholesterol, tryglicerides); (circulating vitamins: folic acid, vitamin B6, vitamin B12); blood glucose, homocysteine]. *Methods.* We studied 932 individuals (365 M; 567 F, with a mean age of 54 years) enrolled within a population study performed in Florence between 2002 and 2004. *Results.* Subjects were divided into 3 classes of physical activity, in relation to the amount of physical activity performed. After adjustment for age, gender, smoking habit, alcohol and total energy intake, by using a general linear model, a U-shaped curve for most of the parameters was observed. In particular, significant results were obtained for HDL-cholesterol and vitamin B6 concentrations. Actually, a moderate amount of physical activity accounted for significantly higher levels of HDL-cholesterol and vitamin B6 (62.9±1.01 mg/dL and 10.4±1.04 ug/L, respectively) respect to both absent or light (60±1.01 mg/dL; 11.5±1.04 ug/L) and intense physical activity (59.8±1.03 mg/dL; 11.4±1.09 ug/L) [(HDL-cholesterol: moderate vs. absent or light: p=0.02; intense vs. absent or light: p=0.9; moderate vs. intense: p=0.03); (vitamin B6: moderate vs. absent or light: p=0.04; intense vs. absent or light: p=0.3; moderate vs. intense: p=0.05)]. *Conclusions.* A moderate amount of physical activity is significantly associated with a more favourable biochemical profile in terms of lipid and vitamins' circulating levels, with respect to both null and intense categories.

C082

CRYPTOGENIC STROKE AND TRANSIENT CEREBRAL ISCHEMIA: RISK FACTORS FOR THROMBOPHILIA AND PARADOXICAL EMBOLISM

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Introduction. Patent foramen ovale (PFO), atrial septal defect (ASD), atrial septal aneurysm (ASA), and intra-cardiac right-to-left shunting (RLS) through a PFO (paradoxical brain embolism) are risk factors for cryptogenic stroke, especially in young adults. *Aim of the study.* To compare the prevalence of PFO, ASD, inherited and acquired thrombophilia, and endothelial dysfunction, between patients with a history of either transient ischemic attack (TIA) or cryptogenic ischemic stroke and patients with a history of venous thromboembolism (VTE). *Methods.* This study involved 69 patients (60 with a history of TIA/cryptogenic ischemic stroke, and 9 patients with a history of VTE), aged <50 years. The two groups were comparable for age, sex, BMI, and other cardiovascular risk factors. Each patient underwent bilateral carotid ultrasound Doppler study, trans-thoracic echocardiography (TTE) with ultrasound contrast (bubble study), and transoesophageal echocardiography (TEE), in order to find out left atrial spontaneous echo contrast or intracavitary thrombi, communication or ASA, ventricular septal defect, PFO, and the presence of intra-aortic atherosclerotic plaques or thrombi, and flow mediated dilatation (FMD) of the brachial artery. We also studied the prevalence of thrombophilia (FV Leiden, G20210A prothrombin mutation, protein C, protein S, antithrombin, hyperhomocysteinemia, anticardiolipin antibodies). *Results.* A RLS through a PFO was identified in 42% of the 60 patients with TIA/cryptogenic ischemic stroke, and in 13% of VTE patients (p=0.095). Thrombophilia factors did not significantly differ between the two groups. 38% of stroke patients and 20% of VTE patients showed endothelial dysfunction (FMD<10%) (p= NS).

Conclusions. Our study shows a high prevalence of RLS in patients with TIA/cryptogenic ischemic stroke, at variance with VTE patients. Factors predisposing to thrombophilia did not differ between the two groups, and their prevalence was lower than cardiac abnormalities. Interestingly, patients with VTE showed a prevalence of significant endothelial dysfunction comparable to that in patients with TIA/stroke.

C083

PREVALENCE OF INTERLEUKIN-1 β -511C/T POLYMORPHISM IN NON VALVULAR ATRIAL FIBRILLATION

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Purpose. Nonvalvular atrial fibrillation (NVAF) is the most common arrhythmia in clinical practice and is associated with a high risk in total and cardiovascular mortality as well as cardiovascular morbidity including congestive heart failure and stroke. There is a growing body of evidences that inflammation may be associated with NVAF and may be involved in the pathogenesis of NVAF. Aim of this study is to evaluate in a case-control study, the association between the interleukin-1β polymorphism -511C/T (IL-1β -511 C/T) and the presence of atrial fibrillation. *Methods.* 456 NVAF patients and 912 matched controls were genotyped by an electronic microchip technology for IL-1β -511 C/T polymorphism. *Results.* The genotype distribution of IL-1β -511 C/T polymorphism significantly differed between NVAF patients and controls [CC 43.9%; CT: 47.8%, TT: 8.3% vs CC 35.4%; CT: 51.6%, TT: 12.9% for NVAF patients and controls respectively, p<0.01]. The prevalence of CT and TT genotype was significantly (p<0.05)lower in NVAF patients (56.1%) than in control subjects (64.6%). In normotensive subjects no significant difference in genotype distributions of inflammatory gene polymorphisms between patients and controls was observed. In subjects in whom hypertension was documented, a significant difference in genotype distributions between NVAF patients (n=265) and controls (n=379) was found. In a dominant model of inheritance, the presence of IL-1β CT or TT genotypes, conferred a significant protection against NVAF at both univariate (IL-1β CT+TT vs CC genotype: OR: 0.70, 95% 0.56-0.88; p<0.01) and multivariate (OR: 0.73, 95% CI 0.56-0.94, p=0.016) analyses. In normotensive subjects the presence of IL-1β CT or TT genotypes was not independently associated with NVAF at both univariate (OR=0.89, 95% CI 0.63-1.25, p=0.49) and multivariate analysis (IL-1β polymorphism: OR=0.95, 95% CI 0.63-1.45, p=0.82). In the subgroup of hypertensive subjects, a significant association between IL-1β polymorphisms and NVAF was observed at the univariate analysis (OR=0.61, 95% CI 0.44-0.83, p=0.002). In the multivariate analysis adjusted for sex, age, traditional cardiovascular risk factors, IL-1 β polymorphism remained a significant and independent protective factor for NVAF (OR: 0.69, 95% CI 0.48-0.99, p=0.048). *Conclusions.* Our results, obtained in a large number of NVAF and controls, and after adjustment for all potential confounders, show that the presence of the IL-1β -511 C/T polymorphisms has an independent protective effect on NVAF. Furthermore, this association was found among hypertensive subjects, but not in normotensive subjects, so suggesting that the protective effect of the inflammatory alleles may be evident particularly in a condition, in which tissue injury or endothelial impaired function are present.

C084

HIGH C-REACTIVE PROTEIN (CRP) SERUM LEVELS ARE NOT ASSOCIATED WITH CRP 1059 G/C AND INTERLEUKIN-6 (IL-6) -174 G/C POLYMORPHISMS IN ATRIAL FIBRILLATION PATIENTS

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Purpose. Nonvalvular atrial fibrillation (NVAF) is the most common arrhythmia. Recently, the involvement of inflammatory processes in NVAF has been demonstrated. *Methods.* we investigated the prevalence of C-Reactive Protein (CRP) 1059G/C and Interleukin 6 (IL-6) -174G/C polymorphisms and high sensitivity (hsCRP) levels in 456 patients with

NVAF and 912 controls. *Results.* CRP GG or GC as well as IL-6 GG or GC genotypes did not conferred a significant protection against NVAF in a multivariate analysis adjusted for age, sex, traditional cardiovascular risk factors and hsCRP levels. After adjustment for age, sex, smoking habit and BMI, hsCRP levels were significantly higher in NVAF patients [3.42 (2.84-4.11) mg/L] than in controls [2.44 (2.26-2.62) mg/L; $p<0.01$]. hsCRP levels were lower in controls carrying CRP 1059 GC and 1059 CC genotypes [2.04 (1.67-2.48) mg/L] than in controls with 1059 GG genotype [2.51 (2.32-2.72) mg/L; $p=0.056$]. This genotype-phenotype association was not found in NVAF patients. No significant genotype-phenotype association between IL-6 -174 G/C polymorphism and hsCRP levels was observed in both NVAF patients and controls. Raised hsCRP levels were significantly associated with NVAF at both the univariate (OR=1.84 95% CI, 1.12-2.39, $p=0.001$) and multivariate analysis after adjustment for potential confounding variables and inflammatory gene polymorphisms (OR=2.5 95%CI 1.1-5.9, $p=0.033$). *Conclusions.* our results demonstrate, in NVAF patients, the lack of association between the CRP 1059 G/C, IL-6 -174 G/C polymorphisms and AF, confirm the role of elevated hsCRP levels in NVAF and extend the relationship between 1059 CC CRP genotype and hsCRP levels in elderly controls.

C085

METALLOPROTEINASE AND INFLAMMATORY MARKER CIRCULATING LEVELS IN HEART FAILURE PATIENTS

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Previous studies on experimental models of heart failure (HF) and on patients suffering from HF have suggested that the persistent inflammatory activity may play a role in ventricular remodeling. Metalloproteinases (MMPs) are an endogenous family of zinc-dependent enzymes that are responsible for matrix remodeling in several diseases, by promoting changes in fibrillar collagen structure and loss of cardiac contractility via cell proteolysis. Recently, it has been reported high circulating levels of MMP-9 in HF and in acute coronary syndromes. Aims of the present study were: 1- to investigate the role of MMP-3 and MMP-9 and inflammatory markers (C-Reative protein and Interleukin-6) circulating levels in patients with HF; 2-to evaluate the relationship between metalloproteinases and inflammatory markers. We studied 79 patients with a diagnosis of HF (63 M/16 F): 74 (43-95 yrs) and 79 healthy subjects as controls, comparable for age and sex (63M/16 F; 74 (40-83 yrs). Twenty-seven patients were in class NYHA II, 30 in class NYHA III and 22 in class NYHA IV. In all HF patients and control subjects, MMP-3, MMP-9 (antigen and activity) and interleukin-6 (IL-6) levels were assayed by immunoenzymatic assay and C-Reactive Protein (CRP) serum levels by a nephelometric high sensitivity method. MMP-3 antigen and activity circulating levels were significantly ($p<0.05$) higher in HF patients than in control subjects [antigen: 56.0 (1.0-1192.0) ng/mL vs 35.0 (0.1-65.4) ng/ml; activity: 28.5 (1.0-375.0) ng/mL vs 2.3 (0.1-25.2) ng/mL]. MMP-3 antigen and activity levels did not significantly differ in relation to NYHA class as well as to the etiology of HF (hypertensive or dilatative) or to the systolic function of left ventricular or to diastolic dysfunction. MMP-9 antigen and activity circulating levels significantly ($p<0.01$) differed between HF patients and control subjects [antigen, HF: 111.9 (17.3-665.0) ng/ml control: 2.0 (0.1-15.2) ng/mL; activity, HF: 728.0 (1.0-3264.0) ng/ml, controls: 13.3 (0.1-45.2) ng/mL, respectively]. MMP-9 antigen and activity levels did not significantly differ in relation to NYHA class as well as to the etiology of HF (hypertensive or dilatative)or to the systolic function of left ventricular. MMP-9 antigen and activity levels were significantly ($p<0.05$) higher in HF patients with diastolic dysfunction (diagnosed on an altered ecocardiographic E/A ratio) [antigen: 163.9 (21.1-665.0) ng/mL vs 87.8 (17.3-665.0) ng/mL; activity, antigen: 1024.0 (64.3-2240.0) ng/mL vs 591.8 (1.0-3264.0) ng/mL, respectively]. CRP and IL-6 levels were significantly higher in HF patients than in controls [CRP: 10 (0.7-21.0 mg/L vs 1.9 (0.8 -5.4) mg/L, $p<0.01$; IL-6: 8.9 (0.9-51.5) pg/ml vs 4.6 (0.5-12.0) pg/mL]. We also demonstrated a significant correlation between inflammatory markers and MMPs levels[CRP-MMP-3 antigen: r=0.44, $p<0.005$; IL-6-MMP-3 antigen: r=0.33 $p<0.005$; CRP-MMP-9 activity: r=0.31, p<0.001; IL-6-MMP-9 activity, r=0.35, $p<0.05$]. These results demonstrated the association between high levels of MMPs, inflammatory markers and HF, suggesting that MMPs may be a marker for cardiac extracellular matrix degradation, a process involved in LV remodelling. Future studies looking at risk factors for HF need to consider inflammation and MMPs as a potential risk factors in their analysis.

C086

FACTORS ASSOCIATED WITH THE SHORT TERM APPEARANCE OF NEW CAROTID ARTERY PLAQUES IN A PREDOMINANTLY ELDERLY POPULATION-BASED COHORT

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Background. Most epidemiological studies that have dealt with cardiovascular diseases have focused on events. Aim of this study was to investigate incident sub-clinical carotid atherosclerosis in elderly people. *Subjects and Methods.* 486 InCHIANTI Study participants free from carotid artery plaques at baseline, underwent a new carotid artery ultrasound examination three years later. We tested the possible association between the appearance of new carotid artery plaques and baseline traditional cardiovascular risk factors, nutritional profile, homocysteine along with related enzymes and vitamins, plasma antioxidants and inflammatory markers. *Results.* In the whole sample three-years incidence of carotid artery plaques was 40.1% (38.5% in men and 41.3% in women). When compared with participants aged less than 65 years, the relative risk of developing new carotid artery plaques showed a linear increase from 5.48 (95%CI 5.02-5.94) for participants aged 65-69 years, up to 9.57 (95%CI 9.12-10.02) for participants aged 80 years or more. Subjects with family medical history of atherosclerosis showed a relative risk of 1.57 (95%CI 1.35-1.79) and subjects with heavy smoke history (pack-year index >20) showed a relative risk of 1.70 (95%CI 1.48-1.93) when compared with subjects with no or light smoke history. Participants with β2-macroglobulin plasma levels in the third tertile (>220 mg/dL) showed a relative risk of 1.40 (95%CI 1.13-1-66) when compared with participants with β2-macroglobulin plasma levels in the first tertile (≤172mg/dL). Finally, subjects with high (>3 µg/mL) or moderate (>=1>=3 µg/mL) plasma levels of CRP showed a relative risk of 2.23 (95%CI 1.89-2.57) and 1.91 (95%CI 1.56-2.26), respectively, when compared with subjects with low (<1 µg/mL) plasma levels of CRP. *Conclusions.* In a predominantly elderly population-based cohort three-years incidence of carotid artery plaques was significantly and independently associated with increased plasma levels of β2-macroglobulin and CRP, and with non-modifiable traditional risk factors, except for gender.

C087

FACTOR XIIIA V34L AND FACTOR XIIIB H95R GENE POLYMORPHISMS: EFFECTS ON THE RISK OF MYOCARDIAL INFARCTION AND ON SURVIVAL

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Background. Factor XIII (FXIII) plays a key role in the thrombus formation being responsible for its resistance. FXIII gene variants affect fibrin structures and have a role in atherothrombosis. *Objective.* To evaluate the effects of two common FXIII gene variants on myocardial infarction (MI) risk and survival. *Methods and Results.* FXIIIA V34L and FXIIIB H95R were evaluated in 406 MI and in 406 controls. Homozygous-LL34 was underrepresented in cases (4.4% vs 7.9%; $p=0.029$) whereas heterozygous-HR95 did in controls (18.5% vs 13.8%; $p=0.09$). STEMI enrolled were 312 (76.9%) and those eligible for primary percutaneous coronary intervention (PCI) were 270 (86.5%). Additional 87 cases underwent secondary PCI. The combined endpoint was the occurrence of death or reinfarction at 1-year. Kaplan-Meier analysis yielded an overall rate for adverse events of 15.3% with lower incidence in the L34-carriers (19.6% vs 8.3%; $p=0.003$), indistinguishable from that of the 312 STEMI (14.7%) being (18.9% and 7.7%; VV34- and L34-carriers respectively; $p=0.01$). Primary PCI-group had the lowest incidence (11.5%) of adverse events (15.4% and 4.9%; VV34- and L34-carriers respectively; $p=0.01$). Significance was conserved in the overall PCI group (18.0% and 6.2%; VV34- and L34-carriers respectively; $p=0.009$). Cases carrying both FXIII vari-

ants had improved survival (log-rank, p=0.01). Minor bleeding complications were found increased in Leu34-carriers (p=0.04) whereas major bleeding did not. Finally, we failed to ascribe to L34-carriers taking aspirin the hypothesized lower risk for MI compared to L34-carriers not-taking aspirin although adverse events in the former were cut by half (7.7% vs 14.3%, respectively; p=NS). *Conclusions.* L34-allele reduces MI risk and improves survival in all the groups analyzed. Coexistence of both FXIII gene variants improves survival in the whole cohort of cases. Pharmacogenetic of FXIII might be of value in the management of cardiovascular patients to select cases at higher risk for future new events.

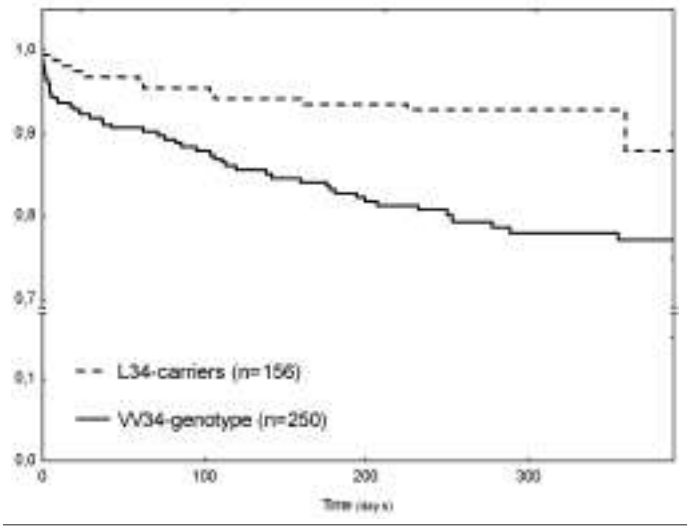

Figure 1.

C088

CARDIOVASCULAR RISK FACTORS AND GLOBAL RISK OF FATAL CARDIOVASCULAR DISEASE ARE POSITIVELY CORRELATED BETWEEN PARTNERS OF 1,009 SPOUSE PAIRS FROM DIFFERENT EUROPEAN COUNTRIES: REPORT FROM THE IMMIDIET PROJECT

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Introduction. Shared environmental factors may put cohabiting partners at similar risk for cardiovascular disease (CVD). We aimed at investigating the within-couple concordance of global risk of CVD. *Methods*. In the framework of the IMMIDIET Project, male-female cohabitant couples, randomly recruited from general practice were studied. 2018 apparently healthy subjects aged 25-74 years from 4 populations were enrolled (Italian, English, Belgian and mixed Belgian/Italian couples). Multivariate within-couple correlations were analysed in the context of the Actor-Partner Interdependence Model. Individual global risk of fatal CVD was calculated using the ten-year risk prediction equations provided by the SCORE project (variables used were: age, sex, systolic blood pressure, total cholesterol, smoking). *Results.* Risk scores of CVD were positively correlated within-couples (r=0.81, r=0.81, r=0.83, for total, coronary and non-coronary risk, respectively, p<0.0001 adjusted for countries. Because of similarity in age between members of couples (r=0.86, p<0.0001), the degree of correlation, although still significant, strongly decreased when further adjusted for age (r=0.19, r=0.21, r=0.25, p<0.0001 for total, coronary and non-coronary risk, respectively). All the variables used in the SCORE equations were positively correlated within-couples (r=0.14, r=0.10, r=0.26, p<0.0001 adjusted for age and country, for systolic blood pressure, total cholesterol and smoking, respectively). *Discussion.* Members of a couple were significantly more likely to have a higher (lower) risk of fatal CVD if their partner belonged to a high (low) risk category, independently of age and country. These findings suggest that being a cohabiting couple is a major factor responsible for the shared cardiovascular risk profile: we should expect that modifying the risk in a member, the risk in the partner should also be modified in the same direction. These concepts can have important public health consequences in targeting screening and addressing prevention campaigns or counselling for cardiovascular disease.

Cell biology of hemostasis

C089

LONG-TERM TREATMENT WITH HIGH-DOSE RECOMBINANT HUMAN ANTITHROMBIN DOES NOT PREVENT ACUTE HUMORAL REJECTION IN PRIMATE RECIPIENTS OF PORCINE XENOGRAFTS

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Background. Fibrin deposition is central to the acute humoral rejection process occurring in the presence of consumptive coagulopathy when pig organs are transplanted into primates. Aim of the study. To assess whether strategies aimed at preventing fibrin formation may extend xenograft survival, we administered high daily doses of recombinant human anti-thrombin (rhAT) to obtain both anticoagulant and anti-inflammatory effects in immunosuppressed primate recipients of porcine kidneys. Method: Six nephrectomized cynomolgus monkeys received a hDAF pig kidney (Novartis) and were treated with GAS914 (Novartis), cyclosporine A, cyclophosphamide, mycophenolate sodium and steroids. Three animals received rhAT (GTC Biotherapeutics, Inc.)(Group A). rhAT was administered at a dose of 500 U/kg twice daily in 2 cases, or at the same dose for the first week and then on any deterioration of graft function in the third case (on-demand administration). The other 3 animals were used as controls (Group B). Results: Some degree of consumptive coagulopathy developed in both rhAT-treated (n=3) and untreated (n=3) primates. No major differences in the coagulation parameters analyzed were observed between the 2 groups. Similarly, no difference in survival was seen between rhAT-treated (20.6±4 days; range: 15-23 days) and untreated animals (17.3±11.6 days; range: 7-30 days), although the rhAT-treated primates had a higher bleeding tendency. Despite the high daily dose of rhAT, considerable fibrin deposition was observed in the graft as early as 2 weeks after transplantation.Conclusions: These results suggest that a high daily dose of rhAT fails to influence survival or prevent fibrin formation and deposition in the graft in our pig-to-primate model. However, the potential role of rhAT administered in combination with heparins or other clotting inhibitor concentrates in this model remains to be determined.

C090

LEPTIN INDUCTION OF HUMAN MONONUCLEAR CELL TISSUE FACTOR

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Obesity is a major modifiable risk factor for cardiovascular disease. Leptin, the hormone synthesized and released primarily by adipose tissue and found increased in obese individuals, has been implicated in the regulation of inflammation and arterial and venous thrombosis. Since expression of tissue factor (TF), the main trigger of coagulation *in vivo*, is abundant in the adipose tissue, we investigated whether leptin could modulate monocyte TF expression. To test this hypothesis, mononuclear leukocytes from healthy volunteers were incubated for 6 h with or without leptin at 37°C. At the end of incubation, cells were disrupted and tested for procoagulant activity and antigen levels by a one-stage clotting assay and by ELISA, respectively. Our results demonstrate that leptin induced TF activity and antigen in a dose-dependent fashion. Leptin exerted its effect at the transcription level, since reverse transcriptase PCR indicated development of TF mRNA in monocytes. The mechanism by which leptin induced TF synthesis resides in an increased migration of the transacting factor c-Rel/p65 into the nucleus, as determined by electro-mobility shift assay. Preincubation with mitogen-activated protein kinase (MAPK) inhibitors indicated the involvement of a p38 MAPK signalling, not of the ERK1/2 pathway, which is known to be implicated in endotoxin-mediated TF expression. In a selected sample of obese patients, loss of body weight by dietary modification led to decreased circulating leptin levels, accompanied by a reduction in plasma TF as well as in TF expression in resting and endotoxin-stimulated mononuclear cells. Thus, we have reported here the original observation that leptin can induce TF expression by human mononuclear cells, suggesting an additional role for this hormone in the cardiovascular risk associated with obesity.

C091

TISSUE FACTOR INDUCTION BY PROTEASE-ACTIVATED RECEPTOR-1 REQUIRES INTACT CAVEOLIN-ENRICHED MEMBRANE MICRODOMAINS IN HUMAN ENDOTHELIAL CELLS

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Protease-activated receptors (PARs) comprise a family of G-protein coupled receptors with a unique proteolytic activation mechanism. PARs have been liked to the regulation of a broad range of cellular function and play an important role in pathogenesis of disorders characterized by chronic inflammation or activation of the coagulation cascade. Signaling through PAR1 and PAR2 shifts the endothelium toward a prothrombotic phenotype, exacerbating the initiating pathophysiological condition. However, there are still considerable gaps in our knowledge of the mechanisms involved in the regulation of tissue factor induced by PARs in endothelial cells. Here we show that PAR1, but not PAR2, is present in endothelial caveolin-enriched membrane microdomains, where is associated to caveolin-1, and that the integrity of these structures is important for accurate PAR1-induced signaling leading to TF induction. Cholesterol depletion of HUVEC by cholesterol sequestring agents induced the relocalization of PAR1 to high-density membranes, and the impairment of TF induction, without affecting PAR2-mediated procoagulant effect. In addition, the caveolin scaffolding domain inhibits TF activity induced by PAR1. In conclusion The inadequate localization of the PAR1 and its signaling machinery outside of caveolin-enriched membrane microdomain can prevent proper continuation of the signal leading to TF induction in endothelial cells.

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C092*

INCREASED VASCULAR WALL ENDOTHELIAL NITRIC OXIDE SYNTHASE (ENOS) LEVELS IN UMBILICAL CORDS FROM GESTATIONAL DIABETIC WOMEN

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Objectives. Nitric Oxide (NO) is a key regulator of endothelial function. Hyperglycemia causes vascular damage, but its effects on NO synthesis and bioavailability are still debated. *Methods.* We obtained umbilical cords from 10 women with gestational diabetes (GD) and from 10 control women (C) and we measured: 1) eNOS protein levels (immunohistochemistry) in umbilical cord tissue 2) basal eNOS gene expression (Real-Time PCR) and protein level (Western Blot), NO production (conversion of [3H]-L-arginine in [3H]-L-citrulline) and nitrotyrosine levels (ELISA) in umbilical vein endothelial cells (HUVEC) cultured from GD and C cords and 3) eNOS gene expression and protein level and NO production in C-HUVEC acutely exposed (24 hours) to 25 mM glucose (HG). *Results*. eNOS protein levels two fold increased in umbilical cords from GD. Consistently, basal eNOS mRNA, protein content and activity were significantly greater in cultured GD-HUVEC (eNOS/GAPDH mRNA= 1.0±0.1 vs 0.6±0.05; eNOS protein = 0.64±0.09 vs 0.37±0.01 AU; NOS activity= 0.20±0.02 vs 0.12±0.03 pmol/mg prot.-1/min-1 in GD- vs C-HUVEC, all $p<0.05$). Nitrotyrosine levels were also 3-fold increased in GD vs C. Exposure of C-HUVEC to HG induced a significant increase in eNOS mRNA, protein and activity (eNOS/GAPDH mRNA= 0.8± 0.05 vs 3.0±0.4; eNOS protein= 0.33±0.04 vs 0.74±0.06 AU; NOS activity= 0.10± 0.03 vs 0.26±0.04 pmol/mg prot.-1/min-1, in C vs HG exposed cells, all $p<0.01$). *Conclusions.* Chronic hyperglycemia may upregulate vascular NO generation *in vivo* in humans. However, NO is likely to react with O2-, thus reducing NO availability and generating potentially dangerous peroxinitrates.

**PREMIO SISET 2006*

C093*

PRO-INFLAMMATORY EFFECTS OF ERYTHROCYTES FROM UREMIC PATIENTS ON CULTURED HUMAN ENDOTHELIAL CELLS: INSIGHT INTO SIGNALING PATHWAYS.

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Background. In End-Stage Renal Disease (ESRD) endothelium may represent a key target for the action of circulating elements, such as modified erythrocytes (RBC) and/or plasmatic factors, that may facilitate inflammation and the vasculopathy associated with uremia. We have previously demonstrated that phosphatidylserine (PS) exposure on the RBC surface from ESRD patients increases RBC-human umbilical vein endothelial cell (HUVEC) interactions and causes decreased Nitric Oxide (NO) production. We postulated that, besides the pro-inflammatory effects due to decreased NO bio-availability, enhanced ESRD-RBC-HUVEC interactions might directly stimulate pro-inflammatory pathways leading to increased vascular adhesion molecule expression. *Methods and Results.* ESRD-RBC-endothelial cell interactions induced a time-dependent up-regulation of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) (measured by Western Blot [WB] and Real-Time PCR), associated with mitogen-activated protein kinase (MAPK) activation and impairment of the Akt/endothelial Nitric Oxide Synthase (eNOS) signaling cascade, measured by WB. In reconstitution experiments, normal RBC incubated with uremic plasma showed increased PS exposure and caused significantly increased VCAM-1 and ICAM-1 mRNA levels when incubated on HUVEC. Interestingly, ESRD-RBC-increased expression of adhesion molecules was prevented by Annexin-V (AnV, able to mask PS on RBC surface), anti-integrin-alfavβ3, anti-Thrombospondin-1 (TSP-1), and PD98059 (a selective inhibitor of MAPK phosphorylation). Moreover, AnV significantly reversed the ESRD-RBC effects on MAPK and Akt/eNOS signaling pathways. *Conclusions.* Our data demonstrate that, possibly via a direct interaction with the endothelial thrombospondin-(alfavβ3)integrin complex, ESRD-RBC-HUVEC adhesion induces a vascular inflammatory phenotype. Thus, intervention targeting ESRD-RBC increased adhesion to endothelium and/or MAPK and Akt/eNOS pathways may have the potential to prevent vascular lesions under uremic conditions.

**PREMIO SISET 2006*

C094

AKT/B-CATENIN PATHWAYS INDUCED BY TOBACCO SMOKE/INTERLEUKIN-1β-COOPERATION REGULATE CYCLOOXYGENASE-2 EXPRESSION *IN VITRO* AND *IN VIVO*

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Cigarette smoking is a major risk factor for atherosclerosis and predisposes to acute cardiovascular events. Exposure to tobacco smoke (TS) increases inflammation and thrombosis. Cyclooxygenase-2 (COX-2), a key enzyme in prostaglandin biosynthesis and β-catenin, a bifunctional protein with dual roles in cell adhesion and transcriptional activation, are both increased in carotid plaques. In this study, we explored whether TS can cooperate with a smoke-increased cytokine, interleukin-1β (IL-1β), to upregulate COX-2 expression *in vitro*, in mouse cardiac microvascular endothelial cells (MMCEC), and *in vivo*, in cardiovascular tissue from atherosclerosis-prone ApoE-/- mice. We derived immortalized MMCEC of normal EC phenotype by lentiviral vector transfection of primary EC with genes for telomerase and SV40T antigen. In MMCEC both dilute saline extracts of TS and IL-1β induced COX-2 protein in a dose- and time-dependent manner, increased phosphorylation of Akt and glycogen synthase kinase-3β (GSK-3β), which in turn decreased β-catenin phosphorylation, preventing proteosomal degradation of β-catenin and permitting β-catenin translocation to the nucleus. Low TS concentrations strongly potentiated the IL-1β response and altered the kinetics. Co-treatment with TS/IL-1β accelerated and strongly augmented Akt/GSK-3β/β-catenin pathway activation, markedly increased endothelial permeability, and disrupted VE-cadherin/β-catenin membrane complexes within minutes. Treatment with LiCl, which inhibits GSK-3β activity and thus abrogates β-catenin degradation, induced nuclear β-catenin translocation and COX-2 expression. Conversely, phosphatidylinositol 3-kinase (PI3-K) inhibitors inhibited TS/IL-1β-induced Akt and GSK phosphorylation, β-catenin translocation and COX-2 expression. Inhibition of β-catenin expression by short interfering RNA (siRNA) strongly inhibited TS/IL-1β-mediated induction of COX-2 and decreased prostaglandin production. We confirmed these *in vitro* results in hearts from ApoE-/- mice that were exposed to cigarette smoke daily during a two weeks period. Our data provide demonstrate that cigarette smoke-mediated COX-2 expression was regulated by PI3K/Akt/GSK-3βb/β-catenin pathway activation. These findings suggest a new mechanism by which tobacco smoke can modulate proinflammatory and proatherosclerotic genes.

C095

EFFECT OF REHABILITATION PROGRAM ON CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND INFLAMMATION IN PATIENTS UNDERGOING CARDIAC SURGERY

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Introduction. Endothelial progenitor cells (EPCs) are bone marrow-derived progenitor cells which are involved in supporting vascular endothelium, so playing a crucial role in the beginning and progression of atherosclerosis. Many regulators of EPCs are currently known; in fact, it was previously demonstrated that potent triggers for EPCs mobilization are statin therapy, erythropoietin, vascular endothelium growth factor, tissue ischemia and regular physical exercise. No data are available regarding the effect of a rehabilitation program after cardiac surgery on EPCs in patients who underwent cardiac surgery. *Aim.* We performed this study in order to assess the variations of EPCs in relation to inflammatory markers in patients who performed a 15 day-rehabilitation program after cardiac surgery [11 CABG=group A and 11 valve replacement=group B]. *Patients and methods.* In 22 patients [13 M/9 F; 72 (57-88) yrs] the numbers of EPCs and the serum levels of IL-6, IL-8 and high sensitivity C-reactive protein (hsCRP) were determined pre-surgery (T1), at the beginning (T2) and at the end (T3) of the rehabilitation program. Peripheral blood EPCs were measured by using flow cytometric and were defined as CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+. IL-6 and IL-8 were determined using commercial ELISA and CRP by nephelometric method. *Results.* With regards to EPCs, we observed a decrease at T2 in both groups. At T3, EPCs increased reaching values higher than T1 in group A, whereas in group B, even if increased with respect to T2, they remained in all patients lower than T1. As far as inflammation, CRP and IL6 levels increased at T2 and then significantly decreased at T3 without reaching T1 values; IL8 levels increased at T2 and remained at similar levels at T3, both in group A and B (Table).

Table.

	Group A (n=11)	*Group B (n=11)*
CD34+KDR+T1 (%)	0.0035 (0.0015-0.0095)	0.0040 (0.0005-0.0170)
CD34+KDR+T2 (%)	0.0025 (0-0.0160)	0.0025 (0.0005-0.0095)
CD34+KDR+T3 (%)	0.0045 (0-0.0105)	0.0025 (0-0.0160)
CD133+KDR+T1 (%)	0.0030 (0.0010-0.0055)	0.0030 (0.0005-0.0160)
CD133+KDR+T2 (%)	0.0020 (0-0.0140)	0.0015 (0-0.0095)
CD133+KDR+T3 (%)	0.0035 (0.0005-0.0075)	0.0020 (0.0005-0.0135)
CD34+CD133+KDR+ T1(%)	0.0025 (0.0010-0.0050)	0.0025 (0.0005-0.0165)
CD34+CD133+KDR+ T2(%)	0.0020 (0-0.0240)	0.0015 (0-0.0095)
CD34+CD133+KDR+ T3(%)	0.0035 (0-0.0065)	0.0020 (0-0.0125)
CRP T1 (mg/L)	8.6(3-9.1)	7(1.10-214)
CRP T2 (mg/L)	44(6.6-110)	72(8.6-187)
CRP T3 (mg/L)	16(2.2-67)	22.5(5.5-54)
IL6 T1 (pg/mL)	4.1(1.8-19.8)	3.8(0.9-22.2)
IL6 T2 (pg/mL)	8.2(2.4-41.2)	16.8(7.1-98.4)
IL6 T3 (pg/mL)	5.4(2.0-21.1)	7.8(5-28.1)
IL8 T1 (pg/mL)	11.8(6-22.6)	10.2(6.5-18.1)
IL8 T2 (pg/mL)	15.9(8.4-29.4)	14.5(7.6-37.2)
IL8 T3 (pg/mL)	13(7.9-19.2)	15.0(8.8-24.4)

A significant correlation was detected between IL8 levels and EPCs both at T1 [CD34+KDR+ and IL8: r=0.38, $p<0.05$; CD133+KDR+ and IL8: r=0.43, $p<0.05$] and T2 [CD34+KDR+ and IL8: r=0.60, $p<0.005$; CD133+KDR+ and IL8: r=0.50, $p<0.001$]. *Conclusions*. A 15-day rehabilitation program is associated with an increase in EPCs, a decrease in inflammation markers such as IL6 and CRP and persistent elevated levels of a cytochemokine with angiogenetic potential such as IL8. These results suggest a mechanism of physical exercise in determining revascularization process after cardiac surgery.

C096

CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND INFLAMMATION IN PATIENTS BEFORE AND AFTER CARDIAC SURGERY

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Endothelial progenitor cells (EPCs) are bone marrow-derived progenitor cells that are supposed to support the integrity of vascular endothelium. Vascular trauma and inflammation produce pro-angiogenic factors that are able to attract circulating EPCs. A single previous study demonstrated an early increase of EPCs after coronary artery bypass grafting associated with a concomitant proinflammatory state. We performed this study in order to assess the variations of EPCs in relation to inflammatory markers in patients with coronary artery disease undergoing coronary artery bypass grafting (group A) as compared with patients with valve disease (group B) undergoing valve replacement. In 30 CABG [23 M/7 F; 71 (27-88) yrs] and in 45 valvular [22 M/23 F; 71 (54-85) yrs] patients the numbers of EPCs and the serum levels of IL-6, IL-8 and high sensitivity C-reactive protein (hsCRP) were determined pre (T1) and post (3-5 days) (T2) cardiac surgery. Peripheral blood EPCs were measured by flow cytometric analysis and were defined as CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+. IL-6 and IL-8 were determined using commercial ELISA assays and CRP by a nephelometric method. EPCs did not significantly differ between group A and B at T1 and at T2. With respect to cardiovascular risk factors, hypertension and gender significantly affected EPCs in group A and diabetes in group B. At T2, EPCs significantly ($p<0.01$) decreased both in group A and B [CD34+KDR+: 0.0022 (0-0.0080)% vs 0.0020 (0-0.0140)%; CD133+KDR+: 0.0018 (0-0.0060)% vs 0.0015 (0-0.0050)%; CD34+CD133+KDR+: 0.0015 (0-0.0060)% vs 0.0010 (0-0.0050)%]. A significant ($p<0.01$) increase of IL6, IL8 and hsCRP was observed between T1 and T2 both in group A [IL6= 4.9(0.4-22.2)pg/mL vs 46(5-118.5)pg/mL; IL8=10(4.7-19.3) pg/mL vs 16.9(6.7-39.7) pg/mL; hsCRp=4.95(0.2-214) mg/L vs 128.5(26-350) mg/L] and B [IL6= 5.7(0.1-62.7)pg/mL vs 26.8(3.7-137.8)pg/mL; IL8=11.2(6.0-22.6)pg/mL vs 20.1(10.8-44.8)pg/mL; hsCRp=9.05(1.3-75) mg/L vs 122.5(36.3-405) mg/L]. Our results demonstrate that EPCs are significantly affected by cardiovascular risk factors and that cardiac surgery per se, independently of the etiology of the disease, is able to determine a significant reduction in EPCs number with the contemporary increase of inflammatory markers.

Platelets: biochemistry, physiology and methods

C097*

CHARACTERIZATION OF PROTEINS RELEASED FROM HUMAN PLATELETS FOLLOWING THROMBIN ACTIVATION

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We studied by a proteomic approach the pattern of proteins released from platelets after thrombin activation. Human washed platelets, from healthy volunteers, adjusted to 2×10^8-10^9 were stimulated with thrombin (0.25 or 0.5 U/mL) at 37°C for 3 min. Reaction was stopped by antiproteases followed by centrifugation to separate platelet pellet from released proteins. These were separated by 2-Dimensional Electrophoresis, the gels were silver-stained and subjected to image analysis. The spots whose intensity increased or decreased in the activated platelet gel in comparison with resting platelet gel were digested with trypsin and subjected to MALDI-TOF MS analysis; peptides from mass spectra of in-gel digest samples were matched against databases such as Swiss-Prot, NCBInr, using the Mascot search engine (Matrix Sciences) for peptide mass fingerprint. By image analysis, 475 spots were detected in a reference 2D gel; 171 and 206 spots matched with spots present in untreated and thrombin-treated platelet gels, respectively. Spots whose mean intensity showed at least a two-fold change in activated platelet gel in respect to resting platelet gel, were analyzed and identified. In 0.25 U/ml thrombin gel, 49 spots showed increased intensity, while 15 had decreased intensity, when compared to resting gel. In the 0.5 U/mLthrombin gel, 19 spots showed increased intensity and 18 had decreased intensity, when compared to resting gel. Transferrin, glutathione-transferase, WD repeat-containing protein 1 isoform 1, thrombospondin-1 precursor and thrombospondin were found among the released proteins by activated platelets. Some of these, such as thrombospondin-1 precursor and transferrin showed multiple peaks spread across a range of 0.5-1 units of isoelectric point (pI) with minor mass changes. This suggests they are splice variants and/or post-translationally modified forms of the same protein. The identification of the whole protein pattern might help understanding the mechanisms underlying thrombin platelet activation.

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C098

HYDROLYSIS OF PLATELET GPV BY A-THROMBIN ON INTACT PLATELETS: EFFECTS OF GPIB AND LIGANDS OF THROMBIN EXOSITE I AND II

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Glycoprotein V (GpV) is a 82 kDa transmembrane glycoprotein non covalently associated with GpIb and GpIX on the platelet membrane. Thrombin cleaves GpV, releasing a 69 kDa N-terminal fragment (GpVs).The functional role of GpV cleavage is not completely understood. In the present study we evaluated the kinetics of the release of GpVs after exposure of human platelets to α-thrombin using an enzyme immunoassay for detection of GpVs (Asserachrom soluble GpV-Diagnostica Stago). Gel filtered platelets were incubated, before thrombin stimulation, with 1) 9 uM HD22, a DNA aptamer which binds to the thrombin anion binding exosite II (ABE-II), 2)15 uM HD1, a DNA aptamer which binds to the thrombin anion binding exosite I (ABE-I), 3) 300 ug/mL LJIb10, an anti-GpIb MoAb, which inhibits thrombin binding to GpIb, 4) 250 ug/mL LJIb1, an anti-GpIb MoAb inhibiting the VWF binding to GpIb, 5) 20 uM C-terminal haemadin peptide (45-57), a ligand of ABE-II, 6) 2.5 uM GpIb N-terminal fragment (1-291), 7) 10 uM NAPAP, a tight binding reversible inhibitor of the thrombin catalytic site. The pseudo-first order rate constant for GpV hydrolysis by 1 nM thrombin, which was 6×10 -4 sec -1, was markedly reduced by HD1 and NAPAP, whereas was not affected by HD22, LJIb10, GpIb N-terminal and C-terminal haemadin. On the contrary, the maximum amount of GpVs released by 1 nM α-thrombin was reduced by about 50% in pres-

ence of C-terminal haemadin, 80% in the presence of HD22, 30% in the presence of GpIb N-terminal, and 40% in the presence of LJ1b10. No effect was observed with LJ1b1.These results indicated that the thrombin binding to GpIb increases the amount of GpV available to proteolysis by thrombin. Furthermore, ABE-II may be partly involved in GpV ligation, whereas ABE-I seems responsible for the molecular recognition of GpV by human thrombin

C099

EFFECTS OF THE SURAMIN ANALOGUES NF864 AND NF449 ON P2X1-MEDIATED RESPONSES OF HUMAN PLATELETS

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It has been shown that P2X1 plays an important role in platelet aggregation and platelet thrombus formation under high shear conditions, making it an attractive molecular target for antithrombotic intervention. Our aim was to test the effects of two suramin analogues, NF864 and NF449, on some parameters of platelet function. Both compounds had previously been shown to be potent and specific antagonists of P2X1 receptors in purified systems. We used normal human platelets that had been washed and resuspended in Tyrode's solution containing CaCl2 2 mM and MgCl2 1 mM. Apyrase (0.5 U/mL), was added to the final suspension to prevent P2X1 desensitization. NF864 and NF449 (up to 10-4 M) did not induce platelet shape change or aggregation. As previously described, the addition of the non-hydrolyzable ATP analogue α,β,methylene-ATP (1 microM), a P2X1 agonist, induced transient platelet shape change, which was completely inhibited by NF864 (10-5M) or NF449 (5x10-6M). However, both NF864 and NF449, at 10-4 M, partially inhibited ADP-induced platelet aggregation, which, in contrast, was not affected by P2X1 desensitization by their previous exposure to α,β,methylene-ATP , suggesting that the two suramin analogues interfere also with the platelet P2Y receptors for ADP. NF864 (10-4 M) inhibited ADP-induced inhibition of adenylyl cyclase (a function that is mediated by the P2Y12 receptor) to a greater extent than NF449. Therefore, the two suramin analogues tested, NF864 and NF449, exhibit a good inhibitory effect on P2X1-mediated platelet activation; however, at high concentrations, they also interfere with the platelet P2Y receptors for ADP.

C100

TYROSINE PHOSPHORYLATION OF IP3 RECEPTOR OVERCOMES THE INHIBITORY EFFECT OF CAMP ON CA2+ MOBILIZATION IN HUMAN PLATELETS

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Platelet activation by stimulatory agents leads to an increase of cytosolic Ca2+, which triggers many intracellular signalling processes. Platelets express two phospholipase C (PLC) isoforms, β and γ, whose activation generates Inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 diffuses rapidly within the cytosol to interact with its receptor (IP3R) to release stored Ca2+ and to generate the initial Ca2+ signal phase. Cyclic AMP (cAMP) elevating agents, such as iloprost, antagonize PLCβ-dependent Ca2+ mobilization evoked by the thromboxane A2 synthetic analogue, U46619, through PKA activation. Conversely, elevation of cAMP fails to affect PLCγ2-dependent Ca2+ mobilization following collagen or convulxin stimulation. In the present study we sought to investigate the reasons for this discrepancy in aspirinated platelets treated with the ADP receptor inhibitors (A3P5PS and AR-C69931MX) and iloprost. Our results demonstrated that iloprost treatment: 1) totally abolished U46619-induced intracellular Ca2+ elevation without affecting IP3 production; 2) unaffected Collagen-induced Ca2+ mobilization and IP3 production; 3) reduced convulxin-induced Ca2+ mobilization but not IP3 production. This led us to hypothesize that PKA inactivates PLCβ-dependent IP3 binding to IP3R. As it has been reported that tyrosine phosphorylation enhances IP3R affinity for IP3, platelets were treated with thapsigargin, that causes tyrosine kinase activation, prior to agonist stimulation. Our data showed a partially restored increase in intracellular Ca2+ (150.5±45.3 DeltanM) in response to U46619 after thapsigargin stimulation despite iloprost treatment that was prevented by the tyrosine kinase inhibitor tyrphostin A23. Thapsigargin did not modify collagen- or convulxin-induced responses. In order to asses whether this phenomenon was, at least in part, due to thapsigargin-induced cytosolic Ca2+ elevation, iloprost-treated platelets were further stimulated with the calcium ionophore ionomycin, before U46619. No intracellular Ca2+ elevation was recorded following U46619 stimulation in this conditions. Our data strongly suggest that tyrosine kinase-dependent IP3R phosphorylation overcomes the inhibitory action of cAMP on Ca2+ mobilization.

C101

POLYPHENOLS ENHANCE PLATELET NITRIC OXIDE BY INHIBITING PROTEIN KINASE C-DEPENDENT NADPH OXIDASE ACTIVATION. ROLE ON PLATELET RECRUITMENT

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Several studies demonstrated an inverse association between polyphenol intake and cardiovascular events. Platelet recruitment is an *in vitro* model to explore platelet activation at the site of vascular injury; however it has never been investigated if polyphenols influence platelet recruitment. Aim of the study was to analyse *in vitro* if two polyphenols, quercetin and catechin, were able to affect platelet recruitment. Platelet recruitment was reduced by nitric oxide (NO) donors and by NADPH oxidase inhibitors and was enhanced by L-NAME, an inhibitor of NO synthase. Quercetin and catechin, but not single polyphenol, significantly inhibited platelet recruitment in a concentration-dependent fashion. The formation of superoxide anion was significantly inhibited in platelets incubated with quercetin and catechin but was unaffected by one single polyphenol. Incubation of platelets with quercetin and catechin resulted in inhibition of PKC and NADPH oxidase activation. Treatment of platelets with quercetin and catechin resulted in an increase of NO and also downregulated the expression of GpIIb/IIIa glycoprotein. This study shows that the polyphenols quercetin and catechin synergistically act in reducing platelet recruitment via inhibition of PKC-dependent NADPH oxidase activation. This effect, resulting in NO-mediated platelet glycoprotein GpIIb/IIIa downregulation, could provide a novel mechanism through which polyphenols reduce cardiovascular disease.

C102

COMPARISON OF TWO POINT-OF-CARE PLATELET FUNCTION TESTS WITH PLATELET AGGREGATION TO IDENTIFY ASA RESPONSIVENESS IN CAD PATIENTS

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Recent studies suggest that coronary artery disease (CAD) patients who do not respond to aspirin (ASA) therapy may be at increased risk of ischemic vascular events. Two new point-of care (POC) systems, PFA-100 (DADE Behring, USA) and VerifyNow (Accumetrics, USA), have been suggested as rapid tools to identify ASA non-responsive patients. Aim of this study was to compare these POC devices with conventional light transmission aggregometry (LTA). PFA-100 Closure Times by collagen+epinephrine (CT/EPI), VerifyNow ASA Assay tests and LTA tests induced by arachidonic acid were assessed in 415 CAD patients undergoing percutaneous coronary intervention and receiving ASA therapy (100 to 325 mg daily). ASA non-responders were defined by PFA-100 as patients with a CT/EPI below the 95th percentile value of the control group (CT/EPI <203 s) or by VerifyNow ASA test as patients with values ≥550 Aspirin Reaction Units as suggested by manufacturer. A cut-off value of 20% of maximal aggregation for LTA by arachidonic acid was used to define ASA resistance. The prevalence of ASA non-responsiveness was 32.5% by PFA-100, 13.3% by VerifyNow ASA test and 31.3% by LTA. In 258/415 (62.2%) samples the results of the 3 tests were concordant. As concerns the comparison between LTA and PFA-100 CT/EPI results, 306/415 (73.7%) samples were concordant (p<0.001). Comparing LTA and VerifyNow ASA results, 322/415 (77.5%) were concordant (p<0.001). At the two POC device comparison, 303/415 (73.9%) samples were concordant (p<0.001), with 39 ASA non-responders and 264 ASA responders. In 280 ASA responders identified by PFA-100 CT/EPI, only 16 pts (0.5%) resulted non responders by VerifyNow ASA. In 360 ASA responders identified by VerifyNow ASA 96 pts (26.7%) resulted non responders by PFA-100. A good agreement was observed among the 3 methods considered to identify ASA responsiveness.

C103

COMPARISON OF VASP PHOSPHORYLATION AND LIGHT TRANSMITTANCE AGGREGOMETRY IN DETERMINING INHIBITION BY CANGRELOR, A PLATELET ADP P2Y12 RECEPTOR ANTAGONIST

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Cangrelor (AR-C69931MX) is a novel antagonist of the platelet ADP P2Y12 receptor. We compared its inhibitory effect by flow-cytometric analysis of vasodilator-stimulated phosphoprotein phosphorylation (VASP-P) and aggregometry. Citrated venous blood from 22 healthy subjects (10 males, 12 females, 28.5 ±6.6 years) was processed as indicated in the VASP kit (Biocytex, Marseille, France). Platelet reactivity index (PRI) was calculated from percent of fluorescence-positive platelets (VASP-P) in the presence of PGE1 with or without ADP (10 μM). PRP aggregation was induced by ADP (1.25, 2.5 and 5 μM). Cangrelor was preincubated 10 min before challenge. Cangrelor induced inhibition of both PRI (IC50: 2.2±1.7 (mean±SD) ln nM) and aggregation (IC50: 0.4±1.6, 2.7±1.6 and 4±1.2 ln nM, at three ADP concentrations), in a concentration-dependent way. No correlation was found between individual IC50, by either method. When individual IC50 by VASP were ranked by increasing order, four of 7 *high responders* by VASP were also identified as such by aggregation with one/two ADP concentrations; one was identified by VASP and all aggregation tests (*very high responder*). Six of 8 *intermediate responders* and 6 of 7 *poor responders* by VASP were similarly ranked by at least one ADP concentration. Among 11 subjects with the lowest and 11 with the highest IC50 by VASP, 10 had IC50 below and 10 above the median against at least one ADP concentration. Three subjects in the lowest, and one in the highest VASP IC50 groups, showed IC50 below or above the median against all ADP concentrations (*very high* and *very poor responder*). Correlation between the methods was poor by individual data, but more accurate when groups, identified according to test response, were compared. Combination of both methods might help identify *in vitro* high or low responders to cangrelor, and possibly other ADP receptor antagonists.

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C104

MONITORING CLOPIDOGREL THERAPY BY A NEW POINT-OF-CARE PLATELET FUNCTION DEVICE IN CAD PATIENTS

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Clopidogrel therapy is daily administered in patients at risk of vascular ischemic events. Activated platelets play an important role to vascular and stent thrombosis and clopidogrel limits this process. This drug, an antagonist of P2Y12 receptors for adenosine-diphosphate (ADP) on platelet membrane, inhibits platelet activation caused by ADP. VerifyNow system (Accumetrics, USA), a new point-of-care (POC) device, has been suggested as a rapid tool to identify clopidogrel non-responsive patients. Aim of this study was to compare this POC system with conventional light transmission aggregometry (LTA). VerifyNow P2Y12 Assay tests - which give results as P2Y12 Reaction Units (PRU) and percentage of inhibition- and LTA tests induced by 2 μMol ADP and by 10 μMol ADP were assessed in 394 CAD patients undergoing percutaneous coronary intervention and receiving clopidogrel therapy (loading dose 300 mg and daily 75 mg). Clopidogrel non-responders were defined by VerifyNow P2Y12 test as patients with values of inhibition <20%. A cut-off value of 70% of LTA by 2 and 10 μMol ADP was used to define clopidogrel non responsiveness. The prevalence of clopidogrel non-responsiveness was 32.2% by VerifyNow P2Y12 test, 7.1% and 22.6% by LTA induced by 2 and 10 μMol ADP respectively. As concerns the comparison between VerifyNow P2Y12 and 2 μMol ADP LTA results, 271/394 (68.8%) samples were concordant (p=0.003) with 16 clopidogrel non-responders and 255 clopidogrel responders. One hundred and twenty-three out of 394 (31.2%) samples gave discordant results, with 111 patients categorized as clopidogrel non responders only by VerifyNow P2Y12 and 12 only by LTA. In 366 clopidogrel responders identified by 2 μMol ADP LTA, 111 patients (30.3%) resulted non responders by VerifyNow P2Y12. As concerns the comparison between VerifyNow P2Y12 and 10 μMol ADP LTA results, 270/394 (68.5%) were concordant (p<0.0001) with 46 clopidogrel non-responders and 224 clopidogrel responders. One hundred and twenty-four (31.5%) samples gave discordant results, with 81 patients categorized as clopidogrel non responders only by the VerifyNow P2Y12 and 43 only by LTA. In 305 clopidogrel responders identified by 10 μMol ADP LTA, 81 patients (26.5%) resulted non responders by VerifyNow P2Y12. A significant agreement between VerifyNow P2Y12 assay and LTA was observed. Biological studies - measurement of the inhibition by ADP of PG-induced platelet cAMP increase or phosphorylation of vasodilator-stimulated phosphoprotein - are needed to evaluate the reliability of this POC system.

Venous thromboembolism: prophylaxis and therapy

C105

DOES ANTICOAGULANT THROMBOPROPHYLAXIS PREVENT PULMONARY EMBOLISM AND DECREASE MORTALITY IN HOSPITALIZED MEDICAL PATIENTS? A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background. Venous thromboembolism, which includes deep vein thrombosis and pulmonary embolism, is the commonest preventable cause of death in hospitalized medical patients. Although anticoagulant thromboprophylaxis is effective in preventing venous thromboembolism, it is underused in hospitalized medical patients. *Purpose.* To assess the efficacy of anticoagulant thromboprophylaxis in reducing pulmonary embolism and mortality and to evaluate the risk of major bleeding complications associated with use of thromboprophylaxis. *Data Sources.* The MEDLINE, EMBASE and Cochrane databases were searched until February 2006, reference lists were reviewed and experts were contacted. Randomized controlled trials that investigated a prophylactic dose of unfractionated heparin, low-molecular-weight heparin or fondaparinux in medical patients were included. *Data Extraction.* Two reviewers independently selected studies and extracted data on study characteristics, quality and assessed outcomes (pulmonary embolism, fatal pulmonary embolism, all-cause mortality, major bleeding). *Data Analyses.* Pooled relative risk (RR) and associated 95% confidence intervals (CIs) were calculated for each outcome. *Data Synthesis.* Ten studies were included, totalling 22,054 patients. Use of anticoagulant thromboprophylaxis was associated with a significant reduction in pulmonary embolism (RR = 0.58; 95% CI: 0.44-0.76; absolute risk reduction [ARR] = 0.59%; number-needed-to-treat [NNT] = 170), and fatal pulmonary embolism (RR = 0.36; 95% CI: 0.20-0.66; ARR = 0.27%; NNT = 370). There was a trend for decreased all-cause mortality (RR = 0.91; 95%CI: 0.82-1.01), and a trend for increased major bleeding (RR = 1.64; 95%CI: 0.86-3.14) in patients who received anticoagulant thromboprophylaxis. *Conclusions.* Anticoagulant thromboprophylaxis is effective in preventing pulmonary embolism and decreasing mortality from pulmonary embolism in at-risk hospitalized medical patients.

C106

INCIDENCE OF VENOUS THROMBOEMBOLISM FOLLOWING GYNECOLOGIC LAPAROSCOPY: A PROSPECTIVE COHORT STUDY

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Background. Information on the incidence of venous thromboembolism (VTE) following laparoscopic procedures is inadequate and there is no solid evidence to guide the use of thromboprophylaxis in this setting. When recommendations for open surgery are followed for patients undergoing laparoscopy, the majority of patients become eligible for thromboprophylaxis, in particular because laparoscopic procedures usually take longer than the equivalent open surgical procedures. Gynecologic laparoscopy is frequently performed in low risk patients. There are currently no clinical studies specifically designed to assess the incidence of VTE in this setting. *Methods.* In a prospective cohort study, consecutive patients undergoing gynecologic laparoscopy underwent compression ultrasonography (CUS) and clinical assessment to evaluate the incidence of clinically relevant VTE. CUS was performed 7±1 and 14±1 days postoperatively and a clinical follow-up was performed at 3 months. No patient received antithrombotic prophylaxis. Patients with malignancy or previous VTE were excluded from the study. *Results.* We enrolled 265 patients, with a mean age of 36.8 years, range 18-74. Risk factors for VTE were detected in 33.3% of patients, the most common being current use of oral contraceptives (15.8%), family history of VTE (9.6%), and varicose veins (6.8%). Most common indications to the laparoscopic procedure were endometriosis in 18.5% of patients, ovarian cysts in 15.7% of patients, unexplained adnexal masses in 13.2% of patients, and infertility under investigation in 8.4% of patients. Mean duration of the procedure was 60.1 minutes (range 10 to 300 minutes). In particular, in 55.5% of patients the duration exceeded 45 minutes. There were no episodes of CUS detected deep vein thrombosis (0%, 95% CI 0-1.58) and no episode of symptomatic VTE (0%, 95% CI 0-1.50) at a 3 month follow up. *Conclusions.* Gynecologic laparoscopy in non cancer patients is a low risk procedure and pharmacologic thromboprophylaxis is not warranted.

C107

THE PAVIA ENDARTERECTOMY PROGRAM FOR CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION:THREE YEAR FOLLOW-UP OF PATIENTS SUBMITTED TO PULMONARY ENDARTERECTOMY

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Chronic thromboembolic pulmonary hypertension (CTEPH) is a rare disease which results from obstruction of the major pulmonary arteries by incompletely resolved or organized pulmonary emboli which have become incorporated into the pulmonary artery wall, eventually causing an increase in pulmonary vascular resistances. A percentage ranging from 0.1 to 4.0 of patients recovering from acute pulmonary embolism develop CTEPH. In our experience, there are two major types of material retrieved from pulmonary vasculature during surgery: the first type, present in approximately 70% of cases, is constituted by thickened, yellowish and relatively firm intima, with no evidence of fresh thrombi. This could represent the result of a single, submassive pulmonary embolism episode. Around 40% of these specimens are retrieved from patients with no history of symptomatic thromboembolic disease. Both DVT and PE are in fact frequently clinically unsuspected. Moreover, more than 50% of the patients with symptomatic deep vein thrombosis and no signs or symptoms of pulmonary embolism do show pulmonary perfusion defects, when submitted to objective tests. The second type, occurring in the remaining 30% of cases, is constituted by yellow, fibrous material associated with thrombi in various stages of organization.

Table 1.

	CVP	*mPAP*	*CO*	*CI*	*PVR*	*PVRI*	*RV-EF*
A: Before-PTA	7±6	48±12	3.3±0.9	1.8±0.5	1125±412	2027±731	15±8
B:Before discharge	5±4	25±10	5.2±1.1	2.9±0.5	289±142	505±234	32±8
C: 3 months	2±2	24±11	5.1±1.4	2.8±0.6	231±198	542±271	32±7
D: 1 year	1±2	23±12	5.0±1.1	2.7±0.6	290±191	531±343	35±8
E: 3 years	2±2	24±12	4.9±1.1	2.6±0.5	317±226	579±393	34±8
p value	A vs. B: ns A vs. C, D, and E: <0.000001 B vs. C, and E: <0.05	A vs. B, C, D and E: <0.000001	A vs. B, C, D and E: <0.000001	A vs. B, C, D and E: <0.000001	A vs. B, C, D and E: <0.000001	A vs. B, C, D and E: <0.000001	A vs. B, C, D and E: <0.000001

Hemodynamic data from 35 patients participating to the Pavia Pulmonary Endarterectomy Program with complete 3-year follow-up: CVP (mmHg) central venous pressure; mPAP (mmHg) mean pulmonary artery pressure; CO (L/min) cardiac output; CI (L/min/m2) cardiac index; PVR (dynes/sec/cm-5) pulmonary vascular resistances; PVRI (dynes/sec/cm-5/m2) pulmonary vascular resistances index; RV-EF (%) right ventricle ejection fraction.

This type possibly represents the evolution of multiple pulmonary emboli, as usually seen in patients with acquired thrombophilia, particularly in the presence of anti-phospholipids antibodies, in whom recurrences may take place despite apparently adequate anticoagulant therapy. Without intervention, CTEPH is a progressive and lethal disease for which there is no effective medical therapy. Pulmonary endarterectomy (PEA) is the treatment of choice. Careful pre- and post-operative management is essential for a successful outcome following PEA. Lung transplantation (LTx) is indicated only in few cases when PEA is not feasible.

In 1994, we started in Pavia a program in which members of a multidisciplinary team work in close interaction with the aim of increase experience in the challenging problems these patients present in the evaluative, surgical, and post-operative phases of their care. So far, 134 PEAs have been performed. Preoperatively, NYHA class distribution was respectively 3-II, 56-III, and 75-IV; mean pulmonary artery pressure and pulmonary vascular resistances were 47±13 mmHg and 1149±535 dynes/sec/cm-5 respectively. The overall operative mortality has been 9.7% (4.5% in 2005). Survival at 3-month, 1-year, and 3-year follow-up was 89.5±2.6, 87.8±2.9, and 83.3±3.5% respectively; this last rate was unchanged up to 10 years. After PEA, mean pulmonary artery pressure and pulmonary vascular resistances were 25±9 mmHg and 322±229 dynes/sec/cm-5 respectively and these results were stable over time. At the 3-year follow-up, 94% of patients were in NYHA class I or II and are treated with oral anticoagulants only. Table 1 summarizes the results of hemodynamic tests collected at three months, one year and three years on the first 35 patients who completed the follow-up program.

C108

THE RISK OF RECURRENT VENOUS THROMBOEMBOLISM AFTER DISCONTINUING ANTICOAGULATION IN PATIENTS WITH ACUTE PROXIMAL DEEP VEIN THROMBOSIS OR PULMONARY EMBOLISM. A MULTICENTER PROSPECTIVE COHORT STUDY IN 1626 PATIENTS

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It has long been recognized that patients with deep vein thrombosis (DVT) and/or pulmonary embolism (PE) are at high risk of recurrent venous thromboembolism (VTE). However, factors accounting for this risk are still controversial. In order to identify conditions associated with an increased risk of recurrent VTE after the withdrawal of anticoagulation, we performed a multicentre prospective cohort study involving two university centers and one hospital centre, all in Italy. 1626 consecutive patients at their first episode of clinically symptomatic proximal DVT and/or PE were followed up to 10 years after discontinuing anticoagulation (median, 50 months). Demographic and clinical characteristics were collected on a standard questionnaire. Recurrent VTE was adjudicated using accepted methods. Recurrent VTE episodes were experienced by 373 patients (22.9%). The cumulative incidence of recurrent VTE was 7.2% (95% CI, 6.0 to 8.5) after six months, 11.0% (9.5 to 12.5) after 1 year, 19.6% (17.5 to 21.7) after 3 years, 29.1% (26.3 to 31.9) after 5 years, and then increased up to 39.9% (35.4 to 44.4) after 10 years. The adjusted hazard ratio for recurrent VTE was 2.30 (95% CI, 1.82 to 2.90) for unprovoked presentation, 2.02 (1.52 to 2.69) for thrombophilia, 1.44 (1.03 to 2.03) for presentation with DVT, 1.39 (1.08 to 1.80) for a duration of anticoagulation < 6 months, and 1.14 (1.06 to 1.12) for every 10-year increase of age. In conclusion, unprovoked presentation, thrombophilia, clinical presentation with DVT, a shorter duration of anticoagulation, and increasing age are associated with a statistically significant increased risk of recurrent thromboembolic events in patients with symptomatic VTE.

C109

DELAY IN STARTING ORAL ANTICOAGULANT TREATMENT (OAT) IN PATIENTS WITH ACUTE VENOUS THROMBOEMBOLISM (VTE): ANALYSIS FROM THE MASTER REGISTRY

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Background. OAT should start early in patients with acute VTE in order to reduce the duration of heparin administration, cost and bleeding complications. *Aim.* To evaluate the delay of starting OAT after acute VTE. *Patients and methods.* MASTER is a multicenter registry of consecutive objectively confirmed VTE. Data about patients, events and treatment regimens were collected by an electronic database at the index event. *Results.* Out of 2093 VTE events analyzed, about 80% of patients received both heparin and OAT. Among the 1865 patients for whom the type of heparin was recorded, 79% received low-molecular weight heparin (LMWH), 18.7% intravenous unfractionated heparin (UH) and 2.3% subcutaneous UH. 68.1% of patients were treated in hospital and 31.9% at home. More patients who started treatment in hospital received both heparin and OAT, if compared with home-treated patients. Excluding those treated with OAT only or with heparin only, more than 90% of patients (92.2% of in-hospital and 82.3% of home-treated patients) started OAT within 10 days, but only 29% during the first day, up to 54.5% within the second and up to 65.9% within the third day. More home-treated patients started OAT on the first day, if compared with in-hospital patients (34.8% vs 27.4%); however, only up to 49.1% of home-treated patients started OAT within the first 3 days vs 70.5% of in-hospital patients. Overall, 70.1% of patients treated with LMWH started OAT within 3 days vs 53.2% of patients treated with UH. Comments: Patients treated with LMWH usually receive OAT earlier than patients treated with UH. Home-treated patients who do not start OAT on the first day have a delay in receiving OAT if compared with in-hospital patients. This delay is probably attributable to difficulties in organizing home monitoring of OAT.

C110

DURATION OF ANTICOAGULANT TREATMENT AND RECURRENCE OF VENOUS THROMBOEMBOLISM IN PATIENTS WITH AND WITHOUT THROMBOPHILIA

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Bertoldi A, for the Warfarin Optimal Duration Italian Trial (WODIT) Investi35

Background and Objectives.

Whether recurrence of venous thromboembolism (VTE) in patients with and without thrombophilia is influenced by the duration of anticoagulant treatment is unclear. The aim of this study was to assess the risk of thromboembolic recurrence after treatment discontinuation in patients with and without thrombophilia treated for three or twelve months of anticoagulation. *Design and Methods.* Patients with a first episode of unprovoked deep vein thrombosis or pulmonary embolism, randomized to three or twelve months of oral anticoagulant treatment, were prospectively followed up to evaluate the incidence of VTE recurrence. Patients were included in this analysis if screened for the following thrombophilic abnormalities: antithrombin, protein C, protein S, resistance to activated protein C and/or factor V R506Q mutation, the mutation 20210GA of the prothrombin gene, homocysteinemia and antiphospholipid antibodies. The diagnosis of VTE recurrence was done by objective tests and adjudicated by a panel unaware of the results of the thrombophilia screening. *Results.* After a median follow up of 57 months, a recurrence of VTE was observed in 79 of 297 screened patients (26.6%). A thrombophilic abnormality was found in 91 patients (30.6%), 48 of 150 patients treated for three months (32.0%) and 43 of 147 patients treated for one year (29.2%). The hazard risk (HR) for recurrence in patients with thrombophilia in comparison with patients without thrombophilia was 1.29 (95% CI, 0.81-2.05, p=n.s.). In patients treated for three months thrombophilia was a risk factor for recurrence (HR=2.03, 95%CI 1.04-3.95, p=0.037). Thus was not observed in patients treated for one year (HR=0.84, 95%CI 0.43-1.63, *p*=n.s.). *Interpretation and Conclusions.* An increased risk of VTE recurrence in thrombophilic patients was observed only in patients receiving three months oral anticoagulation.

C111

ROLE OF FONDAPARINUX IN THE TREATMENT OF HEPARIN-INDUCED THROMBOCYTOPENIA WITH VENOUS THROMBOEMBOLISM

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Heparin-induced thrombocytopenia (HIT) and heparin-induced thrombocytopenia with thrombosis syndrome (HITTS) may develop during anticoagulant treatment in patients submitted to therapeutic or prophylactic regimens. Although HIT is a relatively uncommon adverse event in patients receiving heparin therapy, it bears a significant risk of thrombotic events. Fifty percent of patients, if left untreated, can develop thrombosis. Several molecules have been studied as alternative anticoagulants in patients with HIT, including danaparoid, argatroban, lepirudin. Lepirudin requires dosage adjustments and has potential for antibody formation. Argatroban requires dosage adjustments in patients with hepatic insufficiency. Argatroban increases the international normalized ratio when administered with warfarin, leading to dosage difficulties when transitioning to warfarin therapy. Anticoagulation of

patients with HIT and HITT may be limited by antibodies cross-reactivity with danaparoid and by new generation of antibodies with lepirudin. Fondaparinux is the first of a new class of synthetic antithrombotics: the selective inhibitors of factor Xa.It is the most advanced competitor of low molecular weight heparin (LMWH), which is the reference drug in prophylaxis and treatment of venous thromboembolism. Fondaparinux does not bind to platelet factor 4 and does not react with HIT antibodies in *in vitro* testing. We treated 7 patients who develop HITT (four with both DVT and PE) in the immediate post-surgical period. Three patients were previously submitted to cardiac surgery under extracorporeal circulation (ECC) with unfractionated heparin (UFH) followed by administration of prophylactic dosage of LMWH. The remaining patients had been previously treated with either UHF or LMWH at therapeutic or prophylactic dosage. Patients developed HITT 3 to 7 days after surgery. We administered therapeutic dosages of fondaparinux, i.e. 7.5 mg QD. In two patients, we initially started with 2.5 mg QD, increasing to 7.5 mg QD in the subsequent days, according to their post-surgical bleeding risk. Patients were treated for 6 to 21 days before starting warfarin. Fondaparinux was stopped at an INR equal or superior to 2.0. All patients showed a significant reduction of their thromboembolic burden. All patients but one showed sustained increase of the platelet number. In the remaining patient platelet count remained unchanged. In this patient, treatment was switched to lepirudin and after few days her platelets reverted to close-to-normal levels. No hemorrhagic episodes or adverse events were recorded. Our results suggest that fondaparinux can be utilized with success in the management of patients with HITT. Encouraging results in the use of fondaparinux in the treatment of HIT and HITT, as demonstrated in other recent published case reports, warrant further study in larger controlled trials for this indication.

C112

EDHIT: EVALUATION OF DERMATAN SULPHATE IN PREVENTION AND TREATMENT OF HEPARIN INDUCED THROMBOCYTOPENIA

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Background. Immune thrombocytopenia is a potentially serious side-effect of heparin administration. The incidence of clinically overt heparin induced thrombocytopenia (HIT) is 1.2%. In our country, lepirudin is approved only for treatment of HIT-associated thrombosis. *Aim of the study.* To evaluate the efficacy and safety of dermatan sulphate, an indirect inhibitor of thrombin, in the prevention of HIT recurrence and in the treatment of HIT with or without venous thromboembolism (VTE). *Methods*. EDHIT is a prospective, multicenter, cohort study in 6 Italian centers. The diagnosis of HIT was made according to the following criteria: - drop of platelet count of at least 50% of baseline value and/or platelet count ≤100×10^9/L during heparin administration; - positive ELISA assay for detection of antibodies anti-PF4-heparin-complex. Four groups of patients were enrolled in the study: A, patients with diagnosis of HIT without VTE; B, patients with history of HIT requiring VTE prophylaxis; C, patients with diagnosis of HIT with VTE; D: patients with history of HIT requiring VTE treatment. Dermatan sulphate was administered in prophylactic (600 mg/die the first day, then 300 mg/die) or therapeutic (12 mg/Kg/die, then adjusted to an aPTT ratio 1.3-1.7) regimen. In patients with diagnosis of HIT the efficacy end-points were: rise in platelet count ≥50% of the platelet nadir and/or absence of artero-venous thromboembolic complications. In patients with history of HIT the efficacy end-points were: stability of platelet count and/or absence of arterovenous thromboembolic complications. *Results*. 24 patients were enrolled in the study: 3 patients in group A; 3 patients in group B; 14 patients in group C; 4 patients in group D. In more than 90% of patients was reached the biological/clinical end-points. No patient experienced major bleeding during dermatan sulphate treatment. *Conclusions.* Dermatan sulphate is an effective and safe anticoagulant agent for the prevention and treatment of HIT.

Cancer and thrombosis

C113

THE PERSISTANCE OF RESIDUAL VEIN THROMBOSIS, AFTER AN EPISODE OF DEEP VEIN THROMBOSIS, AND THE RISK OF NEW OVERT CANCER AND CARDIOVASCULAR DISEASE

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Background. We have recently demonstrated that the presence of Residual Vein Thrombosis (RVT), UltraSonography (US)-detected at the 3rd month after an episode of Deep Vein Thrombosis (DVT) of the lower limbs, is an independent risk factor for developing recurrent Venous Thromboembolism (VTE). The management of DVT patients by detection of RVT may, therefore, represent a simple and reproducible method for establishing the individual risk of recurrence and for tailoring the optimal duration of Oral Anticoagulants (OA) (Siragusa S *et al.* Blood 2003;102(11):OC183a). At the present, it is unknown whether RVT may also identify patients at increased risk for cancer and/or cardiovascular disease (CD). Objective of the study. In patients with DVT of the lower limbs, we conducted a prospective study for evaluating the correlation between RVT and the risk of new overt cancer and/or CD. *Materials and methods.* Consecutive patients, with an episode of idiopathic or provoked DVT, were evaluated after 3 months from the index DVT; presence/absence of RVT was detected and patients managed consequently (Figure 1).

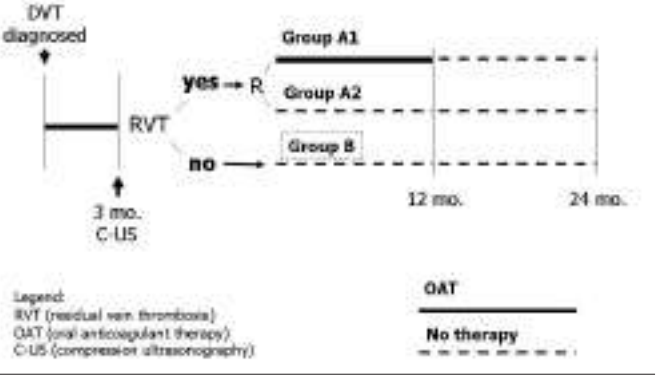

Figure 1.

The incidence of VTE recurrence, overt cancer and new CD was evaluated over a period of 3 years after the index DVT. Survival curves (Kaplan-Mayer) and related Breslow test have been used for statistics. Results. Three-hundred fourty-five patients were included in the analysis (Table 1).

Table 1. Incidence of events over a period of 3 years accordingly to RTV findings.

Group	*Number of patients month of OA from the index DVT*	*Presence of RVT at the 3rd*	*Duration of OA from the index DVT*	*ncidence of I recurrent VTE*	*Incidence of new cancer*	*Incidence of new CD*
Group *A1	142	yes	12 months	11 (7.7%)	8 (5.6%)	7 (4.9%)
Group *A2	91	yes	3 months	16 (17.5%)	9 (9.9%)	7 (7.7%)
Group B	112	no	3 months	1 (0.9%)	3 (2.6%)	4 (3.5%)

*Part of these patients were originally randomized to receive 3 or 12 months of OA.
RVT (Residual Vein Thrombosis); OA (Oral Anticoagulants); DVT (Deep Vein Thrombosis); VTE (Venous Thromboembolism); D (Cardiovascular Disease).

The incidence of recurrent VTE and new overt cancer was statistically lower in patients without RVT than in those with RVT (Table 1, Figure 2); no significant differences were found in the incidence of new CD (Figure 2).

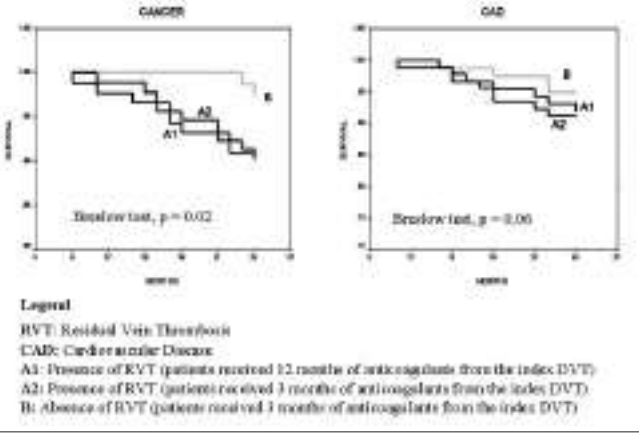

Figure 2. Relationship between RVT and subsequent Cancer and/or Cardiovascular Event.

These data are applicable in patients with idiopathic or provoked index DVT. In patients with RVT, the advantage of prolonging anticoagulation for 12 months was lost at the end of the treatment. *Conclusions.* This is the first study evaluating the relationship between US-detected RVT and the risk of developing cancer and CD; RVT presence, at 3rd month from the index DVT, is an independent risk factor for recurrent VTE and indicates patients at risk for new overt cancer. This risk remains over a period of 3 years, independently whether index DVT was idiopathic or provoked. In these patients, the advantage of indefinite anticoagulation should be assessed in properly designed study.

C114

CLINICAL CHARACTERISTICS OF CANCER ASSOCIATED ACUTE VENOUS THROMBOEMBOLISM: FINDINGS FROM THE MASTER REGISTRY

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Background. Clinical characteristics of acute deep vein thrombosis (DVT) and pulmonary embolism (PE) have been reported to be different in cancer patients and in patients without cancer. However, sparse information is available on this issue in a large cohort of patients representative of the full spectrum of patients with venous thromboembolism (VTE). *Aim.* To evaluate whether clinical characteristics of acute VTE are different in cancer and non cancer patients. *Patient and methods.* MASTER is a multicenter registry of consecutively recruited patients with symptomatic, objectively confirmed, acute VTE. Information about clinical presentation and diagnostic methods, temporary and permanent risk factors, prophylaxis and treatment were captured by an electronic data network at the time of the index event. *Results.* Data on 2119 patients (1056 males) were analysed. In 1541 (72.7%) patients the index event was DVT, in 206 (9.7%) PE and in 372 (17.6%) both PE and DVT; 424 patients (20%) had a cancer. The incidence of bilateral lower limbs DVT was significantly higher in patients with cancer than in patients without cancer (10.6% versus 5.4%; p=0.0004), as well as the rate of proximal DVT (95.7% versus 89.2%; p= 0.0002), ileocaval thombosis (29% versus 18.4%; p=0.0004), upper limb DVT (10.8% versus 5.4%; p=0.0001). The presence of concomitant transient risk factors, incidence of PE (alone or associated with DVT) and rate of use of thromboprophylaxis was not different in cancer patients compared with non cancer patients. The percentage of major bleeding (3.3% versus 1.1%; p= 0.001) and of inferior vena cava (IVC) filter implantation (7.3% versus 4.1%; p= 0.005) were significantly higher in patients with cancer. Oral anticoagulants were less frequently used in cancer patients (64.2% versus 82%; p<0.0001), in whom low-molecular-weight heparins were used more frequently for the long term treatment. *Conclusions.* The incidence of bilateral, proximal lower limbs, ileocaval and upper limbs DVT was higher in patients with cancer than in patients without cancer. Management of the acute phase of VTE in cancer patients was associated with an increased rate of major bleeding and IVC filter implantation.

C115

SHORT-TERM ACENOCUMARINE (A) OR DALTEPARINE (D) FOR THE PREVENTION OF CENTRAL VENOUS CATHETER-RELATED THROMBOSIS (CVCRT) IN CANCER PATIENTS. A RANDOMIZED CONTROLLED STUDY BASED ON SERIAL VENOGRAPHIES

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Background. Timing and frequency of non occlusive (nO) or occlusive (O) CVCrT in cancer patients (pts) remain unclear. In this randomized controlled trial we studied these points and evaluated the efficacy and safety of short-term prophylaxis with A or D in the prevention of CVCrT. *Methods.* Consecutive cancer pts without contraindications to short-term anticoagulation, scheduled for chemotherapy via CVC, were randomly assigned to receive: A 1 mg/day for 3 days before and 8 days after CVC insertion; D 5000 IU 2 hours before and daily for 8 days after CVC insertion; no anticoagulant treatment (NT). All pts underwent venography (V) at day 8 and 30 after CVC insertion and then every two months until CVC removal . The primary endpoint was V detected CVCrT, evaluated as nO or O when it was partially or completely occlusive of the vein lumen, respectively. Bleeding episodes were recorded. Proportions were compared using chi-square test together with odds ratio (OR). We evaluated the modification of following parameters: platelet count, PT, APTT, fibrinogen, anti thrombin, D-Dimer, protein C, protein S, glycocalicin, homocysteine. *Results.* 450 pts were randomized, 348 of whom (120/150 A, 114/150 D, and 114/150 NT) underwent V (median number of procedures 4, range 2-8). Both A and D reduced the frequency of V detected CVCrT (21.9% A vs 55.3% NT, OR= 4.35 (95% CI 2.43-7.69), p<0.001; 40% D vs 55.3% NT, OR= 1.85 (95% CI 1.10-3.13), p=0.02). A was more effective than D (OR= 2.37 (CI 1.34-4.22), p= 0.003). The frequency of O CVCrT was not different in the 3 groups (0.9% A, 5.0% D, 4.4% NT; p= 0.18). Overall, 5.1% of pts with CVCrT were symptomatic, all presenting O CVCrT (42% of pts with O CVCrT were not symptomatic). Most CVCrTs (95.6%) were observed at day 8 after CVC insertion. No major bleeding or pulmonary embolism occurred. *Conclusions.* In this study, Acenocumarine was more effective than Dalteparine in reducing V detected CVCrT. The doses of prophylactic agents used in this study proved to be safe. Symptomatic CVCrT evaluation alone underestimates the actual CVCrT frequency. The first days following CVC insertion are at highest risk for CVCrT. Short term thrombosis prophylaxis appears to be superior to no treatment without the expenses and inconveniences inherent in long-term prophylaxis.

C116

VENOUS THROMBOEMBOLISM IN DIFFERENT TYPES OF CANCER: A PILOT-STUDY

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Background and study purpose. Actual incidence of venous thromboembolism (VTE) in different types of cancer is not known. Aim of this study was to evaluate the incidence of VTE events in a population of heterogeneous cancer patients. *Methods.* From January 1, 2000 to December 31, 2003 we selected the first 100 consecutive patients for each of five most frequent types of cancer in our Province (breast, lung, colon-rectum, stomach, non-Hodgkin lymphoma (NHL). Patients were classified as localised disease (stage I-II) or metastatic disease (stage III-IV). We registered demographic data, presence/absence of VTE risk factors (surgery, chemotherapy, radiotherapy) and symptomatic, objectively diagnosed (ultrasound, V/Q scan, spiral CT) VTE new events (except superficial thrombophlebitis). The follow-up period ended on August 31, 2005. In this preliminary analysis, we only present the frenquency of new VTE events for each group of cancer patients. *Results.* The population included 500 patients, F/M 51/49%, mean age 66.5 years. Overall VTE frequency was 6.4% (32/500 events overall). VTE resulted slightly (but non statistically significant) more frequent in adenocarcinoma com-

pared to non-adenocarcinoma. Hystology best stratified thrombotic risk in NHL patients. VTE frequency was 4/41, 9.8% in large-cell NHL compared with follicular (1/37, 2.7%) and other NHL hystologic types (no events). We registered not statistically significant differences in VTE events among different type of cancers. Chemotherapy alone or in combination was registered in 10/32 (31%) and 24/32 (75%) respectively. Data for surgery alone/in combination were 3/32 (9%) and 18/32 (58%). No events with radiotherapy as the only risk factor were registered while radiotherapy in combination was present in 6/32 cases (19%). Mean follow-up was 35.7 months: no patient was lost to follow-up. *Conclusion.* This pilot study shows that cancer patients are a heterogeneous group concerning VTE risk. An observational, multicenter retrospective study, with both an adequate sample size and a statistical analysis plan, to investigate VTE frequency in different cancer patients is currently ongoing under the sponsorship of the FADOI (Federazione Associazione Dirigenti Opedalieri Internisti-Federation of internal medicine hospitalists).

C117

THROMBOTIC COMPLICATIONS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA.A META-ANALYSIS OF 17 PROSPECTIVE STUDIES COMPRISING 1,752 PEDIATRIC PATIENTS

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Introduction. The risk of thrombosis in children with acute lymphoblastic leukemia (ALL) ranges between 1% and 37%. Epidemiologic studies have usually been hampered by small numbers, making accurate estimates of thrombosis risk in ALL patients very difficult. The aim of this study was to better estimate the frequency of this complication and to define how the disease, its treatment and the host contribute to its occurrence. *Methods.* We performed a meta-analysis of all prospective studies evaluating the incidence of symptomatic thrombosis in children with ALL, published in English, since 1977. Subgroup analyses were performed to evaluate the influence of patient characteristics and treatment strategies on thrombotic complications. Pooled incidence rates (IRs) and 95% confidence intervals (CIs) were calculated using exact method, that accounts for sparseness of individual studies. *Results.* From a total of 100 articles, 67 were excluded for one of the following reasons: case reports (12), no data about the incidence of thrombosis (20), reviews (7), duplicated data (4), no clear definition of the endpoints (2), and studies on adult patients (22). A total of 17 studies were included, comprising 1,752 patients and 91 thrombotic events (IR: 5.2%, 95%CI 4.2-6.4). Most of the events occurred during the induction phase of therapy. Lower doses of asparaginase for longer periods of time were associated with the highest incidence of thrombosis, as were anthracyclines and prednisone (instead of dexamethasone). A meta-analysis of 5 studies evaluating the presence of pro-thrombotic genetic defects showed that the presence of at least one genetic prothrombotic factor was associated with an 8-fold increase in the risk of thrombosis in children with ALL. *Conclusions.* The overall thrombotic risk in ALL children was significant, and the subgroup analysis was able to identify high risk individuals, a finding that will hopefully guide future prospective studies aimed at decreasing this risk.

C118

RETROSPECTIVE SURVEY ON POST-SURGERY OUTCOME IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA

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The incidence of thrombosis and haemorrhage after surgery in Essential Thrombocythemia (ET) and Polycythemia Vera (PV) is unknown. We retrospectively analyzed in a multicenter survey 179 surgical interventions carried out in 64 PV and 87 ET patients (79 males, 72 females, mean age 60). Diabetes mellitus, hypercholesterolaemia and previous venous thrombosis were present in 5%, previous arterial thrombosis in 12%, arterial hypertension in 39% of patients. After diagnosis, antiplatelet drugs were given to 124/151 patients (82%); cytoreductive treatments to 113/151 (74%), warfarin to 15/151 (10%); all PV patients were phlebotomized. In 14/179 surgeries (7.5%), an emergency procedure was performed; 111 (62%) underwent general anesthesia, 53 (29.5%) local, 15 (8.5%) spinal. Ten out of 55 (18%) abdominal interventions were with laparoscopy. 67/179 (37.5%) surgeries were classified as major and 63 (35%) as minor procedures; 30 (17%) were cardiovascular, 16 (9%) orthopedic and 3 (1.5%) neurosurgical. Antithrombotic prophylaxis data were available for 123/179 surgery: in 88 (71.5%) LMWH, in 21 (17%) unfractioned heparin, in 5 (4%) warfarin and in 9 (7.5%) antiplatelet drugs were administered peri-operatively; 137/179 (76.5%) were on cytoreductive therapy (4/137, 2.9% administered as short cycle soon before surgery). 182 clinical outcomes, within 3 months from surgery, were recorded: in 145 (79.5%) cases no events were observed; 9 patients (5%) had AT (1 myocardial infarction, 5 peripheral arterial thrombosis, 3 TIA); 9 (5%) had venous thrombosis (DVT; 2 cases with pulmonary embolism, PE); 15 (8.3%) had hemorrhagic complications (12 major, all requiring packed red cells); 4 (2.2%) deaths surgery-related were recorded. Twenty patients (11%) with major complications were hospitalised (for a mean of 5 days). Despite cytoreduction and antithrombotic prophylaxis, a significative proportion (10%) of PV and ET patients undergoing surgery suffered from thrombotic complications; notably, 6% had major haemorrhages and 4/151 (2.6%) patients died within 3 months from surgery.

C119

PACLITAXEL DOWNREGULATES TISSUE FACTOR IN HUMAN CANCER AND ENDOTHELIAL CELLS: NEW PERSPECTIVES FOR ANGIOGENESIS INHIBITION?

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Tissue factor (TF), in addition to its essential role in hemostasis, is also considered a hallmark of cancer progression. Aberrant TF expression can be observed in tumor-associated endothelial and inflammatory cells, as well as by cancer cells themselves. Paclitaxel, a microtubule-stabilizing compound clinically used in a wide variety of malignancies, combines antitumor and antiangiogenesis properties. Therefore, we investigated whether paclitaxel could modulate TF and some fibrinolytic parameters in the metastatic breast carcinoma cell line MDA-MB-231 and in host's tumor-associated cells, i.e., endothelial and mononuclear cells (MN). To test this hypothesis, paclitaxel was incubated with MDA-MB-231, activated human umbilical vein endothelial cells (HUVEC), or activated human MN at 37Ã‚°C for different time intervals. At the end of incubation, conditioned medium was collected and tested for u-PA, t-PA and PAI-1 antigen levels by ELISA, and cells were disrupted and tested for procoagulant activity by a one-stage clotting assay and TF antigen by ELISA. Both the strong TF activity and antigen constitutively expressed by the MDA-MB-231 cells were significantly reduced in a dose-dependent manner by paclitaxel. TF expression was also reduced in endotoxin (LPS)- and IL-1β stimulated MN and HUVEC. Paclitaxel did not modulate u-PA and PAI-1 release from MDA-MB-231. By contrast, it strongly downregulated PAI-1 levels in LPS- and IL-1β-stimulated HUVEC. Since high doses of paclitaxel have been shown to induce expression of inflammatory genes in monocytes and tumor cells, we tested whether paclitaxel could influence IL-1β and IL-6 release from our cells. Neither the constitutive expression of these cytokines by MDA-MB-231 nor the LPS-induced release from MN and HUVEC were affected. Our data support the hypothesis that the anti-tumor effects of paclitaxel may, in part, be mediated by the capacity of this drug to modulate the procoagulant/fibrinolytic potential of cancer and host cells; moreover, the inhibition of endothelial cell TF could open interesting perspectives for the antiangiogenetic activity of the drug.

C120*

TUMOR CELL-INDUCED ENDOTHELIAL ANGIOGENESIS AND INHIBITION BY ALL-TRANS RETINOIC ACID (ATRA)

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In vivo and *in vitro* studies have shown that ATRA reduces the procoagulant potential of malignant and normal (endothelium and monocytes) cells and this is associated with clinical resolution of disseminated intravascular coagulation in acute promyelocytic leukemia. Particularly, ATRA inhibits Tissue Factor (TF) expression by the endothelium stimulated by tumor cell-derived cytokines. In this study, we evaluated whether ATRA is able to affect the endothelial angiogenesis elicited by tumor-derived products or standard proangiogenic factors. Angiogenesis was evaluated *in vitro* by the capillary-like tube formation assay. Human microvascular endothelial HMEC-1 cells were seeded on Matrigel and incubated for 24 hours with standard proangiogenic factors (VEGF, bFGF and TNFα), or tumor cell conditioned medium (TCM) collected from human breast cancer cell lines MCF-7 and ZR-75-1 and human leukemia cell line NB4, in the presence and absence of 1 μmol/L ATRA. Tube formation was examined under phase-contrast microscopy and tube length determined by an image analysis software. The results show that tube formation observed with control medium was 41% decreased by ATRA ($p<0.05$). All the standard proangiogenic factors induced a significant ($p<0.01$) increase in tube length (80 to 100% mean increment compared to control cells). ATRA significantly inhibited tube formation induced by VEGF, bFGF and TNF-α. TCM from all three cell lines significantly ($p<0.01$) induced tube formation (95 to 172% mean increment). The angiogenic capacity in these cells correlates with TF expression (evaluated as activity and antigen) and with the VEGF levels in the TCM. ATRA significantly ($p<0.01$) inhibited NB4, ZR-75-1 and MCF-7-CM induced angiogenesis by 80%, 61% and 70%, respectively. These results indicate that ATRA can effectively counteract the proangiogenic stimulus of purified as well as tumor-derived angiogenic factors on microvascular EC. As regard to tumor induced angiogenesis, a role of TF and VEGF expressed by TC is also suggested.

**PREMIO SISET 2006*

von Willebrand factor and ADAMTS-13

C121

ADAMTS-13 BINDS PLATELETS IN A SPECIFIC, DIVALENT CATION AND ACTIVATION DEPENDENT MANNER

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ADAMTS-13 cleaves high molecular weight von Willebrand factor (VWF) in order to prevent intravascular platelets adhesion and aggregation as observed in thrombotic thrombocytopenic purpura. ADAMTS-13 is present at low levels in plasma. We therefore surmised that platelets are able to specifically bind the metalloprotease on their surface, hereby concentrating the enzyme where it is most required. With this as background, we studied platelet adhesion to albumin or fibrinogen or VWF or recombinant ADAMTS-13 (each at 10 μg/mL). Washed platelets were let adhere to the wells for 1 hr at 37°C, lysed, and detected assessing optical density at 405 nm after p-nitrophenilphosphate was added. Binding of platelets preincubated with divalent cations to wells covered with ADAMTS-13 was significantly higher than binding to wells coated with albumin ($p<0.001$), was at the same levels of the binding to wells covered with recombinant VWF and approximately half of binding to the wells coated with fibrinogen. Platelets preincubation with EDTA 2mM abolished the binding to ADAMTS-13. Preincubation of platelets with antibodies against αIIbβ3 (7E3, 10 μg/mL) reduced the binding to ADAMTS-13 approximately of 40-50%. Activation of platelets with ADP (10 μM) or collagen (10 μg/mL) increased their binding to ADAMTS-13 ($p<0.001$) as compared to the binding of non-activated platelets. Immunofluorescent studies were then performed to see whether ADAMTS-13 bound to the platelet plasma membrane using a murine anti-human ADAMTS-13 monoclonal antibody (13E2). A positive membrane fluorescent signal was detected using as a source of platelets either a normal donor or a patient with type III Von Willebrand disease, demonstrating that ADAMTS-13 is located on the platelet surface independently from VWF. We conclude that ADAMTS-13 binds to the surface of platelets, the binding is specific, activation and divalent cations dependent, inhibited by EDTA, and not mediated by VWF on the membrane of platelets. αIIbβ3 could contribute to the binding.

C122*

THE FIRST DELETION MUTATION IN THE TSP1-6 REPEAT DOMAIN OF ADAMTS13 LEADS TO A SECRETION DEFECT

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We investigated an Iranian patient with history of recurrent thrombotic thrombocytopenic purpura (TTP) and low plasma levels of ADAMTS13 activity (2.3%). Genetic analysis revealed a 6 bp homozygous deletion at nucleotides 2930-2935 (GTGCCC), in exon 23 of ADAMTS13 gene, leading to replacement of Cys977 by a Trp and the deletion of Ala978-Arg979 residues, in the TSP1-6 repeat domain. To explore the mechanism of ADAMTS13 deficiency, HEK293 and COS-7 cells were transiently transfected using wild type (ADAMT13WT) and mutant (ADAMTS13del6bp) expression vectors. The enzymatic activity of the rADAMTS13WT and mutant proteins, evaluated by quantitative immunoblotting assay (Furlan, 1998) showed 100% and ~10%, respectively. Western blot analysis of the conditioned media and lysate showed a band of ~190 KDa in the medium, corresponding to rADAMT13WT protein; however a fainter band roughly estimated to be 5% of the WT was obtained for mutant protein. Therefore, pulse-chase labelling experiments were performed to evaluate the secretion pathway alteration. After 60' pulse with [35S] methionine, the maximum level of rADAMT13WT in conditioned media was found at 24 hours, but a very weak band of rADAMTS13del6bp was present after 3 hours only and no band being detected after 7 hours of chase. Differential immunofluorescence studies in WT and mutant transfected cells showed that rADAMTS13WT was mostly localized in the perinuclear

area, whereas rADAMTS13del6bp showed less intense staining diffusely throughout the cytoplasm, only a minimal amount of the mutant protein being localized in the Cis-Golgi and ER. This study suggests that the residue Cys977 could be involved in the formation of disulphide bonds responsible for a correct folding of one of the TSP1-like domains of ADAMTS13 and the deletion mutation could lead to uncorrected folding process and an intracellular degradation. This condition causes a secretion defect of the mutant protease reflecting the severe ADAMTS13 deficiency in the patient' plasma.

**PREMIO SISET 2006*

C123

A CASE OF THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP) ASSOCIATED TO ADAMTS-13 GENE MUTATION

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ADAMTS-13 is important in maintaining the normal size distribution of vWF multimers. A severe deficiency of ADAMTS-13 activity, caused by either mutation of the ADAMTS-13 gene or by auto-antibodies to ADAMTS-13, is associated to TTP. We describe the case of 45-year woman first diagnosed with TTP in 1987 during pregnancy. Subsequently, she relapsed three times in 1993, once in 1996, and once in 2000. On each episode, she was treated with plasma exchange and steroid therapy, obtaining the complete remission. In 2001, the ADAMTS-13 testing became available and we prospectively followed the patient for ADAMTS-13 activity and inhibitors and, since 2005, we also measured anti-ADAMTS-13 antibody levels. In 2004 she relapsed after starting interferon for HCV-related chronic hepatitis. Plasma exchange and steroid therapy were resumed, without achieving a durable remission, as she relapsed after one month. She was then given chemotherapy and rituximab, without significant response. From January 2005 on, she is receiving periodic plasma infusion on the basis of platelet count and is continuing on this regimen so far. Measurement of ADAMTS-13 activity during clinical remissions and upon relapses from 2001 showed a severe deficiency (<5%) of this protease, which was associated with no significant inhibitory activity by mixing studies. No pathological levels of anti-ADAMTS-13 antibodies were found. The patient was then identified as a possible carrier of a true constitutive ADAMTS-13 deficiency. The DNA analysis of this patient detected homozygosity for the 3428 C>T in exon 25 of the ADAMTS-13 gene, which predicts the R1123C exchange in the TSP1-8 domain. The inherited nature of severe ADAMTS-13 deficiency was established by family analysis.

This mutation has been previously linked to Upshaw-Schulman syndrome. In our patient the onset of clinically overt disease manifested in the adult age during pregnancy, thus supporting the hypothesis that additional precipitating factors (i.e. infection, surgery, pregnancy) may determine the phenotypic manifestation.

C124

THE NATURAL VWF MUTANT P.R1306W, CAUSING A TYPE 2B VWD, BINDS CHLORIDE IONS WITH LOWER AFFINITY THAN WT VWF AND IS CLEAVED MORE EFFICIENTLY BY ADAMTS-13

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The regulatory mechanisms of VWF/ADAMTS-13 interactions are linked to the high conformational mobility and structure of VWF. Physiological concentrations of NaCl inhibit the hydrolysis of VWF by ADAMTS-13. This effect is due to the specific binding of chloride ions to VWF. Chloride binding to recombinant wild-type (WT) and p.R1306W A1-A2-A3 domains VWF mutant, responsible for a type 2B VWD, was investigated by monitoring the change of the intrinsic protein fluorescence. The temperature-dependent binding of Cl^- to both wild type (WT) and the natural mutant p.R1306W A1-A2-A3 domains of VWF is characterized by a large heat capacity change, ΔCp, equal to -1.05±0.18 Kcal mol^{-1} K^{-1}, and -0.4±0.02 Kcal mol^{-1} K^{-1} for WT and p.R1306W A1-A2-A3 domains, respectively. At any temperature, chloride affinity was higher for WT than for the mutant p.R1306W form. The values of ΔCp for chloride binding to WT and to the p.R1306W mutant imply that in both cases a vast apolar surface area is buried upon chloride binding as a consequence of a folding conformational transition, and that this phenomenon is much more evident in WT than in the p.R1306W mutant. Kinetic experiments using RP-HPLC methods showed that p.R1306W A1-A2-A3 domains are hydrolysed by recombinant ADAMTS-13 more efficiently than the corresponding WT form. Chloride ions inhibit allosterically hydrolysis by ADAMTS-13 of both WT A1-A2-A3 and p.R1306W A1-A2-A3 domains, although less efficiently in the latter case. In conclusion, chloride ions bind to p.R1306W VWF mutant with lower affinity as demonstrated in this study, and the rate of hydrolysis by ADAMTS-13 increases. This can contribute along with the enhanced binding of high molecular weight VWF multimers to platelets, to the loss of these VWF forms as usually observed in these patients.

C125

MOLECULAR MAPPING OF THE CHLORIDE BINDING SITE IN VWF: ENERGETICS AND CONFORMATIONAL EFFECTS ON THE VWF/ADAMTS-13 INTERACTION

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The regulatory mechanisms of VWF/ADAMTS-13 interactions are linked to the high conformational mobility of VWF. Physiological concentrations of NaCl inhibit the hydrolysis of VWF by ADAMTS-13. This effect is due to the specific binding of chloride ions to VWF. The molecular mapping and conformational effects of chloride binding to VWF were investigated by both denaturation and steady state kinetic studies. Urea-induced unfolding was measured in the presence of NaCl, CH3COONa and NaClO4 at pH 8.0, 25°C, for multimeric VWF, the recombinant A1-A2-A3 VWF domains and the 34/39 kDa fragment corresponding to the A1 domain. NaCl increased the stability to urea unfolding of both the A1-A2-A3 and A1 domains, whereas this effect was not observed for full length VWF. Chloride ions stabilize a folded conformation of VWF more efficiently than CH3COO- but less strongly than ClO4-, thus ruling out effects of ionic strength. Spectroscopic evidence was obtained that chloride binds to both the A1 and A1-A2 domain, but not to the isolated A2 domain. The temperature-dependent binding of Cl- to wild type (WT) A1-A2-A3 domains of VWF is characterized by a large heat capacity change, ΔCp, equal to -1.05±0.18 Kcal mol^{-1} K^{-1}. The values of ΔCp for chloride binding to WT A1-A2-A3 domains imply that a vast apolar surface area is buried upon chloride binding as a consequence of a folding conformational transition. Kinetic experiments showed that chloride ions inhibit allosterically hydrolysis by ADAMTS-13 of the A1-A2-A3 and A1-A2 domains, while this effect was absent in experiments using the isolated A2 domain. On the whole, these findings showed that the A1 domain contains the binding site of chloride ions, which control allosterically the availability of the Y1605-M1606 bond to the proteolytic attack of ADAMTS-13 in the A2 domain.

C126

INCIDENCE, CLINICAL-LABORATORY FEATURES AND MANAGEMENT OF ACQUIRED VON WILLEBRAND SYNDROME AND OTHER ACQUIRED DEFECTS OF HEMOSTASIS IN A COHORT OF 240 PATIENTS WITH CHRONIC LYMPHO-MYELOPROLIFERATIVE DISORDERS

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Background. Acquired von Willebrand Syndrome (AVWS) is a rare bleeding disorder with laboratory findings similar to those for congenital von Willebrand disease. The actual prevalence of AVWS in the general population is unknown because large prospective studies on this syndrome are not available. Retrospective data showed that AVWS is especially frequent in lympho- (LPD) or myeloproliferative (MPD) disorders. *Aims and design of the study.* To determine incidence, clinical-laboratory features and management of AVWS and other acquired hemo-

static defects, we have sequentially observed for one year our cohort of patients with chronic LPD/MPD. Exclusion criteria were platelet counts <70,000/uL and any therapies, including non-steroid anti-inflammatory drugs. *Methods.* A bleeding severity score derived from a detailed history of 11 symptoms. Screening tests: bleeding time (BT), prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT) and, if prolonged, PT-PTT-TT 50:50 mixing tests. Additional specific tests: FVIII/VWF activities (AVWS/HA); platelet nucleotides (acquired storage pool defects, ASPD); silice clotting time (SCT), Russel viper venom time (RVVT), anticardiolipin antibodies (ACA) for lupus anticoagulant-antiphospholipid antibodies (LAC/APA). *Results.* Among 458, 240 patients satisfied the inclusion criteria, with percentual (%) diagnosis of MGUS (38), ET (38), CLL (7), PV-CML-IMF (7), HD-NHL (5), MDS M (2), MM (2) and amyloidosis (1). Results are reported in the Table. In one year, severe mucosal (n=21) and non-mucosal (n=13) bleeds in LPD (n=12) or MPD (10) were treated with DDAVP (n=18), FFP/concentrates (n=4), IVIg (n=10), rFVIIa (n=2). *Conclusions.* AVWS and the other acquired hemostatic defects shown here are not so rare (9/16%) and can be severe in LPD/MPD. An early correct diagnosis should improve morbidity and mortality of patients with bleeding complications in chronic LPD/MPD.

Table.

Features	*Lymphoproliferative*	*Myeloproliferative*	*Total*
Case number (%)	122 (51)	118 (49)	240 (100)
Bleeding score (> 10)	30/122 (25)	18/118 (15)	48/240 (20)Abnorm
screening tests	57/122 (48)	22/118 (19)	79/240 (33)
Acquired defects:	21/122 (17)	38/118 (32)	59/240 (25)
1) AVWS	10/122 (8)	12/118 (10)	22/240 (9)
2) ASPD	0/ 122 (0)	19/118 (16)	19/240 (8)
3) LAC/APA	8/ 122 (7)	3/118 (3)	11/240 (5)
4) anti FVIII or X inhibitors	3/122 (2)	4/118 (3)	7/240 (3)

C127

THE VON WILLEBRAND DISEASE (VWD) TYPE 2A (II H): A UNIQUE VARIANT OF VON WILLEBRAND FACTOR (VWF) LINKED TO 3 DISTINCT MUTATIONS

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Type IIH VWD, classified now as type 2A is a unique VWF variant that has been shown to have abnormalities different from other variants previously described (IIC, IID, IIE, IIF and IIG), i.e. the absence of high molecular weight (HMW) VWF in plasma and platelets and the absence of the triplet structure on high resolution agarose (1.6% LGT) gel. The patient is a man who was 30-year-old at the time of diagnosis, with a life long bleeding history (epistaxis, ecchymosis and prolonged bleeding after dental extractions). Although, 5 additional family members were investigated (Am J of Hematol 32:287, 1989), only the propositus showed bleeding symptoms, suggesting a recessive inheritance of the disease. SSCP analysis was performed evaluating all exons of the VWF gene. Three distinct novel mutations, not identified in 100 normal chromosomes, were found: 604 C>T (R202W), 2546 G>A (C849Y) and 2546 G>A (R1583Q). Mutations R202W and R1583Q were found to be on the same allele, since were both identified in 2 propositus'relatives. The absence of HMW VWF in the propositus'platelets suggests a multimerization defect that could be due to mutations R202W (D1 domain) and C849Y (D' domain). In fact, both domains are involved in the multimerization process. However, the VWF multimerization is compromised only in the presence of both defects, since carriers of R202W present a normal multimeric pattern. The absence of a triplet structure, was confirmed in the propositus, his father and his daughter and is perhaps linked to R1583Q mutation. The presence of a mutation C849Y in the D' domain prompt us to investigate the VWF-Factor VIII binding in the propositus, that behaved similarly to a known type 2N heterozygous variant. Future expression studies maybe able to correlate the mutations to the patient phenotype and explain the absence of the triplet structure.

C128

INCIDENCE AND LABORATORY FEATURES OF THROMBOCYTOPENIA IN 43 PATIENTS WITH VON WILLEBRAND DISEASE TYPE 2B: CORRELATION WITH MOLECULAR DEFECTS AND ACQUIRED MODIFICATIONS OF VON WILLEBRAND FACTOR

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Background. Von Willebrand type 2B (VWD) is an inherited bleeding disorder caused by abnormal von Willebrand factor (VWF) that displays increased affinity to the platelet glicoprotein 1b α (GpIba). VWD 2B is due to a group of mutations clustered within VWF A1 domain and is characterized by binding of its high molecular weight multimers (HMW) to platelets often resulting in moderate-mild thrombocytopenia. Even though there are many case reports on thrombocytopenia associated with VWD 2B, retrospective and prospective studies in a large cohort of patients are not available. *Aims and design of the study*. to determine incidence and laboratory features of thrombocytopenia in VWD 2B, we have prospectively observed our cohort of 43 patients (18 families) previously characterized by VWF mutations. *Methods.* Data of platelet count with mean platelet volume (MPV) and morphologic evaluation of the blood smear to search for giant platelets or aggregates were associated with the history of physiologic or pathologic stress conditions such as pregnancy, infections, surgery or use of DDAVP. All patients were characterized by ristocetin induced platelet agglutination (RIPA) in the Platelet Rich Plasma (PRP), ristocetin cofactor activity (VWF:RCo) with VWF antigen (VWF:Ag), multimeric structure of VWF. Mutations within VWF A1 domain were searched for and confirmed by sequencing exon 28. *Results.* Among 43 VWD cases, a platelet count< 140,000 was found at baseline in only 11 (26%), but was observed after stress conditions in 34 cases (79%); no reduced platelet counts was found in 9 patients (21%) from two different families (R1308L, R1341Q).An increased MPV was found in 35 cases but giant platelet and aggregates in only 6 cases. All the phenotypic features were correlated to VWF mutations as reported in the Table. *Conclusions.* Based on these results, thrombocytopenia can be associated in most VWD 2B patients, especially when high levels of mutant VWF are triggered by physiologic and pathologic stress conditions. However, not all VWD 2B show thrombocytopenia and a relatively high degree of heterogeneity of this phenomenon occurs within patients characterized by the same molecular defects.

Table.

	Low Plt (<140×10³)				*Plt Morphology*	
Mutation (n)	*RIPA (mg/mL)*	*VWF:Ag (U/dL)*	*Basal (n)*	*Post stress (n)*	*MPV (μm³)*	*gp/aggr.*
R1306W (15)	0.65	27	4	15	10.3	3
R1308C (5)	0.72	40	2	5	11.5	2
R1308L (5)	0.50	37	0	0	9.1	0
I1309V (6)	0.40	79	2	6	11.8	0
V1316M (3)	0.50	45	2	3	9.2	1
P1337L (4)	0.50	39	0	4	9.5	0
R1341Q (4)	0.67	43	0	0	9.9	0
R1341W (1)	0.70	43	1	1	9.9	0

Nutrition factors/homocysteine

C129*

ADHERENCE TO MEDITERRANEAN DIET AND ANTHROPOMETRIC AND METABOLIC PARAMETERS IN THE MOLI-SAL PROJECT, AN OBSERVATIONAL STUDY IN THE ALTO MOLISE REGION

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Introduction. The Mediterranean diet is considered a healthy eating pattern with protective effects on chronic diseases. To assess the relation between the level of adherence to Mediterranean diet and anthropometric and metabolic variables, in the absence of clinical evidence of cardiovascular disease or diabetes. *Methods.* 536 healthy volunteers (248 men and 288 women, aged 18-96 y) from Alto Molise were studied. Blood pressure, blood glucose, and total cholesterol were measured using automatic devices (OMRON-HEM-705CP, ACCU-CHEK® compact and Accutrend® Roche Diagnostics, respectively). Food intake was evaluated with semi-quantitative food-frequency questionnaire, and a Mediterranean dietary score was created, based on traditional Mediterranean foods (vegetables, fruits, nuts, legumes, fish, meat, cereals, potatoes, dairy products, sweets, olive oil and alcoholic beverages). The score was divided in three tertiles, corresponding to low, medium and high adherence to Mediterranean diet. *Results.* The distribution of males and females in the three categories was similar (47%, 32%, 21% vs 41%, 35%, 24%, p=0.32). Females consumed more frequently cheese, cakes, olive oil, vegetables but less frequently cereals and alcohol than males (p<0.0001). A higher score was positively associated with age (p<0.0001) both in males and females. In men, no association was found between dietary score and anthropometric or metabolic variables. Higher values of diastolic and systolic blood pressure, BMI and W/H ratio were found in females belonging to the highest tertile. However after adjustment for age, only systolic blood pressure remained significantly associated with dietary score. *Conclusions.* The adherence to the Mediterranean diet in a mountain area of Molise depends on age, possibly because younger people tend to follow a westernized diet. The association between higher adherence to the Mediterranean diet and higher systolic blood pressure in females could depend on the higher consumption of cheese or other salted foods.

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**PREMIO "FONDAZIONE POLIDORO"*

C130*

COFFEE CONSUMPTION AND RISK OF CORONARY HEART DISEASE: A META-ANALYSIS

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Introduction. Coffee is among the most widely consumed beverages in the world. During the past decades the relationship between habitual coffee drinking and coronary heart disease (CHD) has been assessed in numerous studies, with conflicting results. Aim of this paper was to systematically examine the published data on the association between habitual coffee consumption and risk of CHD. *Materials and Methods.* A systematic literature search of MEDLINE, EMBASE, Web of Science and the Cochrane Database up to April 2006 was performed using a combined text word and MeSH heading search strategy. Studies were eligible if they had reported estimates of the association with CHD for the different consumption of coffee, as reported by cups per day. *Results.* 13 case-control studies and 9 cohort studies were identified, incorporating 9,487 cases of CHD and 27,747 controls for case-control studies, and more than 370,000 participants followed for a time ranging from 2 to 44 years for cohort studies. The summary odds ratios (ORs) of included case-control studies showed a dose-dependent relationship with the occurrence of CHD, with statistically significant estimates for the highest categories of coffee consumption considered: 1.83; (95%CI 1.49-2.24; p<0.0001) for the highest consumption category (≥5 cups/day), and 1.33 (95%CI 1.04-1.71; p<0.0001) for the second highest category (3-4 cups/day), while no significant association emerged for low daily intake of coffee (<2 cups/day) 1.03 (95%CI 0.87-1.21; p=0.45). The analysis of long-term follow-up cohort studies showed a significant association only between the highest category of consumption of coffee and CHD, with a relative risk (RR) of 1.24 (95%CI 1.04-1.47; p=0.02), whereas no association of an increased risk of CHD for the second and third categories of coffee intake, with RRs of 1.14 (95%CI 0.96-1.36; p=0.14), and 1.04 (95%CI 0.87-1.24; p=0.14) for the second and third highest category respectively, was found. *Conclusions.* The present meta-analysis investigating the association between coffee intake and risk of CHD indicates that a low-to-moderate daily consumption of coffee consumption is not significantly associated with a higher risk of CHD.

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**PREMIO "FONDAZIONE POLIDORO"*

C131

INFLUENCE OF DIETARY FISH INTAKE ON INFLAMMATORY AND RHEOLOGICAL PARAMETERS: AN INTERVENTION STUDY

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Introduction. Fish intake has long been indicated as a protective dietary factor for cardiovascular diseases, due to the beneficial effects of its content of omega-3 polyunsaturated fatty acids (EPA and DHA). Numerous studies have demonstrated that fatty acid profile of cultured fish diet has a strong impact on the fatty acid profile of the lipid deposited in muscle. Aim of this study was to evaluate the influence of short-term dietary intake of fish on biomarkers related to the atherosclerotic process. *Methods.* In 7 dyslipidemic subjects (4 females; 3 males) with a mean age of 53.1 years we evaluated lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and tryglicerides) inflammatory markers (interleukin-6 and interleukin-8), haemorheological profile [whole blood viscosity(WBV), plasma viscosity, erythrocyte filtration rate], platelet aggregation and platelet function on whole blood (PFA) before (T0) and after a dietary intervention with 800 g of Orbetello farmed sea bass (Dicentrarchus labrax) per week for 10 weeks (T1). *Results.* Tryglicerides tend to be lower at T1 (143.8±43.9) than at T0 (170.1±91.3 mg/dL). Moreover, a favourable change within the inflammatory pattern, as seen by lower levels of interleukin-6 and interleukin-8 was observed at T1 (1.6±1.2 pg/mL for interleukin-6 and 12.2± 6.6 pg/mL, for interleukin-8) with respect to T0 (1.9± 1.2 pg/mL and 17.4± 11 pg/mL for interleukin-6 and 8, respectively). With regard to haemorheological parameters, a significant (p=0.04) improvement in WBV at the highest shear rates was reported after 10 weeks of fish dietary intake (WBV 11.040 sec-1: 7.8±0.9 vs. 8.7±1.3; WBV 20.400 sec-1: 4.3±0.07 vs. 6.4±0.4; WBV 94.500 sec-1: 4.3±0.07 vs. 4.5±0.3, for T1 and T0, respectively). *Conclusions.* Dietary short-term intake of fish seems to impose favourable biochemical changes in dyslipidemic subjects, with regard to lower circulating levels of markers of atherosclerosis, such as lipid parameters, inflammatory markers and haemorheological profile.

C132

ALCOHOL DOSING AND TOTAL MORTALITY IN MALES AND FEMALES: AN UPDATED META-ANALYSIS OF 34 PROSPECTIVE STUDIES

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Introduction. Moderate consumption of alcohol is protective against vascular disease, but its association with total mortality is controversial, especially in females. We performed an updated meta-analysis of prospective studies, with the aim to assess the relationship of alcohol doses with mortality for any cause, both in males and females. *Methods.* Articles were retrieved from those listed until December 2005 by PUBMED (www.pubmed.gov), supplemented by references of the selected articles. Seventy-three publications were identified. Two reviewers selected 34 prospective studies on males and females, for a total of 1 015 835 subjects (69% males) and 94 533 deaths. Data were pooled with a weighed, least-squares regression analysis of second-order

fractional polynomial models. *Results.* A J-shaped relationship between increasing amounts of alcohol intake and total mortality was observed in adjusted studies, both in males and in females (Figure). Low to moderate consumption of alcohol (up to an average of 38 grams/day in males and of 18 grams/day for females) significantly reduced total mortality (maximum protection being 18%, 99%CI: 13%-22% in females and 17%, 99%CI: 15%-19% in males), while higher doses increased it. The degree of protection by alcohol was lower in USA as compared to Europe, but in males only. When adjusted and unadjusted data from the same studies were compared, the maximum protection was only reduced from 19% to 16%. *Conclusions.* Women are more exposed than men to all causes of deaths at comparable moderate or high level of alcohol consumption, probably due to different metabolism of alcohol and increasing risk of various cancers. Nevertheless, low alcohol consumption significantly reduces total mortality both in males and in females. Our findings, while confirming the hazards of excess drinking, strongly indicate the existence of significant windows in which the overall healthy effect of alcohol is greater than the harm.

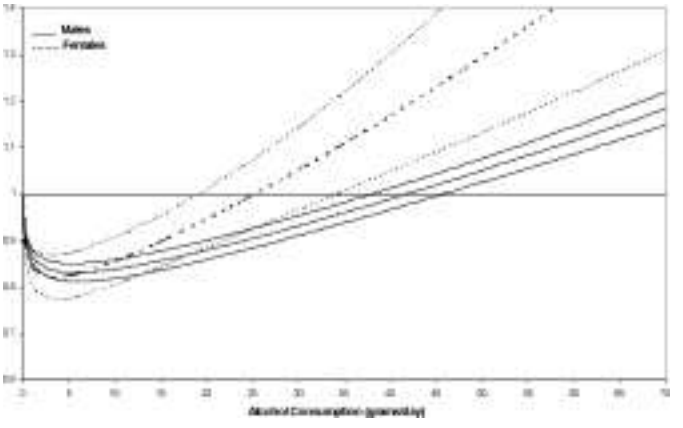

Figure 1.

C133

GENETIC AND ENVIRONMENTAL DETERMINANTS OF HOMOCYSTEINE METABOLISM IN EUROPEAN COUNTRIES AT DIFFERENT RISK OF MYOCARDIAL INFARCTION: REPORT FROM IMMIDIET

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Introduction. Homocysteine (Hcy), of which the levels are influenced by both genetic and nutritional factors, is an independent predictor of CAD. We evaluated genetic and environmental factors that might influence Hcy levels in populations at different CAD risk. *Methods.* 542 Italian subjects (IT, 271 couples), 536 Belgian subjects (BE, 268 couples) 526 English subjects (UK, 263 couples), and 414 subjects from 207 couples living in Belgium and formed by a member of Italian origin (MI) and their spouse of Belgian origin (MB) participated a population-based cross-sectional study: the IMMIDIET study. Adherence to a diet affecting Hcy metabolism was scored using data from semi-quantitative food frequency questionnaires. *Results.* There was a significant difference in the levels of Hcy and of vitamins related to its metabolism among the three countries. English couples showed the lowest levels of Hcy and the highest of folic acid and vitamin B6, both in males and females. Mixed Italian and Belgian females differed in plasma vitamin B12 levels ($p<0.0005$), while males slightly differed in folate levels ($p=0.051$). MTHFR T-allele was also differently distributed among countries (0.31 in UK, 0.31 in BE and 0.45 in IT, $p<0.0001$, and 0.45 in MI and 0.34 in MB, $p<0.005$). In multivariate analysis older age, sex, country, MTHFRC/T genotype, smoking, physical activity, vitamin supplements, and nutritional score were associated to Hcy levels in the 3 populations.

Table. Plasma Hcy and vitamins levels according to gender and population.

	Italians	*Belgians*	*UK*	*p*
Males				
Hcy (µmol/l)	13.9±6.8	13.4±5.4	11.2± 3.4	0.0001
Folate (ng/mL)	5.4± 2.0	6.8±2.2	8.3±2.7	0.0001
Vit. B 12 (pg/mL)	443±183	327±126	405±166	0.0001
Vit. B 6 (pg/mL)	53.9±31.8	77.5±55.5	84.7±67.3	0.0001
Females				
Hcy (µmol/L)	9.7±3.0	11.2±4.0	9.5± 3.3	0.0001
Folate (ng/mL)	6.2± 2.3	7.4±2.7	9.0±2.9	0.0001
Vit. B 12 (pg/mL)	473±193	308±125	423±205	0.0001
Vit. B 6 (pg/mK)	41.9±30.3	64.5±44.3	94.6±118.2	0.0001

However, after addition of vitamin levels to the model, associations of score and physical activity with Hcy levels disappeared and was weaker for smoking and vitamin supplement intake. Similar findings were found in mixed couples. *Conclusion.* Our results support the key effect of folic acid, vitamin B12 levels and MTHFR polymorphism on Hcy levels, in different countries. However, differences among countries in Hty levels cannot be explained by difference in dietary habits, vitamin intake or genetics.

C134

HYPERHOMOCYSTEINEMIA AND MORTALITY AFTER CORONARY ARTERY BYPASS GRAFTING

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Objectives. to evaluate the prognostic impact of hyperhomocysteinemia (HHcy) after coronary artery bypass grafting (CABG) surgery. *Background.* The independent prognostic impact, as well as the possible causal role, of HHcy in coronary artery disease (CAD) is controversial. No previous study specifically addressed the relationship between HHcy and mortality after CABG surgery. *Methods.* We prospectively followed 350 patients who underwent elective CABG between May 1996 and May 1999. At baseline, fasting total homocysteine (tHcy) levels were measured in all participants, and a post-methionine loading (PML) test was performed in 77.7% of them (n=272). *Results.* After a median follow-up of 58 months, 33 patients (9.4%) had died, 25 because of cardiovascular events. HHcy, defined by levels higher than the 90th percentile (25.2 µmol/L) of the population's distribution, was significantly associated to total and, especially, cardiovascular mortality (20% versus 5.9%, $p=0.002$ [log-rank test 9.76]). The PML test had no prognostic value. After multiple adjustment for other univariate predictors by Cox regression logistic models, including statin therapy (the most powerful predictor in uni-/multivariate analyses), high-sensitivity C Reactive Protein (hs-CRP) levels, and all known major genetic (MTHFR 677C>T polymorphism) and non-genetic (B-group vitamin status and renal function) tHcy determinants, HHcy remained an independent prognostic factor for total and cardiovascular mortality (HRs: 3.34, 95% CIs 1.28 to 8.71, $p=0.014$; and 4.58, 95% CIs 1.67 to 12.6, $p=0.003$, respectively). *Conclusions.* HHcy is an important prognostic marker after CABG, independent of modern drug therapy and biomarkers.

C135

HOMOCYSTEINE LEVELS IN AMNIOTIC FLUID: RELATIONSHIP WITH BIRTH-WEIGHT

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Hyperhomocysteinemia could play a role in the placenta similar to that played in adults who are at risk of thrombosis. Moreover, hyperhomocysteinemia in women can be associated with the birth of small for gestational age (SGA) newborns, but there are discrepancies on this issue. To date, there is no biochemical marker predictive of SGA in one pregnancy. We verified whether there is a relationship between homocysteine in amniotic fluid at mid-pregnancy and birth-weight. Amniotic fluid was obtained from 459 healthy women undergoing mid-trimester amniocentesis (17.1+1.2 weeks) because of maternal age. Homocysteine levels were measured in 434 (10 twin) pregnancies. Femur length (FL) and biparietal diameter (BPD) was also measured. Outcomes of pregnancies were recorded. 233 (53.7%) foetuses were males, 201 (46.3%) females. Reference interval were 1.04+0.72 uΔmol/L, (95% C.I. 0.43-2.41). An univariate analysis showed the presence of an association with gestational age, FL, BPD. A multiple linear regression showed that homocysteine levels are significantly associated with FL (p<0.001) and BPD (p=0.011). After excluding twin pregnancies, 31 newborns (7.3%) were classified as SGA. Mean birth-weight was 2390g in SGA, whereas it was 3360g in 393 adequate for gestational age (AGA) (p<0.001). The adjusted mean level of homocysteine was significantly lower in AGA (1.01 umol/L; 95%CI: 0.94-1.08) than that measured in pregnancies that ended with a SGA (1.29 uΔmol/l; 95%CI: 1.05-1.51; p=0.03). These data provide in a large setting reference values for homocysteine in amniotic fluids. Moreover, they suggest that homocysteine levels in amniotic fluids may be higher in pregnancies that end with a SGA newborn.

C136

MODERATE HYPERHOMOCYSTEINEMIA: RELATIONSHIP WITH LIVER FUNCTION PARAMETERS

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While moderate hyperhomocysteinemia (HHcy) is recognized as an independent risk factor for ischemic cardiovascular and cerebrovascular events and for venous thromboembolism, four large placebo-controlled trials of secondary prophylaxis with vitamin supplementation have shown no benefit of homocysteine-lowering strategies. In contrast, analysis of death rates in the US and Canada demonstrated a significant reduction in stroke-related mortality after the introduction of folate fortification. HHcy may share properties of a marker of disease, with no effect on the outcome of homocysteine-lowering treatments, and of a causal factor when reflecting vitamin deficiency in the absence of coexisting diseases. Hypertension and impaired kidney function are associated with HHcy; there is no information on whether impaired liver function is associated with an increase in tHcy levels. Within the frame of the Cremona Homocysteine Study, we have analyzed the relationship of tHcy levels with parameters of liver function. At univariate analysis, adjusted for gender and age within deciles 2 to 9 of the tHcy distribution (7.0-29.1 μmol/L), tHcy levels were negatively correlated with B vitamins (r partial <-0.072, p≤0.003) and positively with AST (r partial 0.196, p=0.0001), ALT (r partial 0.104, p=0.0001). alkaline phosphatase (r partial 0.072, p=0.004) and γ-GT levels (r partial 0.328, p=0.0001). tHcy levels were also positively correlated with cystatin C levels, blood pressure, triglycerides, smoking and alcohol intake (r partial > 0.168, p≤0.009). With respect to subjects with no apparent disease at enrolment, subjects with hypertension (p=0.001), glucose intolerance (p=0.003), hyperlipidemia (p=0.019), COPD (p=0.002) and with liver disease or pancreatitis (p=0.009) had higher tHcy levels, as well as subjects on regular hypotensive (p=0.006) or hypolipemic treatment (p=0.012). At multivariate analysis, in addition to gender, age, folate, vitamin B12 and cystatin C (p=0.0001), γ-GT (r partial 0.332, p=0.0001), AST (r partial 0.200, p=0.0001), and ALT (r partial-0.108), but not alcohol intake, were independent determinants of tHcy levels. γ-GT, AST and ALT levels remained significant independent determinants also when extending analysis to deciles 1 to 10 of the tHcy distribution. These results strongly suggest that impaired liver function is associated with HHcy, independent of vitamin status.

POSTERS

Platelet abnormalities

P001

PREVALENCE OF ANTI-HLA AND ANTI-GPIIB/IIIA ALLO-IMMUNIZATION IN PATIENTS WITH GLANZMANN THROMBOASTHENIA: EXPERIENCE OF A SINGLE CENTER

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Background. Platelet transfusions, the main therapy of Glanzmann Thromboasthenia (GT), can induce an allo-immunization against HLA antigens and GPIIb/IIIa complexes, with a possible reduction of efficacy of subsequent treatments. *Aims.* To investigate the development of allo-antibodies anti-HLA antigens and anti-GPIIb/IIIa complexes in GT transfused patients, and evaluation of efficacy of replacement therapy. *Patients and methods.* From 1975 onwards, we have followed 17 GT patients; 12 type I, 3 type III, 2 not classified; 8 men, 9 women; median age at diagnosis 9.8 years (range 1-44.5); median age at the time of this study, 35.5 years (range 23.6-68.5). Our patients showed at least once in their life the following symptoms: 10/17 epistaxis; 5/17 gastrointestinal hemorrhage; 5/17 oropharingeal hemorrhage; 4/17 muscle hematoma; 2/17 bleeding for traumatic injury; 2/17 hemarthrosis; 2/17 hematuria; 1/17 intracranial hemorrhage; 1/17 hematothorax; 1/17 otorrhagia. Five/9 women experienced meno-metrorrhagia. Ten major and 22 minor surgical procedures have been performed. Two spontaneous deliveries and 3 cesarian sections with 5 live births have been observed; moreover, 2 abortions occurred, 1 spontaneous and 1 voluntary. Globally, 9/17 patients have been transfused with platelets and red blood cells (RBC); 5/17 only with platelets; 2/17 only with RBC. One patient has never been transfused. Platelet transfusions have always been hemostatically effective. Fifteen/16 transfused patients have been investigated for allo-antibodies, anti-HLA and anti-GPIIb/IIIa. *Results.* The positivity for allo-antibodies has been demonstrated in 4/15 patients (27%): isolated for anti-HLA in 2; isolated for anti-GPIIb/IIIa in 1; combined in 1. *Conclusions.* The prevalence of allo-immunization (27%) is inferior to recent literature data (50%). While positivity for anti-HLA (3/15, 20%) agrees with the recent literature data (22%), positivity for anti-GPIIb/IIIa (13%) is inferior (35%). Presence of allo-immunization did not compromise the efficacy of platelet transfusions.

P002

RITUXIMAB IN REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

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We investigated the efficacy of Rituximab, a chimeric anti-CD20 monoclonal antibody effective in B-cell depletion, in resistant ITP patients.

Eleven adult ITP patients (2 males, 9 females; median age 46.3 years; 28.6-67.8) were treated with Rituximab (375 mg/m^2/weekly for four doses). Median time between diagnosis and Rituximab start: 4.1 years (0.2-33.1 months). All patients had already received at least two lines of therapy (median 3; 2-6). Median platelet count at Rituximab start: 10×10^9/L (3-20×10^9/L). Response definitions: complete response (CR), platelet count ≥150×10^9/L; partial response (PR), >50 <150×10^9/L; minimal response (MR), >20 ≤50×10^9/L; no response (NR) ≤20×10^9/L. After completing therapy, patients were evaluated for platelet count after 1 and 3 months, and thereafter every 3 months until relapse or start of a different treatment. Peripheral blood B lymphocytes were evaluated by flow-cytometry as CD19+ cells before treatment, 1 and 3 months after stopping therapy, and then every 3 months up to recovery. Five responses (1 CR, 3 PR, 1 MR; 45%) and 6 NR (55%) were observed. Two relapses occurred 5 and 18 months after response. Median follow-up of all treated patients: 8.7 months (1.8-31.1); median follow-up of all responsive patients: 13.7 months (2.6-18.7). Median baseline value of peripheral blood CD19+ B-cells: 128×10^6/L (58-371). One month after completing therapy, 6/8 evaluable cases showed absence of CD19+ cells and 2/8 showed a count of 9 and 4.4×10^6/L CD19+ cells, respectively. At the last available control (median follow-up: 11 months; 1-28), 8/9 patients had still not recovered the baseline CD19+ cell count (median value: 6×10^6/L; 0-295). Five/11 (45%) ITP patients had an early response (1 CR, 3 PR, 1 MR), persisting in 3 cases. No late responses were observed. The response was independent from the post-therapy CD19+ cell count.

P003

THROMBOCYTOPENIA AND CONGENITAL RADIAL UPPER EXTREMITIES DYSPLASIA: NOT ONLY THROMBOCYTOPENIA ABSENT RADIUS (TAR) SYNDROME, BUT A CONTINUUM SPECTRUM OF DISEASES. A CASE REPORT

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Oliver and Shaw in 1959 described first TAR syndrome. Diagnostic criteria of TAR were posed in 1969 by Hall and coll.: bilateral radii absence with thumbs presence (thumbs are absent in other not TAR radial aplasia). In TAR patients thrombocytopenia is always present below 150000/mcl, symptomatic in over 90% of cases and often transient. TAR might be associated to congenital heart disease (as interatrial septal defect), lower limbs anomalies, epilepsy, intracranial vascular malformation, uterus and ovary agenesis, scoliosis. The genetic basis of TAR syndrome is uncertain. Remains unclear if TAR syndrome is transmitted as autosomal recessive or autosomal dominant condition with variable penetrance. Case associated with consanguinity are rare. We report a case of 16 years old male patient underwent to our observation for thoracic pain. Electrocardiogram, chest x-rays, echocardiogram, cardiac enzymes were normal. At complete blood count he showed as only altered feature platelets 124000/mcl, in two different controls. Biohumoral and autoimmunity tests, viral serologies, abdominal ultrasonography were normal. Bone marrow biopsy showed a reduced megakaryocytes number with a normal c-mpl expression. No karyotypic alterations were found on bone marrow cells. Clinical examination showed bilateral thumbs dysplasia and light scoliosis documented also at x-rays. Patient referred in clinical history surgical intervention for congenital interatrial septal defect in 1993. Parents and other two brothers were normal. Thus diagnosis of thrombocytopenia associated with upper extremity dysplasia was posed. Congenital upper extremity radial dysplasia represents a continuum spectrum of disease extending from radial aplasia and phocomelia to minimal radial dysplasia. These conditions are frequently associated with minimal or mild thrombocytopenia, as in TAR syndrome, and should be kept be in mind in congenital thrombocytopenia diagnosis, also in childhood or in adolescence. Moreover a correct diagnosis is important because this pathologic condition is frequently associated with other organ or systems malformation.

P004

VALPROIC ACID DOES NOT AFFECT PRIMARY HEMOSTASIS

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Valproic acid (VPA) is used in the treatment of epilepsies. One of the reported side effects of VPA is bleeding, which is not completely explained by the mild thrombocytopenia that develops in some treated patients. Small-sized, non-controlled studies, showed that VPA may have additional inhibitory effects on primary hemostasis. Aim of our study was to evaluate the effects of VPA on some parameters of platelet function and on the plasma levels of von Willebrand factor (VWF). 20 patients (median age 30y, range 12-43y, 9 men, 11 women) on long-term VPA treatment for epilepsy and 20 sex- and age-matched controls (14 patients treated with other anti-seizure medications and 6 healthy controls, 30y, 13-41y, 9 men, 11 women) were studied. The following parameters were evaluated: platelet count, PFA-100 closure times, plasma vWF ristocetin cofactor activity, platelet nucleotides, platelet aggregation induced by ADP (2 and 4 μM), collagen (2 μg/mL), U46619 (1 μM) or thrombin receptor activator peptide (10 μM) in citrated platelet-rich plasma. Specific platelet responses that are mediated by the 3 platelet P2 receptors were also studied: 1) P2Y1-dependent platelet shape change

induced by ADP (0.1, 0.5 and 1 μM); 2) P2Y12-dependent inhibition of adenylyl cyclase by ADP (0.1 and 1 μM); 3) P2X1-dependent platelet shape change induced by α,β-methylene-ATP (1 and 10 μM) in PRP in the presence of 0.5 U/mL apyrase, to prevent receptor desensitization. The mean values of none of the above parameters were significantly different between the two study groups. None of the VPA-treated patients had abnormalities of any of the studied parameters. In conclusion, our control study involving 20 patients on chronic VPA treatment, failed to detect abnormalities of platelet function or plasma vWF levels. Therefore, the causes of the observed bleeding complications in patients on chronic VPA treatment remain to be elucidated.

P005

THROMBOTIC THROMBOCYTOPENIC PURPURA: SIX-YEAR RETROSPECTIVE ANALYSIS OF TREATMENTS AND OUTCOMES

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Thrombotic thrombocytopenic purpura (TTP) results from occlusive microangiopathy induced by extremely adhesive large von Willebrand Factor multimers, unusually secreted by endothelium, due to genetic or acquired (autoantibody-mediated) deficiency of the metalloprotease ADAMTS-13. Schistocytic haemolytic anaemia and consumptive thrombocytopenia, with possible severe haemorrhagic diathesis and multiorgan ischemia, are main clinical features. Early recognition and treatment of TTP result in favourable outcome but diagnostic uncertainties and inadequate treatment still have dramatic consequences. We reviewed treatments and outcomes of TTP cases diagnosed at our Centre between 1999 and 2005. Twenty-two patients (17 women, 5 males, mean age 35 yrs, range 24-71) and 29 events were analyzed (6 patients -27%- relapsed, 1 twice). Among women, 8/17 cases were pregnancy-related (47%); other associated conditions were cancer (23%), drug intake (23%) and thyroid disease (18%). Median time between symptom onset and diagnosis was 44 hrs (range 6-148). In two cases relapses were diagnosed by laboratory follow-up. Eight patients, including one relapse, received steroids (prednisone 0.5-1 mg/Kg or equivalent) and fresh frozen plasma (FFP); complete remission was achieved in 5 (62.5%); increase of steroid doses and plasma exchange (PE, at least 7 procedures) were needed in one and two patients, respectively. Fourteen patients and 6 relapses were treated by steroids, FFP and PE started within 24 hrs from the diagnosis, complete remission being obtained in 9 patients (64%) and 3 relapses; two patients and one relapse needed another PE course; vincristin was also added in one relapse (1 mg, four doses in two weeks). Three patients died (14%), but only in one patient death was exclusively due to TTP. In this case-series, it is likely that severity of clinical presentation and time from symptom onset had a role in treatment decision making. Moreover, a poor prognosis, despite early and intensive treatment, is related to severe underlying diseases.

P006

PROGRESSIVE SEVERE RENAL INSUFFICIENCY IN JUVENILE THROMBOCYTOPENIA

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We report a case of rapid and severe renal insufficiency after streptococcal pharyngitis in a 16 years old Romanian male with diagnosis of ITP. Thrombocytopenia was observed at seven years old age. After unsuccessful steroid treatment, patient underwent splenectomy without platelet recovery. Successive bone marrow aspiration showed augmented megakaryocytes, compatible with diagnosis of ITP. The patient arrived in Italy two years ago. Six months ago he was recovered into the Nephrology Department with suspect of glomerulonephritis, because of the onset of haematuria after an episode of streptococcal pharyngitis. Clinical conditions quickly become worse and due to the rapid onset of acute renal insufficiency the patient underwent dialysis treatment. At admission patient showed: platelet count 20.000/uL, bleeding time 7' (14' after disease progression). Blood smears examination showed giant platelets, optical platelet count revealed 120.000 Plt/uL; granulocytes presented cytoplasmatic inclusions. In suspect of congenital thrombocytopenia, due to mild thrombocytopenia and cytoplasmatic inclusions in granulocytes, blood smears were evaluated for granulocytes distribution of the non muscle myosin heavy chain IIa (NMMHC-IIA); the pattern observed was suggestive for MYH9 gene mutation. DNA analysis confirmed MYH9 gene mutation (5818delG). MYH9-related diseases are autosomal dominant macrothrombocytopenias characterized by different associations of clinical and laboratory signs (sensorineural hearing loss, cataract, nephritis, polymorphonuclear Dohle-like bodies). The patient did not show ocular or hearing defects, but unusually rapid and progressive renal failure; previous splenectomy may have played a role in this evolution. Congenital syndromes must be considered in paediatrics thrombocytopenias; accurate blood smear evaluation may be helpful in diagnostic evaluation. Misleading of diagnosis may grow worse clinical outcome of patients.

Platelets: biochemistry, physiology and methods

P007

HOMOCYSTEINE, OXIDATIVE STRESS AND NITRIC OXIDE IN TYPE 2 DIABETES

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Mild hyperhomocysteinemia is considered a risk factor for various arterial thrombosis. The pathogenic mechanisms of homocysteine are still not clear. However oxidative damage may have a role. The aim of this study was to assess the contribution of plasma homocysteine levels to platelet oxidative stress and platelet activation. The study was performed on a group of 34 males with type 2 diabetes and 36 healthy subjects matched for sex and age. Patients and healthy subjects were undergone to laboratory evaluation for plasma homocysteine levels and other metabolic parameters. In both groups of subjects platelet reactive oxygen species, nitric oxide and cGMP levels were measured. Moreover the GSH content in platelets of patients and of controls was assayed. Plasma homocysteine levels were significantly (p<0.0005) increased in patients (9.6±1.4 μmol L-1) compared with healthy subjects (7.6±1.3 μmol L-1). The basal level of reactive oxygen species significantly (p<0.0005) higher in patients than in controls was found. In addition platelets of patients were more responsive to thrombin or collagen as reactive oxygen species formation compared with healthy subjects ones. On the contrary nitric oxide, cGMP and GSH content were decreased in platelets of patients. Results of the present study show that mild hyperhomocysteinemia is associated with enhanced oxidative stress in platelets of type 2 diabetic patients. The oxidative stress may result from elevated platelet free oxygen radical generation, decreased GSH intracellular content and decreased NO bioavailability. Through these mechanisms homocysteine could promote platelet hyperactivity contributing to the development of platelet related vascular complications.

P008

PLATELET ACTIVATION IN RETINAL VEIN OCCLUSION

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Retinal vein occlusion (RVO) is a common vascular occlusive disease affecting elderly but also young patients. The pathogenesis of RVO has not yet been clarified. However degenerative changes of the vessel wall, abnormal perivascular changes and abnormal haematological factors constituite the primary mechanism of vessel occlusion. In these events platelets could play a very important role. The following is a study on platelet response to collagen, to deeply investigate some steps of the signalling pathway involved. Experiments have been carried out on a selected group of 30 RVO patients, which have been compared to a group of 25 healthy subjects matched for age, sex and clinical characteristics. In resting and activated platelets of both groups of subjects p72syk phosphorylation, PLCγ2 phosphorylation and activation, PKC activation, intracellular calcium levels and nitric oxide formation were measured. p72syk and PLCγ2 phosphorylation were determined by immunoblotting techniques. PKC activation was detected following the p47 (pleckstrin) phosphorylation in [32P] loaded platelets. Intracellular calcium was assayed in FURA 2/AM loaded platelets by a spectrofluorimetric method. NOx (nitrite+nitrate) was detected by a spectrometric method. Results have shown that platelets of patients were more responsive to collagen than those of healthy subjects. The difference between the two groups was significant (p<0.0005) at low collagen concentrations (0.5-1.0 μg mL-1). In stimulated platelets of patients increased phosphorylation of p72syk and PLCγ2 was found. Also PKC was more activated in patients. In addition intracellular calcium rise induced by collagen was significantly increased in patients. RVO patients showed a lower basal content of nitric oxide both in resting and stimulated platelets compared to healthy subjects. Altogether these results suggest that the high platelet response to collagen shown in patients might be an important factor in the development of RVO, contributing to the thrombogenic effects.

P009

THE CONTRIBUTION OFα2β1 INTEGRIN IN THE ACTIVATION OF GLYCOPROTEIN GPVI BY COLLAGEN UNDER FLOW CONDITION

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We studied the role of platelet integrin α2β1 and glycoprotein GPVI in stabilizing adhesion and modulating signal transmission of Fluo 3-AM loaded platelets perfused onto a surface of acid-soluble collagen type I at wall shear rate of 250 s-1. We analyzed concurrently the instantaneous velocity and [Ca++]i in single platelets interacting with collagen using a video-imaging method characterized by high-speed image acquisition (25 frames/s) and high performance software. Perfusion of washed platelets resulted in single platelet adhesion that displayed a series of transient [Ca++]i elevations. Two distinct peaks could be defined on the basis of [Ca++]i levels, duration and sensitivity to EGTA. Type αï¿½ like Ca++ oscillations were characterized by a rapid increase of 0.2 to 2 μM, lasting for 0.5-2.5 seconds, EGTA insensitive and prevented by BAPTA-AM. Type γ like Ca++ peaks were characterized by a maximum level >2-3 μM lasting for several seconds (10-60) and stable adhesion to the collagen surface and abolished by EGTA. MoAb against integrin α2β1 prevented platelet adhesion to collagen surface and the appearance of both type α and γ like peaks. Whereas only type γ like peak, which involves a transmembrane Ca++ flux, was inhibited by an anti-GP VI MoAb (Fab 9O12.2). Inhibitors of PI3-K (wortmannin and LY294002), PLC (U73122) and PKC (Ro31-8220) prevented the appearance of both Ca++ peaks and platelet stable adhesion. The concurrent blockage of P2Y1 and P2Y12 ADP receptors did not inhibited either the two Ca++ peaks or platelet arrest time. Similar results are obtained with ASA. Finally, GPVI KO mice platelets interacting with type I acid-soluble collagen elicited the appearance of α like peaks only. Our results demonstrate that α2β1 partecipates in the initial stage of platelet adhesion and activation and regulates the activation of GPVI with no need for secondary mediators.

P010

PLATELET RECEPTORS INTERPLAY IS REGULATED BY SHEAR STRESS

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We studied the role of the GPIb-V-IX complex, integrin α2β1 and GPVI on platelet adhesion and activation to collagen under increasing shear stress. We analyzed concurrently the percentage of adhering platelets and Ca++ oscillations in single platelets loaded with FLUO 3-AM by using a videoimmaging apparatus characterized by high speed image acquisition and a high performance software. Platelets were perfused onto a surface of type I acid soluble collagen which was incubated with control buffer or with equimolar concentrations of recombinant A2 or A1-A3 domains in the presence of an αIIbβ3 monoclonal antibody (MoAb). In some experiments, purified VWF was used with or without an αIIbβ3 MoAb. Experiments were run at increasing shear rates of 600, 940, 1500 and 4500 s-1. Platelet adhesion and activation at 600 s-1 in the collagen control, was similar to that observed with either A2 or A1-A3 domains and were not influenced by addition of an anti GPIb MoAb. Increasing the shear rates from 940 to 4500 s-1 a decrease in platelet adhesion and activation was observed in the collagen control and in that with the A2 domain, reaching >90% inhibition at 4500 s-1, while with collagen + A1-A3 domain, platelet adhesion and activation increased up to 1500 s-1 with a slow decrease from 1500 to 4500 s-1. From 940 to 4500 s-1 an increased platelet translocation velocity was also observed which was abolished by an anti GPIb MoAb. In the presence of collagen plus purified VWF an increase in platelet adhesion and aggregation was observed by increasing the shear rates. Our results demonstrate that under low shear stress conditions collagen receptorsïï¿½ α2β1 and GPVI are those involved in firm platelet adhesion and activation while at higher shear stress, adhesion and activation were obtained by the concerted action of GPIbα and αIIbβ3.

P011

PLATELETS MODIFY LDL THROUGH GP91PHOX-DEPENDENT O2- FORMATION

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Oxidative stress-mediated LDL modification has a key role in initiation of atherosclerotic process. Platelets produce reactive oxidant species upon stimulation with agonist but it is uncertain if they are able to oxidatively modify LDL. Human platelets taken from healthy subjects were incubated with LDL and then stimulated with collagen. Compared to unstimulated platelets, collagen-stimulated platelets induced LDL modification as shown by enhanced conjugated dienes formation, electrophoretic mobility, Apo B 100 degradation and monocytes uptake; activated platelets also induced a marked reduction of vitamin E contained in LDL. A significant inhibition of LDL oxidation was observed in platelets treated with aspirin, an inhibitor of cyclooxygenase, and ETYA, an inhibitor of lipooxygenase. The above reported experiments were also conducted in patients with hereditary deficiency of gp91phox, the central core of NADPH oxidase, and in patients with hypercholesterolemia. Platelets from gp91 phox-deficient patients had produced small amount of reactive oxidant species(ROS) and weakly modified LDL. Conversely, platelets from hypercholesterolemic patients showed enhanced ROS formation and oxidized LDL more than platelets from controls. This study provides evidence that platelets modify LDL via arachidonic acid-mediated NADPH oxidase activation and suggests a role for platelets in favouring LDL accumulation within atherosclerotic plaque.

Antiplatelet drugs

P012

CELECOXIB, IBUPROFEN AND THE ANTIPLATELET EFFECT OF ASPIRIN IN PATIENTS WITH OSTEOARTHRITIS AND CHRONIC STABLE ANGINA

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Purpose. We performed a placebo-controlled, randomized study to address whether celecoxib or ibuprofen undermine the inhibition of platelet cyclooxygenase(COX)-1 activity by aspirin (ASA) in patients with osteoarthritis and stable ischemic heart disease, and explored the pharmacodynamic interaction between ASA and the two drugs on indices of COX-2 activity *ex vivo*. *Methods.* 24 patients chronically treated with ASA (100 mg daily) were co-administered celecoxib 200 mg BID, ibuprofen 600 mg TID or placebo for 7 days. We evaluated platelet aggregation on day 1 (baseline, 4h) and 7 (pre-drug, 4h) with both Born's aggregometer and the Platelet Function Analyzer (PFA) and we measured serum thromboxane(TX)B2 and lipopolysaccharide (LPS)-stimulated prostaglandin(PG)E2 generation as indices of COX-1 and COX-2 inhibition, respectively. *Results.* The co-administration of ASA with ibuprofen was associated with a significant increase of arachidonic acid (AA, 1mM)- and ADP (2 μM)-induced platelet aggregation detectable on day 7, at pre-drug (*p<0.01 vs baseline) (Figure), with a significant increase in platelet aggregability, as detected at PFA by the decrease of closure time (CT) with collagen-epinephrine (CEPI) cartridges (**p<0.001 vs baseline) (Figure), and with a corresponding increase in serum TXB2 (to 1223±1283% of baseline, median 800, range 40-3672; p<0.05 vs baseline). Differently, the co-administration with celecoxib or placebo did not undermine the ASA-related inhibition of platelet COX-1 activity, as assessed by serum TXB2 and by platelet aggregation tests. At steady-state, ibuprofen (> 80%) or celecoxib (>70%), but not placebo, caused a comparable and persistent inhibition of LPS-stimulated PGE2 generation, a marker of COX-2 activity *ex vivo*. *Conclusions.* Ibuprofen, but not celecoxib, interferes with the irreversible inhibition of platelet COX-1 activity by ASA, and translates into a functional failure of ASA to inhibit platelet function, despite comparable suppression of COX-2, in patients with osteoarthritis and stable ischemic heart disease.

Figure 1.

P013

PLATELET AGGREGATION AND THROMBOXANE FORMATION IN PATIENTS UNDERGOING CABG: EFFECT OF TWO DIFFERENT DOSES OF ASPIRIN

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Aspirin is the most widely used antiplatelet agent that prevents the formation of TXA2 by inhibition of COX-1. Despite clear benefit from aspirin (ASA) in patients with cardiovascular disease, evidence of heterogeneity in the individual response has given rise to the concept of aspirin failure to prevent a thrombotic event. *Objective.* To investigate platelet function and the antiplatelet effect of ASA in 44 patients undergoing

CABG and randomly assigned to 100 or 325 mg/d ASA treatment. Methods. Venous blood was collected before, 3 and 5 days after surgical intervention. Collagen induced aggregation of platelet rich plasma (PRP) and TXB2 levels in PRP and in serum were evaluated. Results. Before surgery the two groups of patients were comparable in terms of platelet aggregation and thromboxane B2 levels. After 2 days of ASA treatment, collagen-induced aggregation was inhibited by 38% in both groups of patients and a similar degree of inhibition was recorded 5 days after surgery. 3 days after surgery serum TXB2 levels were reduced by 95% and 90% in patients receiving 325 or 100 mg/d ASA with residual TXB2 of 10.8±2.2 and 24.6±3.7 ng/mL (p<0.02), respectively. No significant differences in the levels of TXB2 were observed after 5 days. Measurement of TXB2 in collagen stimulated PRP showed a 80% reduction in patients treated with 100 mg ASA and 95% in those treated with 325 mg/d. *Conclusions. I*n patients undergoing CABG ASA 325 mg/d led to an almost complete inhibition of TXB2 production, whereas ASA 100 mg/d showed a residual amount of this metabolite. Inhibition of platelet aggregation was comparable with the two treatments.

P014

EVALUATION OF ANTIPLATELET EFFECT OF CLOPIDOGREL IN PATIENTS WITH CORONARY ARTERY DISEASE (CAD): FLOW CYTOMETRY AND AGGREGOMETRY

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Introduction. Clopidogrel, alone or in association with aspirin, has proved successful in treatment of atherothrombotic disease. It acts by blocking the P2Y12 platelet ADP receptor , thereby inhibiting ADP-induced platelet activation and aggregation. However, despite antithrombotic therapy with clopidogrel, 5-10% of patients experience acute or subacute thrombosis after a coronary event or stent implantation. Unfortunately, increasing experiences indicates that a significant proportion of patients do not respond adeguately to clopidogrel. Because failure of antiplatelet therapy can have severe consequences, there is need for a reliable assay to quantify the effectiveness of clopidogrel treatment. *Aim of study.* Compare the performances of two laboratory methods to measure the antiplatelet effect of clopidogrel: platelet aggregometry and quantitative analysis VASP phosphorylation by flow cytometry. *Materials and methods.* We studied 36 patients with CAD treated with a 300 mg clopidogrel loading dose, followed by 75 mg/day clopidogrel in combination with 160 mg/day aspirin. For VASP we used a flow cytometric assay; a platelet reactivity index (PRI) was calculated from the mean fluorence intensity (MFI) in presence of PGE1 alone or PGE1 and ADP simultaneously according to the following calculation: PRI = [(MFI(PGE1) – MFI(PGE1+ADP))/MFIPGE1]×100. For aggregometry test we used ADP 5 mmol/L. Aggregation was expressed as the maximal percent change (MA) in light trasmittance from baseline. *Results.* Considerable diffences were found in the responsiveness to clopidogrel: the PRI was 63 ± 17.9%, and MA was 39.3±28.5%. Percent of resistant patients was 72.2% with VASP test and 33.3% with aggregometry. *Conclusion.* The flow cytometric analysis of VASP seems to be a suitable test to evaluate the efficacy of clopidogrel treatment. However, this assay demonstrated a wide interindividual variability of the inhibitory response of platelets to clopidogrel. Moreover, this study showed that one-third of the patients appeared to be unprotected by combined-therapy (clopidogrel plus aspirin).

P015

POST-TREATMENT PLATELET FUNCTION PREDICTS MYOCARDIAL INJURY IN ACUTE MYOCARDIAL INFARCTION PATIENTS UNDERGOING PRIMARY PCI

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Purpose. We sought to evaluate if platelet function measured after PCI affects the severity of myocardial infarction (MI), measured by markers of cardiac necrosis, in a real world setting, i.e. in patients undergoing primary PCI. A great interest was focused on the phenomenon of aspirin or clopidogrel resistance, but no data are available on patients with MI undergoing primary PCI. Methods. We measured platelet function by both a point-of-care assay (PFA-100) and platelet rich-plasma aggregation by two agonists (arachidonic acid and ADP) in 247 patients with MI after PCI. *Results.* 97 (39.3%) patients were found to be aspirin resistant by PFA-100 (CT/EPI <203 sec.). 98 (39.7%) patients were found to be aspirin resistant by platelet aggregation with arachidonic acid (AA-PA ≥20%), 15/247 (6.0%) were found to be clopidogrel resistant by platelet aggregation with ADP 2 µmol (ADP-PA ≥70%) and 74/247 (29.9%) by ADP 10 µM. CK-MB and cTnI mean peak values were significantly higher in the first tertile of CT/ADP and CT/EPI distribution with respect to the other tertiles and they were significantly higher in aspirin resistant than in aspirin sensitive patients. CK-MB and cTnI peak values were significantly higher in the third tertile of AA-PA, ADP2-PA and ADP10-PA distribution with respect to the other tertiles and were significantly higher in aspirin resistant patients. No significant differences were detected according to clopidogrel resistance by ADP 2 µM, whereas clopidogrel resistant patients by ADP 10 µM had significantly higher CK-MB and cTnI levels. Multivariate analysis revealed platelet function measured by PFA-100 (both CT/ADP and CT/EPI), platelet aggregation by AA and by ADP 10 µM, timeliness of percutaneous revascularization and multivessel disease to be independent predictors of CK-MB and cTnI peak values. *Conclusions.* We found that a persistent platelet activation after revascularization procedure in patients with MI undergoing primary PCI affects the severity of MI independently of the other known clinical, procedural, and laboratory parameters.

P016

ERYTHROCYTE DEFORMABILITY AND WHITE BLOOD CELL COUNT ARE ASSOCIATED WITH PLATELET FUNCTION IN HIGH-RISK VASCULAR PATIENTS

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Recently the phenomenon of aspirin resistance has been object of several studies, but no data are available on the possible role of the haemorheologic parameters in affecting platelets function and resistance to antiplatelet agents. Aim of our study was to evaluate platelet function and haemorheology in patients with acute coronary syndromes (ACS), receiving double antiplatelet therapy with aspirin and clopidogrel. The study population included 301 (231 M/ 70 F; age: 66±13 yrs) consecutive adult patients admitted to the Coronary Care Unit of the Azienda Ospedaliero-Universitaria Careggi, with diagnosis of acute myocardial infarction or unstable angina. We assessed: whole blood viscosity (WBV) at shear rates of 0.512 s-1 and 94.5 s-1, plasma viscosity (PLV) at 94.5 s-1 shear rate, erythrocyte deformability index (DI) and PFA-100 closure times with ADP (PFA/ADP) and epinephrine (PFA/EPI). We considered any PFA-100-EPI result <203 sec (95th percentile of control distribution) to be indicative of aspirin resistance. 104/301 patients (34.5%) had PFA/EPI CTs in the reference range (Group 1) whereas the remaining had values higher than 203 sec. (Group 2). WBV at 94.5 sec -1 s.r. was similar in group 1 and 2 (WBV: 4.43±0.25 vs 4.45±0.61 mPa*sec, respectively). PLV and WBV at 0.512 sec -1s.r. were slightly higher, but not significantly, in group 1 than in group 2 (PLV: 1.47±0.13 vs 1.44±0.15 mPa*sec; p=0.08 and WBV: 23.37±4.6 vs 22.54±3.90 mPa*sec; p=0.07). DI was significantly lower in group 1 with respect to group 2 (4.05±2.93 vs 5.71±3.30, p<0.0001). DI distribution was significantly and inversely associated with PFA/ADP CTs (Q1= 115.71±69.62; Q2=139.86±86.90; Q3=162.33±95.34; Q4=168.39±99.29,p<0.0001). White blood count (WBC) was significantly higher in group 1 than in group 2 (11464±3504 vs 7867±2162, p<0.0001), whereas no significant difference was found in fibrinogen levels between the two groups (462.3±159.9 mg/dL vs 459±159.5 mg/dL). We demonstrated that hemorheological properties are related to the phenomenon of aspirin resistance. In particular, erythrocyte deformability and high WBC are associated with the platelet response to aspirin. In conclusion, these results demonstrated that in patients with acute coronary syndromes the antiaggregant effect of aspirin is modulated not only by the direct action on platelets, but also by the erythrocyte deformability and the white blood cell count.

P017

CLINICAL DETERMINANTS OF PLATELET FUNCTION IN PATIENTS WITH CAD UNDERGOING CORONARY ANGIOGRAPHY AND TREATED BY DUAL ANTIPLATELET THERAPY

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Purpose. We sought to evaluate the prevalence and the clinical determinants of platelet function in a real world setting, i.e. in a large cohort of patients undergoing PCI and treated by dual antiplatelet therapy. Methods: We measured platelet function by both a point-of-care assay (PFA-100) and platelet aggregation (PA) with two agonists (arachidonic acid, AA, 0.5 mg/mL and ADP 2 and 10 μM), leukocyte count and ESR in 868 adult patients with coronary artery disease: 386 with acute coronary syndromes undergoing a primary PCI (group A) and 482 coronary artery disease patients scheduled to undergo an elective PCI (group B). Venous blood samples were obtained after the revascularization procedure (8-12 hrs). *Results.* PFA test detected 265/868 (30.5%) aspirin resistant patients (CT/EPI<203 sec). In a multivariate model, ACS (OR:1.5, 95% CI 1.1-2.1, p=.01) and chronic use of aspirin (OR:0.4, 95% CI 0.3-0.6, p=.0001) were significant and independent predictors of response to aspirin detected by PFA-100. Leukocytes and ESR were significantly higher in patients with aspirin resistance both in patients of group A and B. PA induced by AA detected 271/868 (31.2%) aspirin resistant patients (AA-PA >=20%). In a multivariate model, ACS (OR:1.7, 95% CI 1.2-2.4, p=.001) and chronic use of aspirin (OR:0.4, 95% CI 0.3-0.6, p=.0001) were significant and independent predictors of response to aspirin detected by AA-PA. Leukocytes and ESR were significantly higher in patients with aspirin resistance both in patients of group A and B. 54/868 (6.2%) patients were clopidogrel resistant by ADP 2 μM and 192/868 (22.1%) were clopidogrel resistant by ADP 10 μM. In a multivariate model, previous use of clopidogrel (OR:0.6, 95% CI 0.3-0.9, p=.02) and diabetes (OR:1.5, 95% CI 1.1-2.2, p<.05) were significant and independent predictors of response to clopidogrel detected by ADP-PA. *Conclusion.* Our data demonstrate that diabetes, ACS, chronic use of antiplatelet agents are independent predictor of the platelet response to both aspirin and clopidogrel. Furthermore, we provided the first evidence of a possible involvement of the inflammatory processes in the development of laboratory resistance to aspirin.

Hemorrhagic diseases: diagnosis and molecular defects

P018

EVALUATION OF LATEX IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF FACTOR XIII ANTIGEN

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Introduction. Patients with inherited FXIII deficiencies may exhibit severe bleeding diathesis; acquired FXIII deficiency may occur in several diseases, including inflammatory bowel diseases and acute leukemia. Moreover increased plasma FXIII activity has been find in patients with atherosclerosis, diabetic angiopathy and in chronic leukaemia. *Aim of study.* Evaluate a new latex immunoassay for the quantitative determination of Factor XIII Antigen (FXIII) produced by Instrumentation Laboratory. *Materials and Methods.* This new kit is performed by a latex reagent (a suspension of uniform size polystyrene latex particles coated with rabbit polyclonal antibodies). When a plasma is mixed with the Latex, the coated latex particles agglutinate. The degree of agglutination is directly proportional to the concentration of FXIII Ag and is determined by measuring the decrease of transmitted light caused by the aggregates. We have evaluated the within and between run imprecision, the linearity and the correlation of results obtained using the ACL TOP versus the method in use in our laboratory (Berichrom F XIII) performed on the coagulometer BCT (Dade Behring). The analytical correlation has been performed on 158 samples selected for hepatic pathology, because the frequency of inherited deficiency is rare. *Results.* Statistic analysis of Bland-Altman and linear regression analysis have demonstrated a very good correlation between two methods (y=0.954x-4.0115 r=0.977). Moreover our study has demonstrated good results in terms of within imprecision (CV between 1.7 and 7.4) and between imprecision (CV between 2.4 and 7.8); the linearity has been performed in two ranges, low (54.8 to 1.1%) and high (200 to 70%). *Conclusion.* The new latex method has demonstrated to be a valid help to rule out the FXIII assay also in terms of ease of use and fast turn around time. This makes F XIII as simple as any other test in the Hemostasis laboratory using the IL Coagulometers.

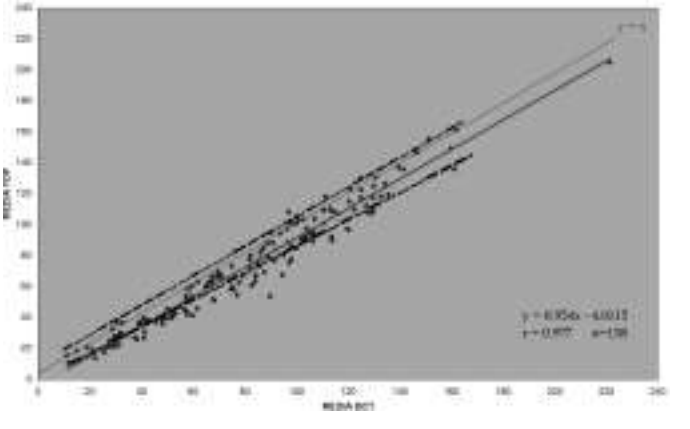

Figure 1. Regressione Lineare FXIII.

P019

A NEW CASE OF COMBINED FACTOR V AND FACTOR VIII DEFICIENCY SUGGEST THAT THE LMAN1 M1T MUTATION IS A FREQUENT CAUSE IN ITALIAN PATIENTS

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Combined factor V and factor VIII deficiency (F5F8D) is a rare autosomal recessive bleeding disorder recognized in more than 100 cases, mutations in LMAN1 and MCFD2 genes accounting for about all cases

of F5F8D. Among them, 15 Italian patients have been reported and in 14 the molecular defect has been identified. We investigated a 3-year-old Italian boy with no family history of bleeding disorders or consanguineous marriage who came to our attention because of abnormalities in routine coagulation studies. A prolonged APTT (ratio:1.95 [normal range: 0.80-1.20]) and PT (ratio 1.82 [normal range: 0.80-1.20]) were recorded, whereas factor V clotting activity was 8.7%, and factor VIII clotting activity 13.7%. His parents showed normal coagulation tests. The sequencing analysis of LMAN1 and MCFD2 genes showed a homozygous methionine to threonine substitution in the LMAN1 gene at the starting codon (M1T). Both parents were found to carry the same mutation in a heterozygous state. The mutation identified in the present study has been already found in 7 out 15 Italian patients analyzed, and in 7 out 10 carrying a mutation in the LMAN1 gene . Although we did not know whether our patient has the same haplotype of other Italian patients presenting with F5F8D, it is conceivable that the mutation has been inherited from a common ancestor. Founder effects have been used to explain the presence of high-frequency Mendelian diseases in many isolated populations. The existence of an ethnic-specific founder mutation, such as the LMAN1 M1T mutation, may contribute to explain the observed higher F5F8D prevalence in the Mediterranean area. Since the LMAN1 M1T mutation has been identified in 50% of F5F8D, we suggest that the search for this mutation should be the first step in the molecular characterization of patients from an Italian ethnic background.

P020

A 10 YEARS CLINICAL EXPERIENCE OF AN ITALIAN GROUP ON INHERITED ABNORMALITIES OF FIBRINOGEN

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Inherited abnormalities of fibrinogen present a highly variability in penetrance and expressivity and clinical manifestations vary from severe bleeding or thrombosis to asymptomatic. This variability makes clinical and genetic counseling more difficult. We report the experience of a clinical group working in specialist centers in Southern Italy on a series of consecutive patients presenting with congenital abnormalities of fibrinogen. Over 10 years, 18 patients were diagnosed to carry a congenital abnormality of fibrinogen. These patients and 26 first-degree relatives were in-depth investigated to fully characterize the nature of their abnormal fibrinogen levels. We found that carriers of low levels of a normal fibrinogen molecule did not become symptomatic until plasma values were detectable. Actually, while all afibrinogenemics had clinical important bleedings, subjects presenting with hypofibrinogenemia remained asymptomatic. In the presence of the synthesis of an abnormal molecule, the clinical phenotype was not strictly related to plasma fibrinogen levels but was associated with the molecular defect, most carriers remaining asymptomatic. Personal and family histories of bleeding and thrombosis are important for the clinical management of patients presenting with congenital abnormalities of fibrinogen. Biochemical and genetic investigations may be an useful guide for decision-making, providing additional steps in the assessment of the risk of patient presenting with low levels of a normal (hypo- and afibrinogenemia) and with an abnormal molecule (dysfibrinogenemia), respectively.

P021

COMPARISON BETWEEN TEG® AND ROTEM® TO EVALUATE PERIOPERATIVE BLEEDING IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFTING (CABG)

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Perioperative bleeding in cardiac surgery is one of the most frequent and costly complications. However, common guidelines for bleeding management are not available. At present, decisional citeria for transfusion re-do surgery are: amount of blood losses, common laboratory coagulation tests and activated clotting time (ACT). When bleeding is supposed not to be surgical , therapy is empirically based on blood, fresh frozen plasma or platelets transfusion. Should bleeding persists despite transfusions the next step is surgical reexploration. Thromboelastography and thromboelastometry can provide prompt and reliable bedside informations to clinicians on the causes of blood losses in critical situations. Previous studies have demonstrated the efficacy of those devices in addressing the indication to transfusions or surgical reexploration. The aim of this study was to compare TEG® with Kaolin and Heparinase assays and ROTEM® with INTEM, EXTEM, HEPTEM, APTEM and FIBTEM assays, to assess the cause of postoperative bleeding after CABG. All patients aged less than 75 years who underwent isolated CABG were included unless the presented exclusion criteria: associated congenital and/or acquired coagulopathies, unstable angina, associated anticoagulant therapy, chronic renal failure, previous PTCA and/or coronary stenting and prolonged intubation after operation. The TEG® and ROTEM® analysis were carried out at the same time before, during and after surgery and the results were related to the clinical manifestation. In our experience both instruments gave overlapping results. We conclude that TEG® and ROTEM® have the same accuracy in the evaluation of perioperative bleeding in CABG patients. However, ROTEM® demonstrated better resistance to accidental impacts, a good reproducibility and a wide range of tests that allow to explore the different pathways of coagulopathies.

P022

HOMOZYGOUS DYSPROTHROMBINEMIA (382 ARG-HYS) ASSOCIATED WITH SEVERE BLEEDING IN A 4 MONTHS OLD INFANT

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We report on a case of severe dysprothrombinemia observed in a 4 month old infant who developed persisting hematomas of the medial face of both thighs. At hospitalization he had marked prolongations of the PT (>4.0) and APTT ratios (2.95), both corrected by 1:1 mixing with NPP. Vitamin K administration did not correct the abnormality, which was due to selective deficiency of FII:C (<1%), with substantial normality of the other vitamin K-dependent factors (54-110%), of FV (71%) and fibrinogen (268 mg/dL). Infusion of PCC led to a correction of PT and APTT ratios and prompt amelioration of bleeding symptoms. Because of repeated bleeding episodes at follow up, the infant is on prophylaxis with PCC on a weekly basis. Plasma samples were obtained from the propositus - before and after the infusion of PCC - and from his parents (first cousins) on separate occasions. In the propositus, FII:C and antigen levels prior to the infusion of PCC ranged from 2% to 4% and from 65% to 76%, respectively; both parents had reduced FII:C (48%-53%) but not antigen levels (76%-86%). When prothrombin was activated with ecarin, the resulting FII:C activities were consistently low, but corresponding amidolytic activities were in the normal range (100%-106%). Plasma F1.2 levels were high in the propositus both before (1.06 nM) and after (1.23 nM) PCC infusion and low-normal in the propositus' parents (0.034-0.187 nM). Activated protein C levels were below 0.5 ng/mL at baseline in the propositus and in his parents, but they increased to 9.0 ng/mL after PCC infusion. Analysis of the prothrombin gene revealed 9 single nucleotide polymorphisms (SNP) in the homozygous state in the promoter region, in introns 1, 5 and 6, and in exons 6 and 10. Of relevance, 2 non synonymous SNPs were observed in exon 10 (382 Arg-Hys) and exon 6 (122 Thr-Met). The mutation in the catalytic site of thrombin has been previously reported, with a very similar phenotype but not in association with severe bleeding. Preliminary data suggest faster des-fragment1-meizothrombin formation with the propositus plasma than with normal plasma upon ecarin activation under conditions preventing thrombin generation.

Hemorrhagic diseases: clinical

P023

THROMBIN GENERATION IN PLATELET-RICH PLASMA FROM PATIENTS WITH CIRRHOSIS

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Coagulation factors deficiencies, thrombocytopenia and thrombocytopathy are associated with cirrhosis. However, bleeding in cirrhotics does not entirely correlate with abnormal coagulation tests. Recently, it was shown that because of the concomitant abnormalities of the procoagulant and the anti-coagulant drives, thrombin generation in plasma from cirrhotics is normal when assessed with assays which include thrombomodulin (the main protein C activator). However, thrombin in-vivo is also generated as a function of platelets suggesting that the occurrence of thrombocytopenia and thrombocytopathy might have implications on thrombin generation. We addressed this issue using an assay that accounts for the contribution of plasma, platelets function and numbers. The study showed that platelet-rich plasma with platelets adjusted by dilution of autologous platelet-rich into platelet-poor plasma to a standard count (100×10^9/L) generates as much thrombin in cirrhotics as in controls (1,063nM-vs.-1,167nM, N.S.). When the platelets were adjusted to correspond to baseline whole-blood counts, cirrhotics generated significantly less thrombin than controls (949nM-vs.-1,239nM, $p<.001$). Furthermore, thrombin generation correlated with platelet numbers (rho=0.50, $p<.001$). In addition, the amount of thrombin generated as a function of the baseline whole-blood patients' platelets counts increased significantly when the numbers were adjusted to 100×10^9/L (953nM-vr.1,063nM, $p<.001$). In conclusion, these observations suggest that the thrombocytopathy (surmised to be associated with cirrhosis) does not affect thrombin generation. On the contrary, severe thrombocytopenia may limit thrombin generation. These findings might justify prophylactic platelets transfusion in those patients with low counts when they bleed spontaneously or after surgery or biopsy. Controlled clinical trials supporting this indication are warranted.

P024

ULTRASONOGRAPHICAL IMAGES OF JOINTS AND PROGNOSIS VALUE IN PATIENTS WITH SEVERE HEMOPHILIC ARTHROPATHY

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Objective. We have wanted to appraise in the hemophilic patient if the appearance of any ultrasonographical image is in relationship to damage of the articular functionality. *Materials and methods.* We have studied 12 patients suffering from serious hemophilia A and B (age included between 2 and 40 years) with echographyc investigations repeated, emphasizing the evolution of the echographyc picture and as the appearance of any signs could have value negative in the natural history of these patients (functional limitation articulate up to the ankylosis). *Signs considered*: 1. articular deposit with interest of the withdrawals of the articular hollow, 2. ypertrophy of synovia, 3. thickening of the articular capsule, 4. deterioration of the articular cartilage with irregularity of his profile, 5. find (in the serious forms) of a double cloth of the synovia, 6. interest of the periarticular tissues. Other important sought sign has been the relaxation of the prerotulea purse with irregular profile (in the case of the knee) communicating with the articular hollow (bursitis). We have appraised the presence of cysts of Baker also (with harvest yperechogenic) and anechogenic areas in the poplitheus hollow (appreciable in patients with severe articular damage) and the increase of the vascular flow in the acute phases. *Results.* All the patients with serious hemofilic arthropathy showed the double cloth of synovia, a relaxation of the prerotulea purse (the articulation of the knee is most stricken) with diffused yperechogenic signal of the popliteus hollow (presence of cysts with yperechogenic material inside). *Conclusions.* The double synovial cloth [marker] together to the microcalcifycations of the soft tissues and the relaxation of the prerotulea purse (bursitises) would result foretell a negative evolution of the Hemofilic arthropathy up to the ankylosis.

P025

ATHEROSCLEROSIS, VASCULAR DAMAGE, THROMBOTIC RISK FACTORS AND CONGENITAL BLEEDING DISORDERS

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Thrombosis in patients with hereditary bleeding disorders is uncommon, nevertheless the co-existence of prothrombotic factors may increase the possibility of developing thrombotic complications in high risk condition such as surgery, activated coagulation factor concentrate infusions, presence of central venous device. *Aim of the study.* Relationships between prothrombotic risk factors and presence of carotid atherosclerosis and endothelial hyperplasia of arterial neck-vessels (investigated by Doppler Ultrasound) in patients with hereditary bleeding disorders. *Patients*. 26 patients (aged between 16 and 71 yrs) were enrolled and stratified according to their age: A <40 yrs (13 pts), B >40 yrs (13 pts). They carried different factor deficiencies: 20 FVIII, 1 FIX, 2 FXI, 1 FVII, 2 FV. Thrombotic factors considered: Homocysteine, AT, PC, PS, ACA, APA, Anti β2 GPI, LA, FV Leiden mutation, FII G20210A mutation, Cholesterol (total and HDL), Lp(a), hypertension (6 pts in B), cigarette smoking (2 pts in A, 3 pts in B), diabetes (2 pts in B). *Results.* A significant association emerged between the presence of carotid atherosclerotic plaques and both PS deficiency (p=0.011) and hypertension (p=0.02). In A further relationships between atherosclerotic lesions and enhanced ACA-APA (p=0.014) and cholesterol levels (p=0.014) were observed. Homocysteine mean levels were higher in A than in B (Homocysteine M/L ±SD A: 17.9±10.2; B: 16.1±5.18; p=0.58). The endothelial hyperplasia at right and left carotid fork in B was significantly higher than in A (p=0.0006 and p=0.026). In A the intimal thickness of both right and left carotid fork was greater in patients with homocysteine >14ΔM/L than in patients with homocysteine <14 M/L (p=0.016 and p=0.041). *Conclusions.* Like in normal people, in patients with hereditary bleeding disorders the presence of both congenital and acquired prothrombotic factors may facilitate the development of atherosclerosis. Particularly hyper-homocysteinemia promotes a significant rise of intimal-thickness in high share-stress sites in younger patients.

P026

COMPLIANCE AND OUTCOME OF A COHORT OF HCV POSITIVE INDIVIDUALS IN A HAEMOPHILIA CENTRE IN SOUTH OF ITALY

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To determine compliance and outcome of a cohort of HCV positive individuals with congenital bleeding disorders the study concerns data from 100 multitransfused patients (p.) over a period of 10 years, between 1995 and 2005. All the subjects were strongly advised for clinical follow up. 34 p. (34%) out of 100 (22 hemophiliacs A, 10 hemophiliacs B and 2 p. with type 3 von Willebrand disease) gave their consent to be observed periodically in order to evaluate the activity of infection by clinical examination, laboratory and echography. 31 (91.1%) but 3 p. had biochemical and virological evidence of active infection. The HCV genotype distribution was 3a (23.5%) followed by 1a (17.5%), 1b (14.7%), 1a/1b (11.7%), types 1, 4 and 2a/2c (5.8%) respectively. 6 (17.6%) out of 34 p. showed echographic signes of evoluted chronic hepatitis, 2 p. resulted affected by hepatocarcinoma. 24 (70.5%) subjects were considered eligible for antiviral treatment. 7 (29.1%) out of 24 p. decided to delay the induction of therapy, 3 p. (13%) declined the treatment. All the 4 p. (16.6%) initially treated with αferon and amantadin resulted not responsive, whilest 7 out of 12 p. (58.3%) who were given pegylated interferon-α-2b in combination with ribavirin showed a sustained viral response after discontinuation of 6-12 months treatment. 1 p. was withdrawn from therapy because of side effects.

P027

HAEMOPHILIA CENTRES: PERFORMANCE MEASUREMENT AND BENCHMARKING

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Reasons. To guarantee appropriate, high level services an evaluation system is required. Literature doesn't provide indicators monitoring Haemophilia Centres (HCs) performance. For this purpose, a study was promoted to design a performance evaluation system based on a set of indicators to be utilized by 8 HCs of Emilia-Romagna Region, an Italian region of 5 millions inhabitants. *Method.* HCs specifications, performance indicators and standards were identified by a multidisciplinary panel including professionals, patients and health authorities. Epidemiological, clinical and performance data by the Regional Haemophilia Registry were utilized for developing the indicators. After literature review and a peer review, a set of significant, measurable and reliable indicators referring to regional strategic plan objectives was defined. *Indicators refer to.* Demand, patient access, service provided, outcome. Data from the best performing HC were used as benchmark. A six monthly report on results was provided to all the stakeholders. *Results.* The system proved to meet the information needs of all the stakeholders. Sixteen objectives, 3 areas of interest, 24 indicators were identified. One year application showed indicators to be successful in measuring targets set by the strategic plan and to be appropriate to compare Centres performances. Differences around 40% emerged between HCs performance. Reasons for not achieving the targets were investigated and actions taken. *Conclusion.* The system proved to be reliable and well accepted by all the stakeholders and was already adopted by other diseases networks. Further developments may allow the application of the system to HCs of other regions.

P028

EXPERIENCE OF SECONDARY PROPHYLAXIS ON 20 ADOLESCENT AND ADULT HAEMOPHILIA PATIENTS

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Prophylaxis is the treatment of choice for children with severe haemophilia. Few studies documented the efficacy, the pharmacoeconomic impact and HRQoL of secondary prophylaxis started in adolescence and adulthood. Three Italian Hemophilia Centers (Parma, Trento and Verona) performed a retrospective study. 20 adolescent and adult patients with severe haemophilia (17HA, 3HB) were switched from on-demand treatment to prophylaxis. We collected data on bleeding frequency, treatment and outcomes, Pettersson score was measured in a subgroup of patients. 10 adolescents started prophylaxis at 12y (10-15) and continued for 5 years (1-14) and 10 adults started at 34y (18-72) and continued for 5.5y (2-11). In both groups we observed decreased joint bleeding, better orthopaedic score, less work/school days lost with improved QoL, delayed progression of Pettersson score, increased annual consumption of clotting factor. The cost for bleed avoided in adults was €1907. In our cohort the cost of prophylaxis has been 31,3% higher than on-demand treatment, but the prevention of bleeds, delayed progression of arthropathy, improvement of QoL appears to counterbalance the high cost also in adolescent age and adulthood.

Table.

	Adolescents		*Adults*	
	On-Demand	*Prophylaxis*	*On-Demand*	*Prophylaxis*
Bleedings	22.1 (5-50)	2.28 (0-10)	40.3 (16-84)	7.3 (0-17)
Joint bleedings	18.9 (4-40)	1.1 (0-3)	33.2 (6-84)	5.7 (0-15)
Orthopaedic score	9 (1-23)	2.4 (0-8)	17.7 (6-37)	10.3 (1-19)
Pettersson score	4 (3-6)	4.25 (3-7)	6 (4-7)	6 (4-7)
work/school days lost	34.5 (0-48)	1.7 (0-8)	27.5 (0-60)	0.75 (0-2)
Clotting factor consumption UI/Kg/y	2384.1 (1457-3916)	3142.1 (1575-4600)	2568 (590-8400)	3371 (2000-6369)

P029

COST-EFFECTIVENESS OF SECONDARY PROPHYLAXIS IN SEVERE HAEMOPHILIA A AND B - A COHORT ANALYSIS OF TREATMENT IN ADOLESCENTS AND ADULTS

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Haemophilia treatment is very expensive; prophylaxis offers better outcomes at higher costs. Differences in short term outcomes and in coagulation factors consumption and costs were evaluated in patients who switched from on demand treatment to prophylaxis. 20 patients (10 adolescents and 10 adults) were studied matching consumption of clotting factor, number of bleedings, days lost from school/work; for on demand treatment data are referred to the last year before switching; prophylaxis was conducted for a mean of 63 months (12-165). Prophylactic average dose/Kg was 25.5 (16-33) 2-3/w of FVIII and 36 (34-39) 1-2/w of FIX. In our cohort the cost of prophylaxis was 31.3% higher than on-demand treatment, but the number of bleedings, the orthopaedic evaluation and the number of days lost from work or school clearly show that prophylaxis is the most effective therapy. Clotting factor consumption per bleeding avoided was 2549 UI (i.e 1907€). Clotting factor consumption per joint bleeding avoided was 3061 UI (i.e 2290 €).

Table.

	Adolescents		*Adults*		*Total*	
	On-Demand	*Prophylaxis*	*On-Demand*	*Prophylaxis*	*On-Demand*	*Prophylaxis*
N. Bleedings	22.1 (5-50)	2.28 (5-50)	40.3 (16-84)	7.3 (0-17)	31.2 (5-84)	4.8 (0-17)
N. Joint bleedings	18.9 (4-40)	1.1 (0-3)	33.2 (6-84)	5.7 (0-15)	26.05 (4-84)	3.41 (0-15)
Orthopaedic score	9 (1-23)	2.4 (0-8)	17.7 (6-37)	10.3 (1-19)	13.32 (1-37)	6.35 (0-19)
Days/y lost from work/school	34.5 (0-48)	1.7 (0-8)	27.5 (0-60)	0.75 (0-2)	31 (0-60)	1.2 (0-8)
Clotting factor consumption UI/Kg/y	2384.1 (1457-3916)	3142.1 (1575-4600)	2568 (590-8400)	3371 (2000-6369)	2476 (590-8400)	3250.70 (1575-6369)

P030

IMMUNE TOLERANCE INDUCTION (ITI) WITH PLASMA DERIVED FVIII IN TWO HAEMOPHILIC PATIENTS WITH INHIBITOR PREVIOUSLY TREATED WITH RECOMBINANT FVIII. DISAPPEARANCE OF THE INHIBITOR AND IMPROVEMENT OF THE QUALITY OF LIFE

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The development of inhibitors is currently the main problem in the treatment of haemophilic people occurring in up to 35% of patients with severe haemophilia A. Patients with Inhibitor show more severe bleeding tendency, with haemorrhagic episodes that are particularly difficult to control. Therefore the most adequate management of patients with inhibitor is rapid immune tolerance induction (ITI). In fact, rapid inhibitor elimination and immuno tolerance induction (ITI) are the preferred way to reduce the high risk of bleeding episodes in patients with haemophilia A and FVIII inhibitors. From 2003, 2 haemophilia A patients with inhibitors, aged 18 and 36 years, were treated using exclusively Hemate P® (ZLB Behring), a plasma derived FVIII concentrate of intermediate purity with high VWF content. Peviously, both the patients had been subjected to ITI with recombinant FVIII achieving a failure. Inhibitor elimination was achieved in all patients after a median time of 4 months. After inhibitor elimination in the first patient it has been assisted to a dramatic reduction of the number of the hemorrhages and those that have been taken have been held under control. In second

patient hemorrhages in the course of the last year have not been taken. The two patients have stayed subjected to test for the appraisal of the quality of the life (QoL) before and after treatment ITI with dedicated questionnaire using both the Sf-36 and haemophilia-related questionnaires with multivaried analysis of factors influencing quality of life (Trippoli S. *et al.*, Haematologica 2001; 86: 722-8). The achieved results show in the two patients enlists a poor QoL before ITI with nearly equal score, while after ITI although the patients show a psychological uneasiness, thinks their state of health acceptable and however improved.

P031

SUCCESSFUL CONTROL OF BLEEDING WITH RECOMBINANT ACTIVATED FACTOR VII (FVIIA) AFTER CARDIAC RE-TRANSPLANTATION IN A PATIENT ON AGGRESSIVE ANTIPLATELET TREATMENT

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Introduction. Bleeding complications after cardiac surgery are an important cause of morbidity and mortality. Standard management of post-operative bleeding includes transfusion of blood products and surgical re-intervention which in turn increases costs and morbidity. Control of post-operative bleeding is thus crucial in modern patient care. *Case report.* In 2003, a 48-yr old woman underwent heart transplantation for dilated cardiomyopathy. She had common cardiovascular risk factors as well as inherited thrombophilia (heterozigosity for prothrombin G20210A mutation and homozigosity for MTHFR C677T and hyperhomocysteinemia). After transplant, the patient was put on statin and aspirin (100 mg/d). However, due to vascular graft disease, in addition to percutaneous coronary intervention, she received aspirin (100 mg) in combination with clopidogrel (75 mg/d). Despite this treatment, her graft coronary disease worsened rapidly, and cardiac decompensation developed. Urgent cardiac re-transplantation was then needed in this patient on double anti-platelet regimen, which was followed by a significant post-operative bleeding, blood loss reaching 3500 mL over a 6-h period. Despite infusion of >4000 mL of blood products (packed red blood cells, fresh-frozen plasma, platelet concentrates), she developed haemorrhagic shock. A 70 μg/kg bolus of recombinant activated factor VII (rFVIIa) was then infused, with an immediate, dramatic reduction of blood losses to 50 mL/h: within 12 hrs the patient recovered her circulatory ad respiratory function. She is now doing well, with a normal cardiac function and no further thrombotic complications. *Discussion.* This is the first reported case of successful bleeding control with rFVIIa after emergency cardiac re-transplantation in a patient undergoing aggressive anti-platelet treatment. Activated rFVIIa appears thus promising in critical care settings where severe post-operative bleeding occurs due to treatment with anticoagulant or antiplatelet drugs.

P032

SINGLE BOLUS OF RECOMBINANT FACTOR VIIA FOR PROPHYLAXIS OF BLEEDING DURING SPONTANEOUS DELIVERY IN A PATIENT WITH FACTOR VII DEFICIENCY

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Few data are available concerning pregnancy and delivery in women with rare coagulation deficiencies. Due to genetic heterogeneity of these disorders, bleeding tendency is variable, often unpredictable and poorly correlated with plasma levels of the deficient factor. Moreover, the rarity of these autosomal recessive disorders, clinically expressed only in homozygotes, makes it difficult to define guidelines for prophylaxis and treatment of bleeding in this setting. In particular, as post-operative/post-partum bleeding is a frequent symptom in these patients, indications for spontaneous or elective Caesarean delivery are debated by haematologists and obstetricians. We report the case of a 25-yr old woman with Factor VII (FVII) deficiency who underwent spontaneous delivery at term of her first pregnancy. Her coagulation defect was diagnosed five years earlier, after an apparently spontaneous severe upper limb haematoma (PT INR 6.9, FVII:C 2%). Despite a history of menorrhagia since the menarche, the patient did not show anaemia or need oral contraceptives (tranexamic acid was irregularly administered). Molecular characterization of gene defect revealed double heterozygosity for two previously described mutations (Gly333Ser/Cys310Phe). Her pregnancy was uneventful, without any abnormality of routine laboratory tests and of foetal growth. After specialist counselling and obtaining the patient's informed consent, spontaneous delivery and haemostatic cover by recombinant FVIIa (NovoSeven, Novo Nordisk) were planned. A single 20 μg/Kg i.v. bolus was injected at advanced labour, and normal PT (INR) was obtained at least over the following 10 hrs. No maternal or foetal complications occurred, or other haemostatic and transfusion requirement were needed. Our report supports the concept that spontaneous delivery in patients with severe coagulation defects is feasible, provided that tight interaction between haematologists, obstetricians and clinical pathologists, together with adequate replacement and transfusional treatment, is always available. Moreover, low-dose rFVIIa is shown to be an effective and safe approach in patients with FVII deficiency.

P033

ACQUIRED INHIBITOR TO FACTOR V IN AN OLD PATIENT WITH NON-HODGKIN LYMPHOMA

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Acquired inhibitors to coagulation factors are uncommon conditions, due to specific auto-antibodies causing the targeted factor deficiency and bleeding tendency of variable severity. Acquired haemophilia A is the most common disorder, however auto-antibodies against virtually all factors have been reported, often related to immunological or lymphoproliferative diseases. A 72-yr old man was referred to our Institution because of recurrent epistaxis and abnormalities of coagulation tests (PT INR 2.5, aPTT ratio 2.73). No previous personal or family history of bleeding disorders, or recent surgery and new drug intake, were reported. Spleen enlargement, with a large focal lesion, and pancytopenia (leukocytes 2800/μL, haemoglobin 9 g/dL, platelet 82,000/μL). A lymphoid infiltrate of uncertain interpretation was observed at bone marrow biopsy. Vitamin K administration did not improve coagulation tests. Factor (F) V activity was 8%, whereas FVII, FVIII, FIX, FX and prothrombin were also slightly reduced. No correction of PT and aPTT was shown when the patient's plasma was mixed 1:1 with normal pooled plasma, immediately or after 2 h incubation at 37°C, suggesting the presence of an immediate acting inhibitor. Bethesda assay confirmed the presence of FV inhibitor (2.3 BU). Fluorodeoxyglucose F-18 positron emission tomography whole-body scan revealed abnormally high uptake in the spleen and in a slightly enlarged supvraclavear lymph node. Fine needle aspiration biopsy at this site, covered by two 60 μg/Kg recombinant Factor VIIa (NovoSeven) injections, enabled the diagnosis of large B-cell (CD 5-, CD22+, lambda+) non-Hodgkin lymphoma. Complete remission was achieved after 6 courses of CEOP regimen. Interstingly, coagulation abnormalities progressively improved during chemotherapy and so far Factor V inhibitor was no longer detectable (follow-up: 5 months). In this patient the onset of an acquired coagulation disorder was an useful diagnostic and prognostic marker of derangement of the immunologic network due to an underlying, apparently indolent, lymphoprolferative disease.

P034

CATAMENIAL HEMOPTYSIS FROM ENDOBRONCHIAL ENDOMETRIOSIS IN A CHILD WITH VON WILLEBRAND DISEASE TYPE 1

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We describe a twelve years old female with catamenial hemoptysis recurring over four months, two years after menarche. Hemoptysis occurred within the 1 or 2 day of menses, lasted for three days and stopped spontaneously. Her clinical history was positive for nose-bleeding since she was six years old with a frequency of three episodes per year. Physical examination was normal. Laboratory findings,focused to rule out coagulation disorders, revealed low plasmatic levels of von Willebrand Factor antigen and ricof (40%) compatible with diagnosis of von Willebrand disease (type 1). However the coagulopathy did not

explain the periodic recurrence of hemoptysis, so we detected on a probable diagnosis of Rendu Osler Weber syndrome. Instrumental examinations such as rhinofibroscopy, thoracic CT scanning, esophagogastroscopy and hepatic ultrasound color doppler, did not reveal any teleangiectasic lesions and artero-venous visceral malformations. At the onset of the next menses, the patient underwent flexible fiberoptic bronchoscopy that showed striking diffuse hyperaemia and a tiny submucosal red spot at the left bronchial tree, in correspondence of pyramid. Cytologic evaluation of the brushing specimens demonstrated clusters of small cuboid cells consistent with endometrial origin. Catamenial hemoptysis is a cyclic pulmonary hemorrhage synchronous with female menses. It is a rare clinical disorder associated with the presence of intrapulmonary ectopic endometrial tissue. To our knowledge this is the first case of endobronchial endometriosis described in childhood. We cannot asses if the association with the von Willebrand disease could be responsible for the early starting of the hemoptysis.

P035

HEMARTHROSIS DUE TO A RARE CAUSE OF HEMORRAGIC DIATHESIS: EHLERS DANLOS SYNDROME

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We report a rare case of hemarthrosis complicated by severe anemia related to a congenital connective pathology: Ehlers Danlos syndrome. A thirteen year old boy fell down during deambulation, perceiving a "crack" at both knees. Then he felt strong local pain and wasn't able to retake erect posture and deambulation. Previous pathologic and pharmacological history was negative. The patient practiced sport and never reported analogue events. Physical examination of knees showed a tumefaction imputable to an articular effusion with positive thermotouch and functional impotence. Palpation allowed to appreciate dislocation of patella associated to rotula tendon rupture confirmed by radiography, ecography and magnetic resonance. Hematochemical data at admission hadn't any flogistic and hematological alterations. He underwent a therapeutic arthroscopy that revealed the haematic nature of effusion. Afterwards he underwent an operation of re-insertion of each tendon on inferior rotula pole. In the following days an unexpected and ongoing haematic effusion together with a severe anemia (hemoglobin = 7.4 g/dL) requiring erythrocytes transfusion. We screened coagulation disorders in order to explain severe bleeding with no results. We therefore come back to more detailed history and physical examination. His mother referred that the child often enjoyed himself showing contortionist positions. Moreover she had joint hypermobility too and reported uterine rupture during delivery. Detecting on phenotypic features of patient, we noticed hypermobility of other joints. Furthermore patient had over-extensible and velvety skin, and showed a delay of surgical injury cicatrization. Considering cutaneous and articular alterations we suspected a connective pathology and basing our supposition on two major criteria (over-extensible skin and joint hypemobility) and on a minor criterion (familiarity) we diagnosed the Ehlers- Danlos syndrome, hypermobile variant (type III). So hemarthrosis and anemia had been considered as the consequence of an excessive local laceration for tissue fragility, inserted in a contest of congenital connectivopathy.

P036

SUCCESSFUL IMMUNE TOLERANCE INDUCTION IN AN ADULT A HAEMOPHILIAC WITH LATE ONSET HIGH-RESPONDING INHIBITOR

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Inhibitors to Factor VIII (FVIII) occur in approximately 30% of patients with severe A haemophilia, in most cases within the first 20 exposition days (ED) to FVIII concentrates. Inhibitors after 150 ED or more have been rarely reported. Induction of immune tolerance (ITI) to exogenous FVIII is presently considered the only approach able to eradicate/reduce inhibiting activity and restore FVIII treatment. Optimal regimen and type of FVIII concentrate are still open issue concerning ITI, as well predictors of successful outcome. We report the case of a 36 yr-old severe haemophiliac who developed a high-responding (HR) inhibitor after long-term exposition (>500 ED) to the same plasma-derivative FVIII concentrate (Emoclot D.I., Kedrion). The inhibitor was diagnosed (historical peak 18 BU) as treatment of bleeding became progressively less effective, and activated prothrombin complex concentrate was needed for bleeding management. With the exception of several intensive treatments for knee haemarthroses, no other putative acquired risk factor for inhibitor development (surgery, infection or other drug intake) was reported. Due to venous access problems, the patient was reluctant to start ITI. Inhibitor titre permanently >5 BU and the need for orthopaedic surgery led to start ITI 22 months after inhibitor detection (baseline titre 5.6 BU), using a low-dose regimen (50 UI/Kg every other day) of the previously employed FVIII concentrate. Seven months later, inhibitor titre was significantly reduced, being undetectable at 10th month of ITI. *In vivo* FVIII recovery was 85% at 11th month and always >80% thereafter; normal FVIII half-life was shown since the 12th month (always >8 hrs). FVIII doses were reduced at prophylaxis regimen (30 UI/Kg), which is still ongoing. This report highlights the need for careful inhibitor follow-up also in long-term treated patients. Moreover, attempting ITI is recommended for all HR inhibitor patients, also in the presence of negative predictors of success.

P037

LONG-TERM PROPHYLAXIS WITH PROTHROMBIN COMPLEX CONCENTRATE IN A PATIENT WITH MILD HAEMOPHILIA B, FACTOR X DEFICIENCY AND LIVER CIRRHOSIS

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A 55 yr-old man with a history of mild hemophilia B was referred to our Institution because of increasing severity of bleeding tendency, including two haemorrhages from gastro-oesophageal varices. On admission the patient, who had a history of chronic hepatitis C, showed a clear-cut clinical picture of cirrhosis with severe portal hypertension. Unusually prolonged PT and reduced Factor (F) X levels (10%) led to the diagnosis of combined mild FX deficiency, confirmed in his two sons. In several occasions FIX concentrate was unable to stop joint bleeding and the patient needed hospitalization and fresh frozen plasma or prothrombin complex concentrate (PCC) infusions. Prophylaxis with 40 IU/Kg FIX concentrate twice or thrice weekly did not reduce his bleeding tendency, with progressive joint disability. Prophylaxis with 30 IU/Kg PCC (Uman Complex D.I., Kedrion) twice weekly was started on April 2003, when a new gastric bleeding occurred. The patient did not show any sign or risk factor for cardiovascular disease nor thrombophilic abnormalities. To reduce bleeding and hospitalization frequency and to enable physical therapy, a similar schedule was also prescribed at home. Clinical and laboratory follow-up was carried out weekly and then monthly; mild increase of D-Dimer was detected between 4 and 24 hours after PCC infusion. No further significant bleeding episode or any adverse event occurred and the patient's quality of life significantly improved, despite the worsening of liver function and the diagnosis of two hepatic cell carcinoma lesions. The patient followed such regimen until July 2005, when he successfully underwent to orthotopic liver transplantation. PCC prophylaxis has been helpful to manage the bleeding tendency (increased by the coexistence of more genetic and acquired coagulation abnormalities) in this patient, also enabling to undergo OLT, the only therapeutical approach able to restore his severe haemostatic and general clinical impairment.

Anticoagulant drugs

P038

REORGANIZATION OF AN ANTICOAGULATION CLINIC USING A TELEMEDICINE SYSTEM: DESCRIPTION OF THE MODEL AND PRELIMINARY RESULTS

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Objective. Over the last decade, the indications for oral anticoagulant therapy have been expanded considerably, resulting in a significant increase in the number of patients followed at an anticoagulation clinic (AC). Some organizational problems, in particular related to the healthcare staff and patients, have led to the need for decentralizing the healthcare activity using a telemedicine system. The aim of our project was 1) to reduce by 30% over a period of 3 years the number of patients who attend daily the AC, 2) to maintain the same quality levels defined as time spent in the therapeutic range and number of complications; and 3) to assess patient satisfaction through an open questionnaire. *Methods.* A net supported program (TaoNet) was installed at the AC to collect and analyze patient clinical data, connected with the peripheral health units, where clinical data and prothrombin time (expressed as international normalized ratio), reported on portable monitors (Coagucheck), are obtained and sent in real time to the AC. After clinical validation, in a few minutes the AC sends the appropriate dose adjustment as well as clinical advice to the peripheral health units. *Results.* The project was launched in January 2002. Nine peripheral health units are available at present to the public, managing 664 out of a total of 2520 patients (26.35%) under treatment. An increase in the time spent in the therapeutic range was observed compared with the previous 12 months (AC = 62%, peripheral health units = 70%; $p< 0.05$, Table 1). No differences in major complications were observed (Table 2). Patients expressed their overall satisfaction, as evaluated through an open questionnaire. *Conclusions.* This new organization provides the following advantages: 1) direct communication between the peripheral health units and the AC; 2) an improvement in the time spent in the therapeutic range; 3) an improvement in patient satisfaction and quality of life; 4) continuing patient record update; and 5) time gained by the healthcare staff.

Table 1. Number of control visits per year and time spent in the therapeutic range, and trend of patients referring to the peripheral health units as compared with results obtained 6 months previously at the anticoagulation clinic (AC) of Cremona.

	AC	Peripheral health units	Peripheral health units	
			12 months before	12 months after
No. patients	2520	664	664	664
Follow-up (patient-years)	5040	1328	664	664
No. visits per year	17	16	16	16
Time in the therapeutic range (%)	62.0	70.0	65.5	70.0

Table 2. Number of bleeding and thromboembolic complications compared with the results from the ISCOAT study.

	ISCOAT	AC	Peripheral health units		
			12 months before		12 months after
No. patients	2745	2520	664		664
Follow-up (patient-years)	2245	5040	202		202
Major bleeding (% patient-years)	1.25	1.30	0		0.30
Minor bleeding (% patient-years)	6.20	4.50	0.32	0.96	
Thrombosis (% patient-years)	3.50	2.80	0	0	

AC= anticoagulation clinic.

P039

LOW MOLECULAR WEIGHT HEPARIN AND BLEEDING IN PATIENTS WITH SEVERE RENAL INSUFFICIENCY: A META-ANALYSIS

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Background. Dose adjustment or laboratory monitoring of low molecular weight heparin (LMWH) is commonly recommended in patients with severe renal insufficiency but the basis for this recommendation is unclear. *Purpose.* To compare the risk of major bleeding in LMWH-treated patients with creatinine clearance [CrCl] ≤ 30 versus > 30 mL/min using standard weight-adjusted therapeutic doses, empirically adjusted doses, or prophylactic doses of LMWH. *Data sources.* Electronic databases (MEDLINE, EMBASE, Cochrane Library) searched up to December 2005, reference lists and contact with experts. Study selection. Studies that included non-dialyzed patients with varying degrees of renal function who were treated with LMWH and which reported major bleeding. *Data extraction.* Two reviewers selected studies and extracted data on patient characteristics, renal function, LMWH treatment and major bleeding. *Data synthesis.* Eighteen studies using 3 LMWHs (15 studies using enoxaparin, 2 tinzaparin and 1 dalteparin) were included. In 12 studies involving 4971 patients, LMWH significantly increased the risk of major bleeding in patients with CrCl ≥30 compared with > 30 mL/min (5.0% vs. 2.4%, pooled relative risk (RR) 2.34, 95% confidence interval [CI]: 1.46, 3.74; p=0.0004; number needed to harm [NNH] = 38). Increased risk of major bleeding was evident in studies using therapeutic dose of enoxaparin (8.3% vs. 2.4%, RR 2.96; 95% CI: 1.63, 5.37; NNH = 17) but not in studies using empirically adjusted enoxaparin (0.9% vs. 1.9%, RR 1.06; 95% CI: 0.23, 4.97). There were insufficient studies to assess the major bleeding risk using tinzaparin, dalteparin and prophylactic doses of enoxaparin. *Conclusions.* Non-dialysis dependent patients with CrCl≥30 mL/min who are treated with standard therapeutic doses of enoxaparin have an increased risk of major bleeding. Empiric dose adjustment of enoxaparin may reduce the risk of bleeding and merits further evaluation. No conclusions can be drawn regarding other LMWHs.

P040

A COMPARISON BETWEEN UNFRACTIONATED HEPARIN AND ENOXAPARIN IN CARDIAC SURGERY

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Purpose. We evaluated the impact of enoxaparin and unfractionated heparin (UFH) on haemostatic parameters among patients (pts) with unstable angina undergoing coronary artery bypass grafting (CABG). *Methods*. 42 pts with unstable angina and 3-vessel coronary artery disease were randomized to receive enoxaparin (n=21) 100 UI/Kg×2/day sc or UFH (n=22) 180 UI/Kg×2/day sc, starting 3 days before and until 12h before CABG. We collected venous blood samples (baseline, 30 min, 6h and 12h after the study drug administration) the day before CABG and performed the Enox-test or aPTT with a Rapidpoint Coagulation Analyzer. aPTT was also measured with a traditional coagulometer on citrated plasma. Haemoglobin (Hb), haematocrit (Hct), platelet count (Plts) and number of transfused blood units were determined before and after CABG. *Results*. Clinical and surgery-related characteristics, including on-pump time (120±44 min in enoxaparin vs 121±46 min in UFH group) and total surgery time (297±63 min in enoxaparin vs 294±67 min in UFH group), were similar in both groups. The aPTT and the Enox-test time, measured in pts treated with UFH and enoxaparin, respectively, increased slightly 30min after the administration, significantly after 6h, and normalized after 12h. Values of aPTT measured with the Rapidpoint Coagulation Analyzer correlated with those measured with the traditional coagulometer (R=0.85; p<0.0001). Haematological parameters significantly (p<0.001) decreased after CABG in both groups: Hb 13.2±1.2 –> 10.6±1.1 g/dL (Figure); Hct 39.4±3.6 –> 31.9±3.7%; Plts 208±41 –> 158±37×10//μl. The percent change (% Delta) of these parameters was similar in the two treatment groups (Figure). Number of transfused blood units/pt was similar in the two groups (0.7±0.6 in the enoxaparin group vs 0.8±0.7 in the UFH group).

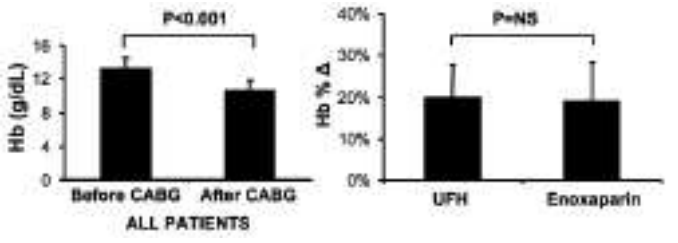

Figure 1.

Conclusions. Enoxaparin and UFH, when stopped >12 h before surgery, are associated with a normalization of haemostatic parameters at the time of surgery and comparable bleeding.

P041

SAFETY AND EFFICACY OF XIMELAGATRAN: META-ANALYSIS OF THE CONTROLLED RANDOMIZED TRIALS FOR THE PROPHYLAXIS OR TREATMENT OF VENOUS THROMBOEMBOLISM

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Background. Ximelagatran (XM), an orally direct thrombin inhibitor, has been approved for a short time in Europe for Venous Thromboembolism (VTE) prophylaxis in orthopedic surgery at fixed doses and without laboratory monitoring. Aim of the study was to evaluate safety and efficacy of ximelagatran in a metaanalysis of prophylaxis and/or treatment randomized controlled trials. *Methods.* Computerized searches of MEDLINE and EMBASE were performed; clinical trials were also hand-search. Eligibility of the studies and extraction of data were independently performed by two authors using a standard form. Absolute risk (AR) of events for XM and Odds Ratio (OR) for its comparison with LMWH and coumarins were calculated. Subgroup analysis was performed for ximelagatran regimen, comparator agent, type of surgery, starting time of prophylaxis. *Results.* Twelve studies and 16,992 patients were meta-analysed. Ximelagatran showed an absolute risk of major VTE of 4.04% and 1.69% and of major bleedings of 1.68% and 1.03% in prophylaxis and treatment trials, respectively. In prophylaxis trials, a significant excess mortality (OR: 2.5; 95% CI: 1.02-6.13) and an excess in major bleedings (OR: 1.41; 95% CI: 0.93-2.14) was found in the whole ximelagatran group. No evidence of treatment effect for major VTE was seen in the comparison with LMWH (OR: 1.01; 95% CI: 0.52 - 1.97). The cohort of patients treated with 24 mg b.i.d. showed similar results. An increase in the absolute risk of bleeding (from 1.04% to 3.03%) was found between post and preoperative administration of ximelagatran. Major VTE risk was increased when ximelagatran was compared to b.i.d. LMWH. *Conclusions.* Besides any consideration regarding erratic hepatotoxic side effects, XM shows a interesting risk to benefit ratio for prevention and treatment of VTE. The best postoperative starting time and the true effect on cardiovascular mortality remain to be assessed.

P042

RANDOMIZED COMPARISON OF DIFFERENT INR TARGETS FOR ORAL SURGERY IN PATIENTS ON VITAMIN K ANTAGONISTS

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Background. Frequently patients on oral anticoagulant therapy (OAT) undergo procedures of oral surgery after INR values are set below the therapeutic range, to avoid excessive post-operative bleeding. Reduction of OAT intensity may engender a risk of thromboembolism. We designed a prospective, randomized study to compare the outcome of oral surgery in patients operated upon conditions of reduced INR values, compared with patients maintained in their usual therapeutic ranges. All the procedures were carried out by the same surgeon.Patients and methods. 131 consecutive patients on long-term OAT because of mechanical heart valves (45% of cases), atrial fibrillation (30%) or venous thromboembolism (25%). 511 dental extractions (in average 4 teeth per patient), 6 fixture insertions and 6 exeresis of cystic neoformations were performed after patients were randomized to two treatment groups. Group A (mean age 64+ 11 years): OAT dosage was reduced during the 72 hours preceding surgery, in order to reach INR values between 1.5 and 2.0 (target 1.8) on the day of surgery; the mean INR value actually reached in this group was 1.77+0.26. Group B: (mean age 61+12 years), OAT dosage was not reduced, but local hemostatic agents (tranexamic acid and oxidized cellulose) were used; the mean INR in this group was 2.89+0.42 on the day of surgery. Results. Relevant bleeding, defined as patients requiring supplementary local hemostatic measures, was observed in 10 cases (15.1%) in group A (reduced dosage) and in 6 (9.2%) in group B (unchanged dosage), n.s. We did not report any thrombotic complication. *Conclusion.* This randomized study show that in patients on OAT who undergo oral surgery is not necessary to stop or to reduce its intensity because simple and inexpensive measures for local hemostasis are sufficient to limit bleeding complications when INR is in the therapeutic range.

P043

IMMUNOASSAY CAN BE NEGATIVE IN HIT AT PRESENTATION

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We report a case of heparin-induced thrombocytopenia (HIT) with multiple thrombosis and negative immunoassay at presentation. *Case report.* a 74 year-old man underwent surgical intervention for colon carcinoma. Platelet count before surgery and 3 days after was above 250.000/mmc. Enoxaparin prophylaxis started 4 days after surgery. Despite a drop in platelet count to 55.000/mmc on day 4 after the beginning of enoxaparin, prophylaxis was continued. The patient suffered from retinal central vein thrombosis and acute popliteal artery obstruction treated by Fogarty balloon on day 9 and 12, respectively. On day 12 a haematological consultancy was performed; heparin was stopped and lepirudin started in the suspect of HIT. Although PTT-ratio remained above 2 there was a progression of arterial thrombosis to gangrene and the patient underwent leg amputation on day 19. Warfarin was started 5 days later and the patient discharged. Diagnostic testing: a serologic assay detecting antibodies that recognize PF4 bound to heparin (enzyme immunoassay, AsserachromHPIA) was performed on day 12 and 29 since the beginning of enoxaparin; the former assay was negative while the latter converted to strongly positive. *Discussion.* The reported case was treated as HIT on clinical ground in spite of negative immunoassay at diagnosis. The same test became strongly positive 17 days after discontinuation of heparin and 10 days after the last arterial thrombosis. We speculate that anti-PF4/heparin antibodies may be undetectable in plasma because cleared by high binding to the target. Recently Rauova L. demonstrated that high platelet-associated PF4 levels are patient-dependent and lead to increased expression of HIT antigens thus modulating their pathogenic potential. We conclude that a single negative immunoassay does not exclude diagnosis of HIT if platelet kinetic and symptoms are suggestive. Since a positive laboratory test is required to use lepirudin in Italy, an alternative test or repetition of immunoassay should be considered.

P044

PITFALLS IN COAGULATION TESTS DETERMINATION

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Background. Most automated coagulometers load plasma samples from centrifuged primary tubes. In some instruments the plasma presence is checked by means of surface liquid sensors that allow the correct sampling independently from liquid levels, sample sizes and containers. We report on *false* incoagulable results in oral anticoagulant therapy (OAT) control due to a pre-analytical phenomenon highlighted by instrument-related analytical conditions. *Materials.* Plasma samples from six patients in OAT, collected in vacutainer containing 109 mM sodium citrate (Becton Dickinson). Centrifugation at 2000 g for 10 min. Thromboplastin reagent: Recombiplastin (IL). *Methods.* PT-INR determination with ACL9000, ACL7000 (IL) and by tilt-tube manual

technique. *Results.* Plasma from six patients, analyzed in different days in a two-month period, gave INR >8 on ACL9000 but in the expected range values when tested on the ACL7000 or after plasma transfer into cups. In at least three samples, two distinct plasma phases were appreciable (upper phase about 400 uL) by simple visual inspection of the tubes. INR determination: upper phase INR > 8, lower phase INR = 1.59-4.92. Results were confirmed also by prothrombin time determination by tilt-tube manual technique. Protein concentration: total protein upper/lower phase = 4.1/7.1 g/dL; relative protein components: very similar composition. No evidence for drug or clinical conditions association could be identified among the six patients. *Comments.* Pre-analytical variables together with specific instrument-related analytical conditions were found to affect coagulation time determination. The gradient protein concentration detected in some samples cannot be explained on the basis of the available information. The different liquid detector systems of ACL9000 (surface sensors) and of ACL7000 (fixed depth sensors) may cause differences in INR results between instruments of the same family. Incoagulable prothrombin times in the control of oral anticoagulant therapy need a careful evaluation of the actual anticoagulation level and should be carefully managed.

P045

LESS CLINICAL SEVERITY OF CEREBRAL ISCHEMIC EVENTS IN AF PATIENTS ON WELL CONDUCTED OAT

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Stroke associated with atrial fibrillation (AF) is especially large and disabling. It is well known that oral anticoagulant therapy (OAT) is effective in reducing stroke and embolism in AF patients. However, despite OAT, ischemic events do occur in some patients. Studies specifically addressing the identification of risk factors for ischemic events during well-conducted OAT are not available. In this study we prospectively investigated the role of classic risk factors and homocysteine levels in the occurrence of ischemic complications in 392 AF patients on OAT. The quality of anticoagulation levels and the occurrence and severity of thrombotic events were recorded. During follow-up [955 patient/years(pt/yrs)] 23 patients [11 M, 12 F; mean age 76 yrs (66-85)] had ischemic complications (rate 2.4×100 pt/yrs). Eight patients (35%) had TIA, 9 patients (39%) had minor stroke with full recovery, 5 patients (22%) had stroke with neurological sequaele, and in 1 patient (4%) stroke was fatal. INRs related to the ischemic events were <1.9 in 7/23 patients and ï‚1.9 in 16/23 patients; the patient with fatal stroke had INR=1.5. No difference was observed among patients who had and had not an ischemic complication in relation to the quality of OAT measured as time spent within, above and below INR therapeutic range. The presence of history of previous ischemic events, of hypertension and of homocysteine plasma levels over the 90th percentile were all associated with an increased risk of ischemic events during OAT [OR =3.7 (1.5-9.4 95% CI), OR=4.9 (1.4-17.1 95% CI) and OR= 5.9 (2.1-16.6 95% CI), respectively].

P046

EVALUATION OF PREFERENCES ON ANTICOAGULANT TREATMENT (OAT) IN PATIENTS RECEIVING VITAMIN K ANTAGONISTS: A CONJOINT ANALYSIS EXERCISE

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Background. Patients' preferences should be taken into account in the development of new drugs. *Aim.* To assess patients' preferences on relevant attributes of hypothetical anticoagulant drugs. *Methods and Patients.* A Conjoint Analysis Exercise was applied in our Anticoagulation Clinic to 97 patients randomly selected among those on OAT for more than 6 months and to 140 consecutive patients on OAT for less than 2 weeks. Patients were asked to choose, in a 9 pair-wise comparison, between 2 hypothetical anticoagulant drugs. Each drug was characterized by 6 attributes (with different levels), previously selected by means of a questionnaire administered to 20 patients and 6 doctors. The attributes and the levels were: patient's expenditure for treatment (€0 vs €15 vs €75/month), pharmaceutical formulation (tablets once daily vs tablets twice daily vs subcutaneous injections once weekly), frequency of controls/visits (twice monthly vs once monthly vs once every 6 months), dose adjustment (required vs not required), interactions with drugs/food (attention required vs not required), minor bleedings (few vs no). *Results.* A monetary value was assigned to each attribute: a significant discrimination was reached for all attributes, except for interactions with drugs/food and dose adjustment. Patients' willingness to pay (WTP) was acknowledged in decreasing order for: tablets once daily, visits once monthly, no minor bleeding, no attention required to interactions with drugs/food. Overall, patients' expenditure was perceived as a relevant attribute. Patients on OAT > 6 months showed higher WTP for once monthly visit and no interactions with drugs/food; patients on OAT <2 weeks for no minor bleedings. *Comments.* Patients on OAT prefer an oral, once daily administered drug, which does not require any personal expenditure. Once monthly control of treatment was a well accepted attribute.

P047

ANTI-INFLAMMATORY ACTION OF A NEW LOW MOLECULAR WEIGHT HEPARIN-LIKE DERIVATIVE IN AN ACUTE EXPERIMENTAL INFLAMMATORY MODEL

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Heparin has several biological actions independent of its well-known anticoagulant activity: its ability to modulate the flogistic processes is clinically relevant, considering the role of the inflammatory state in the appearance and progression of atherosclerosis. The K5 polysaccharide obtained from E.Coli strain 010:K5:H4 has a structure alike to N-acetylheparosan, the precursory polymer of heparin. The aim of this study was to evaluate the effects of a low molecular weight heparin-like derivative: K5-N OS epi LMW, administered intrapleurally at the different doses (0.1, 0.5, 1 mg/kg b.w.) on an *in vivo* model of carrageenan-induced pleurisy in the rat. Compared with the exudates, fluid volume and number of cells collected from the pleural cavity of the sham-operated rats, injection of carrageenan induced a significant increase in polymorphonuclear cell count. Pre-treatment of rats with K5-N OS epi LMW (1 mg/ Kg b.w.) significantly (p<0.01) reduced the volume of the pleural exudates(-38.4%) and the number of PMN within the exudates (-93.5%). In addition, pre-treatment of rats with K5-N, OS epi LMW, as well as with celecoxib (1 mg/Kg b.w.), significantly inhibited (p<0.01) the carrageenan-induced iNOS expression, and iNOS activity (-50%), as well as NO production (-49%). The carrageenan-induced IL1-β and TNF-α levels were found to be significantly lower in the pleural exudates of rats pre-treated with K5-N OS epi (0.5 and 1 mg/kg b.w.) in comparison to those observed in rats treated only with carrageenan. Similarly PGE2 levels in the exudates were significantly lower in the exudates obtained from carrageenan -treated rats that had been pre-treated with K5-N OS epi at 0.5 or 1 mg/kg b.w. These results indicate that the K5-N OS epi LMW is able to inhibit both the expression and the production of inflammatory mediators and offer a new therapeutic approach for the management of various inflammatory diseases.

P048

PT INR: COMPARISON BETWEEN TWO DIFFERENT POINT OF CARE SYSTEMS

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Introduction. Portable coagulation systems are used to perform PT-INR test on capillary blood. These instruments can be used for monitoring oral anticoagulant therapy (OAT) in home patients or in patients followed in peripheral health units. *Aim of the study.* Evaluation of INRatio portable monitor compared with Coagucheck and PT-INR provided by central laboratory reference system. *Method.* INRatio (Dasit, Milan, Italy) based on impedenziometric system using a human recombinant thromboplastin and internal quality control; - coagucheck S (Roche Diagnostics, Basel, Switzerland) using freeze-dried rabbit thromboplas-

tin with an ISI of 1.84; - reference system: magneto-mechanical coagulation analyzer STA-R (Roche Diagnostics) for PT-INR determination from venous blood specimens, using rabbit thromboplastin with an ISI of 1.31 (Neoplastin Plus Stago Paris, France) and recombinant thromboplastin (Recombiplastin IL Milan) in the same analytical session. The blood venous specimens were collected from 80 OAT patients (INR range 2-4) and 20 healthy volunteers; capillary INR was performed by the two portable monitors. Comparison of the different system was performed by linear regression and Bland and Altman method. *Results.* The coefficient correlation among different systems is good ranging between 0.88 and 0.93. The better correlation resulted between Coagucheck system and recombinant Thromboplastin, as showed by confidential bias limits and regression linear lines. Despite these correlations, the Bland Altman method showed an overestimation of INRatio results in comparison with other methods, particularly for longer PT. However the 95% results are included between ± 2 DS, corresponding to ± 0.5 INR. *Discussion and Conclusion.* Results showed good correlation between the three different systems. The disagreement showed by Bland Altman method probably depends on thromboplastin calibration and their different sensitivity. In fact Bland-Altman results vary in function of which reference thromboplastin is used. INRatio system is simple to use and sufficiently accurate; therefore it is a good system for PT/INR determinations in selected patients at peripheral health units. The company should communicate ISI value of thromboplastin used in portable monitors and calibration model as well as assure a quality control system. Before use a portable monitor, we suggest to evaluate the agreement with reference system.

Table.

Equation	*r*	*Linear Regression Conf Bias (0.05)*	*Bias*	*Bland & Altman d+2s*	*d-2s*	
Neoplastin vs Coaguchek	0.89*	y=0.55+0.77x	0,24	±0.06	-0.54	0.57
Neoplastin vs INRATIO	0.88°	y=0.81+0.56x	0,26	±0.09	-1.30	0.49
Recombiplastin vs Coaguchek	0.93^	Y=0.58+0.67x	0.18	±0.06	-0.34	0.73
Recombiplastin vs INRATIO	0.88^	Y=0.86+0.46x	0.22	±0.11	-0.39	1.62
Coaguchek vs INRATIO	0.88°	y=0.54+0.65x	0.30	±0.09	-1.25	0.41

p=0.28 p<0.001° p=0^*

P049

PROTHROMBIN TIME: COMPARISON BETWEEN DIFFERENT REAGENTS

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Introduction. Prothrombin time is test used for laboratory monitoring of patients on oral anticoagulant therapy. The International Normalized Ratio (INR) was introduced to get over the great variability in PT results among different laboratories. Therefore all reagents should be calibrated to provide identical results. *Aim of the study.* compare the PT INR and PT Ratio results performed with STA-R System (Diagnostica Roche) using five different reagents. *Method.* The blood venous specimens were drawn from 80 OAT patients (INR range 2-4) and 20 healthy volunteers into citrate coagulation tubes (0.109M). Sample tubes were centrifugated at 3000 r.p.m. for 10 min and then analyzed by STA-R using five different reagents. - Magneto-mechanical coagulation analyzer STA-Rack Diagnostica Stago Roche; - Neoplastin Plus (Stago Roche Paris, France) with ISI=1.23- Tromboplastin S (Dasit, Italy) with ISI=1.68; - Recombiplastin (IL) with ISI=0.88; - Neoplastine CI (Roche) with ISI=1.81; - Helena (Manchester Reagent) with ISI=1.6. *Results.* Analysis was performed after recalculation of ISI by specific plasma. The results were evaluated by coefficient correlation and linear regression equations and described by graphic representation according to Bland and Altman method. The results are showed in Table 1- 2. Coefficient correlation of PT-INR is resulted between 0.82 and 0.98; the better correlation was between the two thromboplastin of Roche company. Coefficient correlation of PT-Ratio was all above 0.90. In both cases Bland and Altman method has shown poorer agreement between results and marked dispersion of values around average differences. *Discussion.* The results showed poor agreement of PT-INR and PT-Ratio values obtained with different reagents. The disagreement increases systematically with longer PT and the recommended analytical bias (-0.20 INR units) is exceeded in most cases. These results indicate a non-complete standardization of current calibration methods and confirm, as already previously demonstrated, the different sensitivities of thromboplastin reagents. We suggest a constant collaboration between companies and users to improve the agreement among results the quality and sensitivity of reagents.

Table 1.

	r	*Linear regression Equation*	*Bias*	*Bland & Altman Conf. Bias (0.05)*	*d*		*d-2s d+2s*
Recombiplastinvs Tromboplastin	0.96	y=0,52+0.61x	0.12	± 0.06	-0.48	-1.06	0.10
Recombiplastin vs Neoplastine CI	0.86	y=0,70+0.59x	0.23	± 0.07	-0.31	-0.97	0.41
Recombiplastin vs Neoplastin	0.87	y=0,66+0.65x	0.22	± 0.06	-0.21	-0.73	0.47
Recombiplastin vs Helena	0.83	y=1.12+0.28x	0.23	± 0.25	-1.63	-3.39	0.27
Tromboplastin vs Neoplastine CI	0.88	y=0.31+0.95x	0.33	± 0.07	0.17	-0.45	0.85
Tromboplastin vs Neoplastin	0.88	y=0.29+1.02x	0.34	+/- 0.07	0.27	-0.31	1.02
Tromboplastin vs Helena	0.82	y=1.07+0.41x	0.36	±0.22	-1.18	-2.69	0.49
Neoplastine CI vs Neoplastin	0.98	y=1.06x	0.12	±0.02	0.10	-0.09	0.40
Neoplastine CI vs Helena	0.94	y=0.63+0.48x	0.22	±0.19	-1.32	-2.63	0.05
Neoplastin vs Helena	0.96	y=0.60+0.45x	0.15	±0.19	-1.39	-2.81	0.04

p<0.001

Table 2.

	r	*Linear regression Equation*	*Bias*	*Bland & Altman Conf. Bias (0.05)*	*d*		*d-2s d+2s*
Recombiplastinvs Tromboplastin	0.96	y=-1.74+2.51x	0.21	± 0.10	0.93	-1.01	1.88
Recombiplastin vs Neoplastine CI	0.93	y=-2.41+3.12x	0.26	± 0.11	1.06	-0.009	2.13
Recombiplastin vs Neoplastin	0.95	y=-0.89+1.81x	0.22	± 0.08	0.71	-0.05	1.48
Recombiplastin vs Helena	0.92	y=-0.77+1.52x	0.27	±0.09	0.43	-0.26	1.11
Tromboplastin vs Neoplastine CI	0.97	y=-0.25+1.23x	0.06	±0.01	0.13	-0.03	0.29
Tromboplastin vs Neoplastin	0.96	y=0.37+0.70x	0.07	± 0.03	-0.21	-0.48	0.05
Tromboplastin vs Helena	0.91	y=0.51+0.54x	0.10	±0.06	-0.51	-0.94	-0.09
Neoplastine CI vs Neoplastin	0.97	y=0.52+0.55x	0.04	± 0.03	-0.34	-0.70	0.006
Neoplastine CI vs Helena	0.93	y=0.64+0.43x	0.07	±0.06	-0.63	-1.12	-0.15
Neoplastin vs Helena	0.97	y=0.18+0.79x	0.08	±0.03	-0.27	-0.51	-0.04

p<0.001

P050

EFFICACY AND SAFETY OF LOW MOLECULAR WEIGHT HEPARIN DURING WARFARIN UNDERDOSAGE IN PATIENTS WITH VENOUS THROMBOEMBOLISM

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Background. Venous thromboembolism (VTE) is a common and serious disorder. A first course of unfractionated (UH) or low molecular weight heparin (LMWH) followed by at least 3 months of oral anticoagulant therapy (OAT) is the treatment of choice in patients with acute VTE episodes. The first three months of treatment represent the more critical period because of poor anticoagulation quality and the incidence of VTE recurrences is higher in this period. For this reason, at the Haemostasis and Thrombosis Centre of Cremona we introduce a clinical protocol for the management of OAT patients that include the use of LMWH any time we observe a poor anticoagulation quality. *Methods* Patients underwent OAT for unprovoked or provoked VTE less than three months with poor anticoagulation quality (INR <2,0) were enrolled. LMWH (nadroparine) was associated to OAT using therapeutic or prophylactic dosage for INR value less than 1.5 and INR between 1.5-1.95, respectively. Efficacy and safety of this approach was evaluated comparing the incidence of thrombotic and major bleeding events with the previous three years. *Results.* Between June 2002 and June 2005, 1/378 (0,26%) VTE recurrence comparing with 5/353 (1.41%) thrombotic events in the previous three years we observed. No differences between major bleeding events were found. *Conclusion.* The association of LMWH with OAT in VTE patients with low INR reduce the incidence of VTE recurrences without increase of bleeding events.

von Willebrand factor and ADAMTS-13

P051

VON WILLEBRAND DISEASE: A REAPPRAISAL OF CASES USING THE PROVISIONAL CONSENSUS CRITERIA

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Background. Von Willebrand disease (vWD) is the most common inherited bleeding disorder. Due to the heterogeneity of vWF defects, a correct diagnosis may be difficult. Recently, Sadler et al.(1) published the provisional consensus criteria selected by the Subcommittee on vWD which may provide a point of reference. *Aim of the study.* To reappraise our cases using the provisional criteria: 1. Two bleeding symptoms or 1 requiring transfusion or one recurring 3 times. 2. One first-degree or 2 second-degree relatives with a significant bleeding history. *Methods.* This retrospective study includes 61 patients registered from February 2004 up to August 2005 at our centre. Each patient had a review of their medical records. Data on blood group type, agglutination assay, personal and family bleeding history, drugs, FVIIIc and vWF levels were collected. Comparisons were made using the chi-squared test. *Results.* Results are summarized in Table 1 and 2.

Table 1.

Criteria	*Blood group 0*	*non-0*	*TOT*
No	38*	7	45
Yes	8	8	16
46	15	61	

**p<0.05*

Table 2.

Criteria	*Test pos.*	*Test neg.*	*TOT*
No	*24*	*21*	*45*
Yes	*13*	*3*	*16*
37	*24*	*61*	

VPP=35% ($IC_{95\%}$:20-50%),VPN= 87% ($IC_{95\%}$:74-100%).

Seventy-three percent of patients (45/61) did not meet the criteria. Among them we found one case of acquired vWD caused by a monoclonal antibody and three patients on valproate. The number of patients with blood group 0 was higher in those without criteria (p<0.05). Among those who fulfilled the criteria we found two women on estroprogestinic treatment whose tests were negative. Our tests showed a low positive predictive value (35%) and a high negative predictive value (87%). *Conclusions.* These findings suggest that these criteria seem an useful tool in the diagnosis of vWD. They highlight that we need separated references values for 0 and non-0 blood group. Moreover, our VPP should be improved through a more accurate selection of patients to test, seeking for interfering factors (e.g. drugs, lympho-myeloproliferative diseases).

References

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P052

PROSPECTIVE STUDY ON THE BEHAVIOR OF THE METALLOPROTEASE ADAMTS13 AND OF VON WILLEBRAND FACTOR AFTER BONE MARROW TRANSPLANTATION

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Thrombotic microangiopathies (TMAs) are rare but serious complications of bone marrow transplantation (BMT). Clinical manifestations are similar to those of thrombotic thrombocytopenic purpura (TTP), but prognosis is generally poorer despite plasma exchange. The enzymatic activity of the plasma metalloprotease ADAMTS13, that cleaves ultralarge thrombogenic multimers of von Willebrand factor (VWF) derived from activated endothelial cells, is very low or undetectable in patients with classic TTP, and protease deficiency is thought to play a mechanistic role in the formation of platelet thrombi in the microcirculation. This is the first prospective study to evaluate the incidence of TMA in 46 consecutively recruited patients undergoing autologous or allogenic BMT and to explore in parallel the behavior of ADAMTS13, VWF antigen and VWF multimer size. The incidence of post-BMT TMA was 6% (3/46), all cases occurring after allogenic BMT. Compared to baseline values plasma ADAMTS13 activity was significantly reduced in patients undergoing BMT, particularly after the conditioning regimen (mean values: 50±22 vs 77±32%; $p<0.0001$). In the 3 patients who developed TMA ADAMTS13 decreased after conditioning, but in one instance only it was very low (8%). VWF antigen levels progressively increased starting after the conditioning regimen (228±75 vs 178±76% on baseline, p=0.002). The mean proportion of high molecular weight VWF multimers did not change in the various stages of BMT, even though ultralarge multimers were transient found in same cases with and without TMA. Hence, the measurements evaluated in this study are not clinically useful to predict the occurrence of post-BMT TMA.

P053

EXPRESSION STUDIES OF TWO MISSENSE MUTATIONS (D141Y AND C275S) IN THE VON WILLEBRAND FACTOR (VWF) PROPEPTIDE ASSOCIATED WITH TYPE 3 VON WILLEBRAND DISEASE (VWD)

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Type 3 VWD is a rare autosomal recessive disorder, characterized by unmeasurable levels of VWF in plasma and platelets (VWF:Ag <0.01 IU/mL). Eight missense mutations have been identified in our laboratory in type 3 VWD patients. Five of them are in the VWF propeptide, essential to promote the VWF multimerization and storage. We performed expression studies of two mutations (D141Y and C275S) to confirme their relationship with VWD. Both patients were compound heterozygous, one carries mutation D141Y and a deletion of 4 nucleotides (2266-2269), the other a C275S and a nonsense mutation (W202X). The missense mutations were introduced into the pSV-WVF vector by site direct mutagenesis. Mutated vectors were expressed in COS-7 cells alone and co-expressed along with the wild type (WT). An ELISA method was used to evaluate the expressed rVWFs. Assuming rVWFWT as 100%, a markedly reduced secretion was detected in cell media, 13.9% for rVWFD141Y and 14.2% for rVWFC275S, while in cell lysates the amount of mutant rVWFs were slightly increased (118.2% and 127%). As expected, hybrid rVWFs (mutants and wild type), mimicking the heterozygous form, resulted in a milder reduced secretion (44.8% rVWFWT/rVWFD141Y and 39.4% rVWFWT/rVWFC275S), although the amount of hybrid rVWFs were higher in cell lysates (139.5% and 144.3%). Multimer analysis of rVWFs showed that both variants strongly impaired the multimerization process since only dimers were present in the cell media. However, both hybrid rVWFs presented a full set of multimers, similarly to the rVWFWT, suggesting that the mutated rVWF did not compromise the multimerization process of the rVWFWT subunits. In conclusion, these experiments showed that the mutations D141Y and C275S of VWF gene result in a quantitative deficiency of VWF in plasma, due probably to a secretion pathway defect associated with intracellular degradation.

P054

THE THROMBOSPONDIN-1 N700S POLYMORPHISM DOES NOT ALTER VON WILLEBRAND FACTOR MULTIMER SIZE IN PATIENTS SUFFERING AN ACUTE MYOCARDIAL INFARCTION

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An uncommon missense variant of thrombospondin-1 representing an asparagine (Asn-700) to serine substitution (Ser-700) has been identified as a potential genetic risk factor for myocardial infarction (MI). Thrombospondin-1 (TSP-1) is a glycoprotein stored in human platelet α granules and has been shown to regulate von Willebrand factor (VWF) multimer size. The TSP-1 domain responsible for VWF-reducing activity was localized to the calcium-binding C-terminal sequence at position Cys 974. Considering the proximity of the N700S TSP-1 polymorphism and the demonstration that the Ser-700 polymorphism alters the calcium binding affinity of this domain, we investigated whether altered VWF-reducing activity of Ser-700 polymorphism underlies the observed prothrombotic phenotype. Plasma samples were analyzed from a cohort of patients (N=31) who survived an acute myocardial infarction at age £45 compared with age-matched controls (N=19). The relative percentage of high molecular weight VWF multimers did not differ significantly between individuals homozygous for either Ser-700 or Asn-700 ($p=.20$). Similarly, the relative percentage of high molecular weight multimers was 31.2% for the Ser-700 homozygous MI patients (N=21) versus 31.1% ($p=.95$) in the Asn-700 healthy controls (N=10). These findings were confirmed using the collagen binding assay to assess VWF activity relative to antigen concentrations. The mean ratio of CBA/Ag was .98 in the Ser-700 patients versus 1.0 in the Asn-700 control group ($p=.22$). The VWF reducing capacity of TSP-1 was also assessed *in vitro* using recombinant TSP-1 N700S constructs containing the last EGF-like repeat, all type-3 repeats (calcium-binding domains), and C-terminal sequence. Incubation of Ser-700 peptide, Asn-700 peptide, or platelet purified full-length TSP-1 with ultra-large VWF (using either TTP plasma or recombinant ultra-large VWF) did not result in any significant reduction of VWF multimer size under static conditions. In conclusion, the association between coronary artery thrombosis and the TSP-1 Ser-700 polymorphism is not mediated through altered VWF reducing activity.

P055

LOCALIZATION AND FUNCTION OF PLATELET ADAMTS-13

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The hemostatic activity of von Willebrand factor (VWF) is strongly dependent on its multimeric size, with the highest activity in â??unusually large' multimers secreted from endothelial cells. The multimeric size is regulated by a plasma metalloprotease, ADAMTS-13. Since 15-25% of circulating VWF is stored in platelets, the presence and function of ADAMTS-13 in platelets could be an important issue to be investigated. In our study, we confirmed the presence of ADAMTS-13 mRNA in the human platelets as previously reported by Suzuki M. et al (2004). Total mRNA isolated from platelet lysates was reverse-transcribed with specific primers spanning from exon 2 to 5 of ADAMTS-13 cDNA. The immunoblot analysis on platelet lysates, using a monoclonal antibody against ADAMTS-13 CUB domains (13E2/75), revealed a band with an apparent molecular weight of ~200 kDa. Moreover, we demonstrated that platelet ADAMTS-13 is enzymatically active, being able to cleave recombinant VWF by roughly 10% compared to plasmatic ADAMTS-13, dependent on the amount of the enzyme and inhibited by EDTA. Immunofluorescence analysis revealed that ADAMTS-13 in resting platelets was mainly localised at the plasma membrane region. Merge fluorescence studies showed no apparent colocalization of ADAMTS-13 with alfaIIbβ3 or VWF, suggesting that the protease is not detectable in the a-granules. Interestingly, The expression of ADAMTS-13 increased upon platelet activation by TRAP (20 micromolar) and

appeared to spread even in the pseudopods. In conclusion, this study confirmed that ADAMTS-13 is present in human platelets, enzymatically active under static conditions and detectable at the plasma membrane region with immunofluorescence analysis. The exploration of the membrane skeleton and cytoplasmic actin filaments located just under the plasma membrane might clarify the location of ADAMTS-13 and its role in the local regulation of platelet function at the site of vascular injury and thrombus formation.

P056

A TYPE 2B VON WILLEBRAND DISEASE (VWD) PATIENT WITH A FULL SET OF MULTIMERS LINKED TO THREE MUTATIONS (V1229G, N1231T, P1266L) IN THE A1 VON WILLEBRAND FACTOR (VWF) DOMAIN AND A MILD PLATELETS SECRETION DEFECT

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The patient was a 26 years-old woman when she required transfusion because of severe bleeding after abdominal surgery. She was found to have normal/borderline VWF activities (VWF:Ag 64%, VWF:RCo 54%, VWF:CB 57%) with an enhanced affinity for the platelet glycoprotein Ib α (0.6 mg/mL RIPA) and a borderline/normal platelet count (103,000-172,000/μL). Despite a normal VWF multimeric pattern in her plasma, she was found to carry 5 distinct nucleotide substitutions (3686T>G, 3692A>C, 3735G>A, 3789G>A and 3797C>T) on the VWF exon 28 encoding for the A1 domain. Two of these substitutions were silent, whereas the remaining caused the following amino acid changes: V1229G, N1231T and P1266L. The mutations were found also in her father, paternal grandfather and uncle confirming that all defects were carried by a single allele. The three missense mutations have already been reported: V1229G, N1231T together were found in a patient classified as type 1 (Thromb Haemost 1998;79:709), whereas P1266L was identified in the type 2B New York and Malmo (J Clin Invest 1993;91:77). The fact that all five substitutions found in this patient correspond to the pseudogene sequence, sustain the possibility of a gene conversion between the VWF gene and pseudogene, as reported by others (Thromb Haemost 1998, 79:709 Blood 2001;98:248). The patient was the only one among other three family members with the same genetic defect who presented bleeding symptoms. In some patients with type 2B Malmo, additional risk factors for bleeding such as impaired collagen-induced platelet aggregation have been reported (J Thromb Haemost 2004;2:2055). Further investigation performed in our laboratory, indeed showed a mild platelet secretion defect when platelets were stimulated with ADP. Our data confirm that VWF defects can be complex within VWD families and that bleeding tendency can vary according to additional defects of haemostasis.

P057

IMPAIRED COLLAGEN BINDING ACTIVITY (VWF:CB) OF VON WILLEBRAND DISEASE (VWD) TYPE 2B

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Among the different tests used for VWD diagnosis ,VWF:CB has been proposed as additional test to further characterize different VWD variants, despite the fact that has not been standardized yet. In our laboratory we perform VWF:CB with collagen from two different sources: VWF:CB(I) (equine type I from Nycomed) and VWF:CB(III)(human type III from Southern Biotechnologies). Among 83 samples from different VWD patients and 22 normal controls, we found that in VWD type 2B patients the VWF:CB(I) was significantly more reduced than VWF:RCo. (Table 1). In all VWD 2B patients the VWF:CB(I)levels were lower than VWF:RCo, even in patients with normal VWF multimers . The VWF:CB (III) behaved differently and appeared to reflect only the loss of high molecular weight multimers.(HMWM) The VWF:CB (I)/Ag ratios of patients with type 2A and 2B VWD with abnormal multimers were very similar, even though the VWF multimeric structure of 2A is more defective than that of type 2B VWD patients , whereas the VWF:RCo/Ag ratios were clearly different. These data suggest that type 2B VWF variants might also have a reduced VWF:CB not only related to the loss of HMWM.

Table 1.

VWD Type (n)	VWF:RCo/Ag	VWF:CB(I)Ag	VWF:CB(III)/Ag	RIPA mg/mL
N (22)	0.9±0.18	1.04±0.2	1.03±0.2	1.00±0.1
1 (20)	0.96±0.2	1.09±0.3	0.99±0.1	1.3±0.15
2M (20)	0.20±0.1	0.80±0.2	0.82±0.1	1.7±0.2
2A (14)	0.17±0.1	0.12±0.1	0.38±0.2	2.1± 0.7
2B (21)*	0.48±0.2	0.13±0.1	0.58±0.3	0.59±0.13
2B (8)**	0.66±0.2	0.5±0.1	0.75±0.2	0.47±0.13

** Abnormal multimers ** Normal multimers*

P058

AUTOSOMAL RECESSIVE VON WILLEBRAND DISEASE ASSOCIATED WITH COMPOUND HETEROZYGOSITY FOR A NOVEL NONSENSE MUTATION (2908 DEL C) AND THE MISSENSE MUTATION C2362F: DEFINITE EVIDENCE FOR THE NON-PENETRANCE OF THE C2362F MUTATION

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The missense mutation G7335T (C2362F) in exon 42 of von Willebrand factor gene has been almost exclusively detected in the Veneto Region in families with recessive von Willebrand disease. It is not clear whether the mutation in heterozygosity is able to induce bleeding and to cause significant laboratory VWF abnormalities. A novel null mutation (2908del C in exon 22) of the von Willebrand factor (VWF) gene was identified in compound heterozygosity with the C2362F in a propositus from a new family with autosomal recessive von Willebrand disease (VWD). The propositus, referred at age 2 for severe epistaxis and prolonged bleeding after a tongue bite, had factor VIII:C 14-21 IU/dL, VWF Antigen 3-8 IU/dL and Ristocetin Cofactor activity < 3 IU/dL. Multimeric pattern showed the lack of triplet pattern and a faster mobility of central band. In the propositus' family 5 subjects were heterozygotes for the C2362F mutation and 5 were heterozygotes for the cytosine deletion. Bleeding score in 28 heterozygotes for C2362F, assessed with a detailed questionnaire, was similar to what is observed in normal controls. In conclusion, the mutation C2362F is frequently observed in compound heterozygosity with null alleles in patients with recessive VWD in the Veneto region and cause bleeding only in the compound heterozygous or homozygous state.

P059

A FLOW-CYTOMETRIC METHOD FOR THE STUDY OF VON WILLEBRAND DISEASE

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von Willebrand disease (vWD) is a heterogeneous disorder for the diagnosis of which several laboratory tests and repeated analysis of patients and family members are required. Type 1 vWD is characterized by a partial quantitative defect of von Willebrand factor (vWf) with associated mild hemorrhagic and bleeding symptoms. Patients with type 1 vWD undergoing invasive procedures are commonly treated with desmopressin (DDAVP) which transiently enhances vWf plasma levels; given the wide variability in the individual response, DDAVP usually tested for efficacy before therapeutic use by determining plasma vWf (vWf:Ag; vWf:RiCof) and Ristocetin Induced Platelet Aggregation (RIPA). Aim of our study was to validate a flow cytometric test in the diagnosis of type1 vWD and in the assessement of the therapeutic efficacy of DDAVP. Flow cytometric analysis of ristocetin-induced vWf binding to platelets was performed in PRP samples of patients with type1 vWD and control subjects. In a subgroup of patients the test was applied before and one hour after DDAVP infusion (0.3 μg/Kg). Results were compared with those obtained with the measurement of vWf:Ag, vWf:RCof, RIPA and PFA-100 (coll/epi and coll/ADP). Ristocetin-induced vWf binding to platelet GPIbα, as evaluated by flow cytometry, was significantly reduced in patients with type1 vWD respect to healthy subjects. The mean fluorescence intensity (MFI) after stimulation with ristocetin 1.5 mg/mL was 11.0±1.8 in patients with reduced vW:Ag (n=15; p<0.001)

and 11.7±1.4 in patients with reduced vWf:Rco (n=21; $p<0.01$) vs.18.9±1.5 in healthy controls (n=10). vWF binding significantly correlated with vWf:Ag, vWf:RCof, RIPA and PFA100 collagen/EPI (r2=0.29, 0.37, 0.13, 0.4, p<0.05 at least respectively). vWf binding to platelets in patients with type1 vWD increased significantly after DDAVP infusion (from 7.4±1.5 to 18.0±2.7, $p<0.001$,n=9) and correlated with the rise of vW:Ag and vW:RCof (r2= 0.69 and 0.5, respectively, $p<0.05$). The measurement of ristocetin-induced binding of vWf to platelets by flow cytometry is a sensible, simple and rapid test for the diagnosis of type1 vWD and for the monitoring of DDAVP infusion.

P060

BIOCHEMICAL CHARACTERIZATION OF INFUSION VOLUME REDUCED HAEMATE P®/HUMATE-P®

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Introduction. Von Willebrand Factor (VWF)/Factor VIII concentrates, such as Haemate® P/Humate-P®, are used in the treatment of von Willebrand disease (VWD), as well as in selected cases of hemophilia A. As certain treatment protocols like inhibitor treatment for hemophilia A patients do require the infusion of high dosages, an infusion volume reduced (i.v.r.) Haemate® P/Humate-P® was developed increasing the product concentration and decreasing the infusion volume while maintaining the product integrity and characteristics. *Materials and methods.* Vials of eight i.v.r. Haemate® P/Humate-P® batches were reconstituted using i.e. 5 mL (250 IU F VIII), 10 mL (500 IU F VIII), or 15 mL (1.000 IU F VIII) of water for injection. These lots were compared analytically with 18 lots of the current Haemate® P/Humate-P®. Assay reagents resp. kits used were F VIII deficient plasma / Pathromtin SL (Dade Behring), BC von Willebrand reagent (Dade Behring), and Clotimmun-AHG (Dade Behring). *Results.* The biochemical characterization demonstrated that the i.v.r. product contains twofold increased concentrations of the active ingredients VWF and FVIII compared to current Haemate® P/Humate-P®. The VWF:RCo concentrations e.g. were measured as 120 and 159 IU/mL in mean for the 250/500 and 1000 IU presentations, respectively. To our knowledge these represent the highest concentrations available now for a VWF/F VIII concentrate. In addition, it could be shown that the favorable high VWF:RCo/F VIII:C ratio of about 2.4 could be maintained also for the volume reduced product. The mean VWF:RCo/VWF:Ag ratio of 0.81 and the high molecular weight multimer content of i.v.r. Haemate® P/Humate-P® are equivalent to current Haemate® P/Humate-P® and close to that of normal human plasma, what has been reported to be important for achieving an efficacious primary haemostasis. *Conclusions.* In summary, the biochemical investigations performed provide reassurance that the key characteristics for i.v.r. Humate-P®/Haemate® P remain unchanged in comparison to the current product. In particular, the high molecular weight VWF multimers, as well as the favorable VWF/FVIII ratio are not changed.

Nutrition factors/homocysteine

P061

RETINAL VEIN OCCLUSION (RVO) AND HYPERHOMOCYSTEINEMIA

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Retinal vein occlusion (RVO) is a relatively common disease which is usually associated with visual loss of variable degree. The occlusion may be localized in large, medium, and small-calibre veins. The disease is often associated with common systemic vascular disorders such as hypertension, arteriosclerosis and diabetes, but an infiammatory aetiology is possible. Over the past years a limited number of studies has evaluated the possible role of different haemostatic factors in the pathogenesis of RVO, but conflicting results have been reported. The hyperhomocysteinaemia (HHcy) is an established vascular risk factor conferring a 60% rise in relative risk of cardiovascular disease with a total homocysteine (tHcy) rise of 1SD above the normal population mean. The aim of this study is to define the real role of HHcy in the retinal vein occlusion. We studied 45 patients with RVO, 19 males and 26 females, median age 56 years (range 24-78). Eigtheen patients showed a significant increase of the fasting homocysteine (median 25 µmol /L, range 18-50, normal values <15 µmol/L), 13 patients showed, after methionine load, an increase of the values of the homocysteine above the limits of the reference range. Overall, in 31/45 patients (68,8%) a hyperhomocysteinaemia was shown. In addition, 3 patients with hyperhomocisteinaemia had also a congenital thrombophilia (one patient with the Factor V Leiden mutation, 3.2%, and one patient with the G20210A prothrombin mutation, 3.2%), versus one patient with the Factor V Leiden mutation in the group with homocysteine values in the normal range. Between the patients with hyperhomocysteinaemia three of them had un essential hypertension as major cardiovascular risk factor versus four patients with arterial hypertension or diabetes in the group with homocysteine in the normal range. In our experience, the evaluation of the homocysteine, fasting and after methionine load, may be useful in the setting of the RVO; however, further studies are necessary to elucidate the role of the hyperhomocysteinaemia in the RVO.

P062

HAEMOSTATIC EFFECTS OF PHYTOESTROGEN GENISTEIN IN POSTMENOPAUSAL WOMEN

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Genistein is an isoflavone phytoestrogen derived from the soyban which has a weak estrogenic activity. To now its haemostatic effects are not well known. In this single-blind placebo-controlled trial we enrolled 312 healthy postmenopausal women with osteoporosis or osteopenia. 159 patients (mean age 55,10±4,15 yr; BMI 24,50±3,01 Kg/m^2) received genistein (54 mg/day) and 153 patients (mean age 54,70±4,78 yr; BMI 25,01±4,14 Kg/m^2) received an identical placebo-tablet. Both groups received a supplementation of calcium and vitamin D.

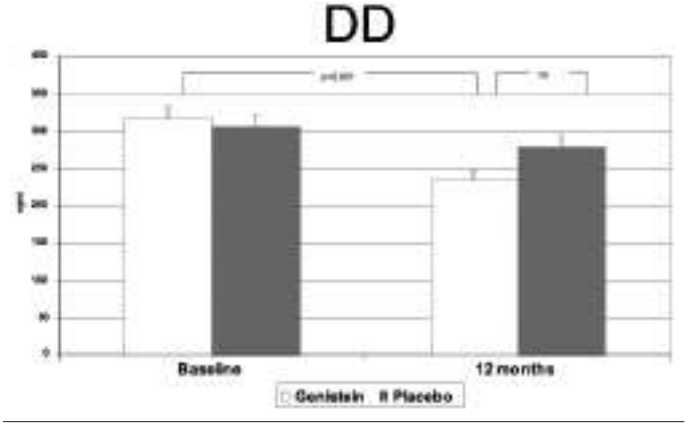

Figure 1.

Plasma levels of D-dimer (DD), plasminogen activator inhibitor-1 (PAI-1) and prothrombin fragment 1+2 (F1+2) were measured at baseline and after 12 months of treatment. Baseline characteristics of the two groups were similar. Compared with placebo, genistein decreased significantly DD (p<0.001), but did not affect PAI-1 and F 1+2 plasma levels. In conclusion the results of our study do not support effects of genistein on activation of the haemostatic system, but on the contrary the significant decrease of DD could indicate a possible favourable impact of genistein in hypercoagulable states.

P063

EVALUATION OF POLYMORPHISMS OF GENES INVOLVED IN FOLATE METABOLISM IN PATIENTS AFFECTED BY DOWN SYNDROME

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Down syndrome (DS) is characterized by an extra copy of genes located on chromosome 21. The cystathionine β-synthase (CBS) and the reduced folate carrier genes (RFC1) map on chromosome 21 and are involved in homocysteine/folate metabolism. We evaluated total fasting homocysteine (tHcy) plasma levels and the prevalence of polymorphisms in methylenetetrahydrofolate reductase (MTHFR C677T, A1298C), methionine synthase (MTR A2756G), methionine synthase reductase (MTRR A66G), CBS (844Ins68) and RFC1 (A80G) genes in a large series of subjects with DS (n=131, 54% males, median age 5.0 years, range 0.25-24 years) and in healthy control subjects (n=90, 53% males, median age 7.0 years, range 1-23 years). Increasing age had a strong positive influence on tHcy levels. After correction for age and gender, tHcy levels were significantly lower in DS subjects than in controls (median values 5.8 μmol/l versus 7.2 μmol/l, p=0.002). Allele frequencies for MTHFR677T, MTHFR1298C, MTR2756G and MTRR66G were 0.44, 0.36, 0.13, 0.45 and 0.45, 0.33, 0.14, 0.44 in DS and control subjects, respectively (p> 0.4). The CBS 844Ins68 mutation (heterozygous) was detected in 23 DS subjects (18%) and in 17 controls (19%, p = 0.8). Wild type and mutated homozygotes for the RFC1 A80G variant were 54 (41%) and 20 (15%) among DS subjects and 38 (42%) and 28 (31%) among healthy controls (p=0.08). However, the frequency of subjects heterozygous for the RFC1 A80G polymorphism was significantly higher among DS (44%) than controls (27%, p=0.02). No polymorphism showed a relation with tHcy levels either in DS or in controls (p>0.21). Plasma folate levels were similar in DS and controls (p=0.95). In both DS and controls, increasing age (p<0.0001) and MTHFR 677TT genotype (p=0.04) had a strong negative influence on plasma folate levels. Two DS subjects and one control had tHcy levels greater than 20 μmol/l: two of them had the MTHFR 677TT genotype and all had plasma folate levels lower than 1.4 ng/mL.

P064

INCREASED HOMOCYSTEINE LEVELS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: A POSSIBLE LINK TO THE THROMBOEMBOLIC DISEASE

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Introduction. Epidemiological findings demonstrate that Chronic Obstructive Pulmonary Disease (COPD) is an independent risk factor for arterial and venous thrombosis, but mechanisms explaining this association are poorly understood. Because COPD patients often have nutritional abnormalities, we hypothized that a poor B vitamins status and, as a consequence, higher total homocysteine (tHcy) values may be observed in COPD. These alterations could, in turn, contribute to the increased vascular risk. *Methods.* In a case-control, cross-sectional study, 42 patients with well documented COPD (stages II-IV of GOLD classification) were compared with 29 non-COPD control subjects matched for sex, age, cardiovascular risk factors and previous vascular events. Levels of folic acid, vitamin B12, vitamin B6, high-sensitivity C-reactive protein (hs-CRP), plasma lipids and creatinine, and arterial blood gases, were measured; tHcy was assayed by high-performance-liquid chromatography. Results. Compared to controls, patients with COPD had increased tHcy (20±16 versus 12±3 μmol/L, p=0.002) and hs-CRP levels, and decreased folate (2.6±1.7 vs 3.3±1.4 nmol/L, p=0.03), vitamin B6 (41.2±34 vs 56.6±33.7 nmol/L, p=0.03) and vitamin B12 (334±143 vs 458±426, p=0.2). Cigarette pack-years (OR 1.11, 95% CI 1.02-1.21, p=0.01) and tHcy (OR 1.43, 95% CI 1.02-1.99, p=0.03) were the only significant correlates of COPD status compared to the normal group with logistic regression analysis. Multiple linear regression analysis showed that low levels of folate (p=0.02) and vitamin B12 (p=0.03) and increased values of triglycerides (p=0.000) were significantly associated with increased tHcy levels within the COPD group. *Conclusion.* Patients with COPD have decreased levels of B vitamins and, hence, significantly higher tHcy plasma concentrations. Hyperhomocysteinemia may also qualifiy COPD patients with increased metabolic risk (higher levels of triglycerides). These complex abnormalities likely concur to the strong thrombotic risk in COPD.

P065

HYPERHOMOCYSTEINEMIA AND THROMBOSIS: THE DIAGNOSTIC ROLE OF POST METHIONINE-LOAD TEST

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Hyperhomocysteinemia (HHcy) is an indipendent risk factor for thromboembolic diseases. This condition is modulated not only by dietary habits (particularly folate, B6 and B12 deficiencies) but also by tossicological, hormonal and genetic factors. Moreover, the major cause of mild HHcy is usually a defect of methylene tetrahydrofolate reductase (MTHFR). The aim of this study was to evaluate the association between HHcy and thrombotic disease and/or thrombotic familiarity, in absence of other coagulation parameters altered. Samples for basal Hcy measurement and for genotyping (MTHFR C677T and A1298C variants) were taken from 57 patients and from 42 healthy blood donors. Moreover, we evaluated Hcy concentration after oral methionine loading, to reveal latent HHcy (abnormal Deltha-Hcy defined at more than 20 μmol/L). Venous blood samples for Hcy measurement were taken from each subjects before and 4 hours after oral methionine loading (0,10 g/Kg body weight). Samples were drawn into tubes containing K3-EDTA, centrifuged within one hour after collection or immediately placed on ice until assay, performed within 6 hours. The Abbott IMX Hcy assay was used for Hcy measurement. The MTHFR variants were determined using polymerase chain reaction (PCR) and hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes (CVC Strip Assay by Nuclear Laser Medicine). Patients with previous thromboembolism disease and MTHFR homozygous 677TT mutation presented the median tHcy concentrations (24.48 ±18.21 μmol/L DS) significantly (p lower than 0,05) more elevated to those of controls (7.06 μmol/L). Hcy levels after methionine loading are strongly and positively associated with MTHFR homozygous 677TT mutation. Both patients and controls with MTHFR heterozygous 677CT genotype don't show methionine intolerance. These preliminary data confirm that basal and post methionine-load Hcy levels have an important role in thrombophilic screening. Moreover, the post methionine-load test, particularly in the screening of subjects with border-line plasma Hcy, seems to be useful to explore the functional defect independently from the conditions that cause it.

P066

VASCULAR AND CONNECTIVE TISSUE MANIFESTATIONS IN FIVE ITALIAN PATIENTS WITH HOMOCYSTINURIA

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Objective. Homocystinuria is a metabolic disorder due mainly to cystathione β-synthase (CBS) deficiency producing an increased urinary excretion of homocysteine and methionine. Differential diagnosis between homocystinuria and Marfan syndrome is based on the dosage of plasma and urinary homocysteine. Among the major clinical features of homocystinuria there are vascular and connective tissue manifestations such as deep venous thrombosis, ectopia lentis and skeletal alterations. In the present study we investigated the clinical manifestations of 5 Italian homocystinuric patients, performed mutation screening analysis on cysthationine-β-syntase (CBS) gene and searched for genotype/phenotype correlation. *Methods.* The CBS gene analysis was performed by denaturing high performance liquid chromatography (dHPLC) and direct sequencing of the heteroduplexes. *Results.* We detected one

novel (missense mutation A157P) and 7 known mutations of CBS gene. The missense A157P is present in 3/300 controls suggesting the possibility that is either a frequent mutation or a rare polymorphism. In our patients homocysteine appears to be the only known factor responsible for venous thrombosis events. *Conclusion*. Our clinical data suggest that the connective tissue manifestations in homocystinuric patients include also cardiovascular and skin features, increasing the overlapping with Marfan phenotype. The detection of cardiovascular manifestations suggests a long term follow up of these patients with aging.

P067

EFFICACY OF OMEGA-3 FATTY ACIDS IN LOWERING LIPOPROTEIN (A) LEVELS: A PRELIMINARY STUDY

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Lipoprotein (a) [Lp(a)] is a unique lipoprotein particle in which the protein component consists of apolipoprotein B bound by a disulfide bond to apolipoprotein (a). Apolipoprotein (a) is closely homologous with plasminogen, so that a negative interaction with fibrinolysis is considered to be the mechanism linking increased Lp(a) levels to thrombosis. Actually, several studies have shown that high levels of Lp(a) play an important role in atherosclerotic diseases wehreas few and inconsistent data are present on the possible therapy able to lower Lp(a). In fact, no clear effect of reduction for common major drug classes has been demonstrated. Recently, a preliminary paper indicated a possible lowering effect for omega-3 fatty acids. Aim of our study was to evaluate the effect of omega-3 fatty acids (2 g/die for 3 months) on Lp(a) levels in 23 patients (14M/9F) (age: 55 (33-74) yrs) who reported to have high Lp(a) levels (>300 mg/L). These patients had either a history of venous thromboembolism (n=6) or atherothrombosis (n=17). Venous blood sampling were obtained at baseline (T0), and after 1 (T1), 2 (T2) and 3 (T3) months of therapy. Lp(a) median values resulted to be decreased after 3 months of therapy with omega-3 fatty acids [T0: 928 (461-2120) mg/L vs. T3: 745 (405-1554) mg/L] with a similar pattern shown at the intermediate measurements [T1: 859 (503-1512) mg/L; T2: 769 (385-1483);]. Delta value of decrease of Lp(a) between T0 and T4 was 141.3±75.6 mg/L with a mean reduction of 26.3%. Globally, Lp(a) levels decreased in 13/23 (56.5%) patients at T4. In particular, at T1, Lp(a) levels decreased in 14/23 patients (60.9%): in 10/14 this decrease persisted until the end of therapy (T4); in 4 patients Lp(a) levels returned similar to the baseline. In 3 patients omega-3 fatty acids decreased Lp(a) levels only after two months (T2). These preliminary results demonstrated a possible beneficial effect of omega-3 fatty acids in decreasing the levels of Lp(a). Larger and controlled trials are needed to confirm this preliminary data.

P068

IS ADHERENCE TO MEDITERRANEAN DIET ENOUGH FOR LOWERING LIPID PARAMETERS AMONG A HEALTHY ITALIAN POPULATION?

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Introduction. During the last 5 years an increasing body of evidence have accumulated on the association between adherence to mediterranean diet (MD), calculated through specific diet-scores, and health status. On the frame of an epidemiologic study conducted in Florence, Italy between 2002 and 2004 we evaluated the association between 2 different scores (a score of adherence to MD and a score of adherence to a healthful life (HL) which includes abstinence from smoking and a moderate physical activity) and circulating parameters linked to chronic diseases. *Methods.* Nutritional and biochemical profiles were studied in 932 individuals (365 M; 567 F) with a median age of 47.5 years. *Result.* Subjects who reported a greater adherence to MD were found to be more frequently males, married and over 45 years. A general linear model, by dividing the study population into quartiles of scores, was performed. After adjustment for age, gender, body mass index, total energy and alcohol intake as well as for smoking habit and physical activity we observed no influence of adherence to MD on circulating levels of biomarkers. On the contrary, as HL score is concerned, an inverse association between circulating levels of lipid parameters, mainly total cholesterol, LDL-cholesterol and triglycerides and higher score of adherence to healthy life, after adjustment for all the possible confounders, was reported. In addition, a significant difference between the highest and the lowest quartile of HL score for homocysteine plasma levels was observed (p=0.04). *Conclusions.* A healthful life which include not only a high adherence to MD but also to lifestyle factors is able to lower lipid parameters and homocysteine in a clinically healthy Italian population.

P069

HOMOCYSTEINE AND INFLAMMATION IN HEART FAILURE

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Recently, experimental data have demonstrated the presence of high levels of proinflammatory cytokines in heart failure (HF), suggesting an inflammatory hypothesis in the pathophysiology of HF. In addition, data are available on the possible role of homocysteine (Hcy) levels in the ventricular remodeling of patients with heart failure. Aims of our study were:1) to investigate the role of Hcy and inflammatory markers in patients with HF; 2) to evaluate their relationship with markers of ventricular remodeling such as metalloproteinases (MMP). We studied 79 patients with a diagnosis of HF (63 M/ 16 F; 74 (43-95 yrs) and 79 healthy subjects as controls, comparable for age and sex (63 M/ 16 F; 74 (40-83) yrs). We determined fasting Hcy levels by FPIA method; interleukin-6 (IL6) by an ELISA method; C reactive protein (CRP) levels by a nephelometric high sensitivity assay; and MMP3 and 9 (total activity) by immunoenzymatic assay. Hcy levels were significantly higher in patients with respect to controls [14,2 (6.5-41.5) μmol/L versus 8.7 (5.1-24) μmol/l; p<0.001]. Hcy levels were significantly higher in class NYHA IV [16.7 (9.9-33.4) μmol/L] with respect to class III [13.9 (6.5-41.5) μmol/L; p<0.05] and class II [12.5 (7.9-32.6) μmol/L; p<0.01]. Hcy levels were significantly different in relation to the etiology of HF (hypertensive: 17.9 (7.4-41.5) μmol/L; dilatative: 15.3 (6.5-27.8) μmol/L; ischemic: 12.7 (6.7-33.4) μmol/L. In addition, Hcy levels were significantly higher in patients with severe systolic dysfunction of left ventricular measured by the ejection fraction (EF): EF >40%= 12.5 (6.7-30) μmol/L; EF 30-40%= 13.6 (6.5-41.5) μmol/L; EF <30%=15 (8.2-33.4) μmol/L; p for trend <0.05. Finally, Hcy was significantly higher in patients with diastolic dysfunction (diagnosed on an altered ecocardiographic E/A ratio): 15.5 (6.5-41.5) μmol/L versus 12.6 (6.7-27.8) μmol/L; p<0.01. CRP and IL6 levels were significantly higher in patients than in controls [CRP:10 (0.7-21) mg/L versus 1.9 (0.8-5.4) mg/L, p<0.01; IL6: 8.9 (0.9-51.5) pg/mL versus 4.6 (0.5-12) pg/mL, p<0.01]. We demonstrated a significant correlation between Hcy and IL6 (r=0.31; p<0.01), Hcy and CRP (r=0.54; p<0.001). We documented a significant correlation between Hcy and MMP-3 and -9 only in the subgroup (n=34) of patients with hyperhomocysteinemia (i.e. Hcy levels above 15 μmol/L) (r=0.46; p=0.009). These results demonstrate a high prevalence of hyperhomocysteinemia in HF, which is associated with the impairment of systolic and diastolic ventricular function and with the etiology of HF. The evidence of a proinflammatory state in HF which is associated with Hcy levels and the first *in vivo* demonstration of a correlation between hyperhomocysteinemia and MMP, which are involved in the ventricular remodeling, prompt clinical trials addressed to evaluate the use of vitamin supplementation and anti-inflammatory therapy in HF patients

P070

A NEW METHOD FOR DETERMINATION OF PLASMA HOMOCYSTINE BY ISOTOPE DILUTION AND ELECTROSPRAY TANDEM MASS SPECTROMETRY

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Perturbation of plasma's redox status and of tissue's aminothiol is an important indicator of chronic oxidative stress. A prooxidant state may accompany hyperhomocysteinemia in cardiovascular disease, in antioxidant deficiencies, and renal failure. High homocystine's levels could warrant an antioxidant intervention. Therefore, the levels of homocystine have physiological significance and may be helpful in the diagnosis of various pathophysiological conditions. For these reason, we

describe a new analytical determination method of homocystine in human plasma. The method utilises liquid chromatography coupled to ionspray tandem mass spectrometry. Quantitative analysis was achieved using as an internal standard homocystine-d8. These compounds were analysed using LC-MS-MS. Autosampler injections of 1 µl into a 5 µl sample loop were made using an isocratic elution phase. Mass spectrometer operated in the selected reaction monitoring (SRM) mode. All data were acquired in positive ion mode. Homocystine and homocystine-d8 were detected through the transition from the precursor to the product ion (from m/z 269.3 to m/z 90.0, and m/z 277.3 to m/z 94.0, respectively). The method is extremely sensitive, with limit of detection in the range of 6fmol/L. The interassay and intraassay coefficients of variation for homocystine were 6,22% and 3,4%, respectively. The accuracy of the assay was evaluated through recovery experiments. The accuracy for the added homocystine ranged from 85 to 110%. High specificity of tandem mass spectrometry coupled with a fast chromatographic process is suitable for a rapid and reliable assay of homocystine, without multistep sample derivatization.

P071

EFFECT OF THREE DIFFERENT WINE POLYPHENOLS ON HUMAN PLATELET FUNCTION

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Resveratrol, quercetin and gallic acid are three compounds representative of three different polyphenol families, found in several plant foods, especially in red wine. The aim of this study was to compare the possible effect of these compounds on human platelet function *in vitro*. Platelet aggregation was induced in PRP by arachidonic acid (AA 0.8 -1.2 mM), the stable TxA2 mimetic U46619 (0.6-1.5 µM), and collagen (1-2 µg/mL). TxB2 released by AA-stimulated platelets was measured by EIA. Data are means±SEM (n=3-6). Resveratrol or quercetin dose-dependently inhibited AA-induced aggregation with IC50s of 44±18 and 130±3 µM and U46619-induced platelet aggregation with IC50s of 94±13 µM and 266±34 µM, respectively. Resveratrol also prevented collagen-induced platelet aggregation (IC50: 56±6 µM). At the highest concentrations tested of resveratrol and quercetin, TxB2 was reduced to 2.6 and 6.5% of control values (1.1±0.1 µg/mL). Gallic acid, up to 1 mM, did not interfere with platelet function, but restored AA-induced platelet aggregation (more than 70%) and TxB2 production to (57±20% and 100% of control), when incubated (37°C, 10 min) with PRP before resveratrol or quercetin. In conclusion, resveratrol and quercetin inhibit both AA- and U46619-induced platelet aggregation, resveratrol being more effective and also able to inhibit collagen-induced aggregation. Gallic acid may prevent the inhibitory effect of resveratrol and quercetin on AA-induced platelet function. These results confirm the antiplatelet activity of some dietary polyphenols, but also show, for the first time, possible interactions between different polyphenols at the level of platelet function. Our data, though preliminary, suggest that the overall anti-platelet and possibly antithrombotic effects of common dietary interventions - such as low to moderate wine consumption - might be more complex than foreseen up to the present time.

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P072

VENOUS THROMBOEMBOLISM DOES NOT CAUSE HYPERHOMOCYSTEINEMIA

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Although the association of hyperhomocysteinemia with venous thromboembolism (VTE) is well established, it is still unclear whether or not it is causal. Moreover, some investigators hypothesized that, if a cause-effect relationship exists between hyperhomocysteinemia and VTE, this could be inverse, in that VTE may cause hyperhomocyeineimia, rather than being a consequence of it (e.g., Frederiksen *et al.*, Blood 2004). This hypothesis is based on the fact that the association appears unequivocal only from the results of retrospective case-control studies, while prospective cohort studies gave conflicting results. In addition, the association with VTE risk of the mutation in the gene encoding for methylene tetrahydrofolate reductase (MTHFR), C677T, which causes hyperhomocysteinemia, is uncertain. VTE can develop in concomitance with transient, triggering risk factors, such as surgery, trauma, pregnancy/puerperium (secondary VTE), or in their absence (idiopathic VTE). We reasoned that these pathogenic characteristics of VTE offer the ideal opportunity to test whether the hypothesis that hyperhomocysteinemia causes VTE is realistic or should be definitely abandoned. In fact, if the hypothesis were true, VTE should cause hyperhomocysteinemia independently of whether it is idiopathic or secondary. Therefore, we compared the plasma total homocysteine (tHcy) levels before and after an oral methionine load (3.8 g/m^2 body surface area) in patients with previous episodes of idiopathic VTE (n=190) to those of patients with previous secondary VTE (n=391) and healthy controls (n=1328). The table shows that both fasting and PML plasma tHcy levels were significantly higher than those of healthy controls in patients with idiopathic VTE, while they were comparable to normal in patients with secondary VTE. Therefore, the results of our study conclusively rule out the hypothesis that VTE causes hyperhomocysteinemia. Whether hyperhomocysteinemia causes VTE or is a marker of unknown factor(s) that is causally related to VTE is unknown and cannot be established by our study.

Genetic factors and thrombosis

P073

PREVALENCE OF GENETIC PROTHROMBOTIC RISK FACTORS: ANALYSIS OF 2170 CONSECUTIVE CASES

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Introduction. The Factor V Leiden (FVL) and the G20210 prothrombin gene mutations (FII G20210A) are well-established risk factors for venous thromboembolism. There are geographic differences in the prevalence of these mutations: in Italy are 2.0 and 3.0 percent, for FVL and F II G20210A, respectively. The aim of our study was to evaluate the prevalence of these genetic prothrombotic mutations in 2170 patients with history of venous or arterial thrombosis, or women with unexplained recurrent spontaneous abortions or other obstetric complications, and in healthy subjects with familial history of thrombotic disorders or women candidates to oral contraception. *Methods*. We screened for genetic thrombophilia 2170 consecutive subjects (Males: 733 Females: 1437) between January 2002 and December 2005. *Results and Conclusion*. FVL and FII G20210 mutations were found in 407/2170 (18.7%) patients (M:138 F:269) with a median age of 42 years (2-82): 199 with vascular disorders (107 with a history of venous thrombosis or pulmonary embolism, 7 with myocardial infarction, 29 with cerebral vascular disease, 21 with venous cerebral thrombosis, 21 with miscarriages or other unexplained obstetric complications, 14 with history of peripheral arterial occlusion) and 208 healthy women or asymptomatic relatives of patients with vascular disorders. In the group of 2170 patients: 226 (10.4%) were carriers of FVL (217 heterozygotes and 9 homozygotes) and 211 (9.7%) were carriers of FII G20210A (201 heterozygotes and 10 homozygotes). 16 had both mutations. 199 (48.9%) patients with at least one genetic prothrombotic defect showed thrombotic disorders. Our study confirms the importance of genetic thrombophilia screening. We also found an increased prevalence of these defects in comparison with the prevalence reported in the literature: if the selection of patients could partially explains this observation, it's our opinion that further and larger prevalence study could verify important geographic differences between Italian regions.

P074

PRE-ECLAMPSIA IN A WOMAN WITH MULTIPLE GENETIC THROMBOPHILIC MUTATIONS

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We report the case of a 25 year old woman, primipara, previously healthy, admitted at 29th week of gestation with headache, abdominal pain, hypertension (>160/100 mmHg on four occasions before admission) proteinuria (> 3g/L in a 24-hour sample). One week prior to admission she also presented peripheral edema, headache and oliguria. Ultrasonographic examination showed no placental abruption or fetal growth restriction. The coagulation and other laboratory parameters were normal. She was initiated on nifedipine and furosemide for control of blood pressure and intravenous albumin. An emergency cesarean section was performed four days after delivery because a decreased umbilical artery flow. The patient gave birth to a healthy baby and became normotensive after birth. The examinations for thrombophilia showed: heterozygosis for the prothrombin G20210A mutation, homozygosis for the plasminogen activator inhibitor (PAI-1) 4G/4G and heterozygosis for the -455G/A β-fibrinogen gene polymorphism. Two years later she had her second pregnancy. The woman had been in good general health. She received prophylactic administration of Nadroparin 5700 IU s.c. daily, from 10 weeks gestation until the post-partum. Blood pressure, coagulation parameters, liver and renal function were normal during this pregnancy. A caesarian section was performed at 38th week and the child was healthy. Pre-eclampsia is defined as blood pressure greater than 140/90 mmHg on at least two occasions in women normotensive and proteinuria: the etiologic mechanisms are still unknown. Some studies supported the association with hereditary thrombophilia because the development of chronic intravascular coagulation. Several genetic mutations could be associated with pre-eclampsia and other pregnancy complications. In our experience, the patient developed pre-eclampsia during her first pregnancy, but her second pregnancy was uncomplicated after prophylactic administration of Nadroparin. It could suggest that not only Factor V Leiden and Prothrombin G20210A but also other genetic mutations should be investigated as risk factors for pre-eclampsia.

P075

RECURRENT PREGNANCY LOSS AND THROMBOPHILIA: OUR EXPERIENCE

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Factor V Leiden (FVL G1691A), prothrombin (FII G20210A) and methylenetetrahydrofolate reductase (MTHFR C667T) mutations are the most common genetic thrombophilias known to predispose to risk factors for venous thromboembolism. Recent studies have suggest that these defects are associated also with an increase risk of recurrent pregnancy loss. The aim of our study was to evaluate the presence of these risk factors in 84 young women referred to our laboratory, with an age between 22 and 45 years, with at least 2 unexplained miscarriage in absence of antiphospholipid antibodies syndrome and no previous thromboembolism events. The DNA mutations were detected by polymerase chain reaction, followed by digestion with specific restriction enzymes (PCR-RFLP). Physiologic coagulation inhibitors (antithrombin III, protein C and protein S) were in the normal range. Fourteen heterozygous carriers of the Factor V Leiden and nine heterozygous carriers of G20210A prothrombin mutation were detected, which represent the frequencies of 16,6% and 10,7% respectively. The MTHFR C667T mutation was observed in 59 patients (45 heterozygous and 14 homozygous carriers) which represents the frequencies of 53,6% and 16,6% respectively. Although our samples examined are limited these data suggest that the screening for the FVL, FII G20210A and MTHFR C677T mutations is useful in women who have experienced recurrent pregnancy loss. Further studies are necessary to evaluate the diagnostic and therapeutic approach for the women with recurrent abortions.

P076

INCREASED PREVALENCE OF THE FACTOR V LEIDEN MUTATION IN SOUTHERN ITALIAN BLOOD DONORS

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The Factor V Leiden (FVL) and the G20210 prothrombin mutations (FIIG20210A) are genetic risk factors for thromboembolism. Geographic differences in the prevalence of these mutations have been identified: FVL is more prevalent in the north (7%) than in south of Europe (3%), whereas FIIG20210A is more common in southern (3-7%) than in northern Europe (2-5%). In Italy the prevalence reported is 2.0 and 3.0 percent, for FVL and FIIG20210A, respectively. The increased prevalence of genetic thrombophilia observed in these years in our patients with thromboembolism (FVL: 10.4%; FIIG20210A: 9.7%), suggested us to assess if these disorders could be more frequent in healthy calabrian population than in the rest of Italy. We investigated 183 unselected blood donors (males: 117, females: 66; median age: 43 years). All donors were caucasian, recruited in the province of Reggio Calabria, in health and without history of vascular disease: we found a FVL prevalence of 6,1% (11/183). The prevalence of FIIG20210A was 3.2% (6/183). We observed an increase of FVL, but not of FIIG20210, in comparison with data reported about prevalence of prothrombotic defects in Italy. Our result seems as high as observed in several reports about FVL prevalence in Greece and Middle East. The European distribution of FVL is according to the hypothesis of a single origin of the mutation appeared in Middle East during the Neolithic Age and spread towards the North Europe with the farmers migrations; some authors are analysing another model of FVL diffusion in the Mediterranean area consequently to the Phoenicians colonizations. We think that it could be a possible reason of high prevalence reported in our study about southern Italian healthy individuals. If confirmed, the evidence of differences between Italian regions should lead to further studies for the geographic risk identification and for evaluation of the correct prophylaxis treatment.

P077

FACTOR V LEIDEN AND PROTHROMBIN MUTATION IN WOMEN WITH A HISTORY OF HELLP SYNDROME

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The HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome is one of the leading causes of maternal morbidity and mortality in the western world. The earliest clinical signs and symptoms of this disorder are protean, and thus it may be mistaken for other conditions, from the benign to the life-threatening. The diagnosis may often be delayed if the symptoms are attributed to a viral syndrome, gastritis, or a musculoskeletral problem. The disorder can be difficult to distinguish from other severe hepatic or hematologic syndromes seen in pregnancy. Severe pre-eclampsia, haemolytic uremic syndrome, thrombotic thrombocytopenic purpura, and acute fatty liver of pregnancy can all produce clinical manifestations and laboratory studies similar to the HELLP Syndrome. The association of thrombophilia and obstetrical complications is documented and well consistent with the hypothesis of an insufficient placental perfusion due to fibrin deposition as a major underlying pathophysiological mechanism. The association of factor V and factor II mutations with preeclampsia and HELLP Syndrome, and a possible role of the two thrombophilic mutations in the pathogenesis of the diseases have been previously investigated. The results, however, are still inconclusive and contradictory. We have tested 11 women with HELLP Syndrome for thrombophilic coagulation parameters: antithrombin III, protein C, protein S, lupus-like anticoagulant, anticardiolipin antibodies, activated protein C resistance, homocysteine, and detection of the G1691A mutation (Factor V Leiden) and of the G20210A mutation in the Factor II gene. In all women the coagulation parameters studied were normal. On the contrary, mutations investigation showed five women with Factor V Leiden, three women with prothrombin mutation, one woman with Factor V and Factor II mutations and two women without mutations. One limitation of our investigation primarily revolves around the retrospective and non controlled nature of the study, with a possible bias of patients' selection. Further studies are necessary in order to clarify the role of congenital thrombophilia in HELLP Syndrome.

P078

LACK OF ASSOCIATION BETWEEN PC LEVELS AND POLIMORPHISM OF NQO1 (NAD(P)H:DEHYDROGENASE QUINONE 1)

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Background. An A/G polymorphism of the NAD(P)H:dehydrogenase quinone 1 (NQO1) has been recently described as the most important genetic determinant of Protein C (PC) levels in the general population (Buil, 2004). *Aim of the study.* To confirm the correlation between this polymorphism and PC in a large epidemiological investigation. *Methods.* We evaluated two groups of subjects in the Vicenza Thrombophilia and Atherosclerosis (VITA) Study: 1) 114 subjects representing the subjects with the lowest, age-adjusted , 1st percentile of PC amidolytic activity; 2) 120 subjects with normal PC level (control group). In 5 subjects belonging to the first group, a mutation in the PROC gene has been previously identified using a DHPLC screening and subsequent DNA sequencing. All the 234 subjects were genotyped for the rs1437135 SNP (HCV2091258 ID Celera database) by an allele specific PCR and subsequent agarose-gel electrophoresis. *Results.* Of the 234 subjects, 7 were homozygous for the G allele (GG), 130 homozygous for the A allele (AA) and 97 heterozygous (GA). The overall mean PC levels were: 75.5 U/mL in GG subjects, 78.4 U/mL G/A, and 79.9 in AA subjects (p=0.89 by variance analysis). The allele frequencies were not differently distributed among the subjects belonging to the first percentile compared to those with normal PC levels (p=0.70, Fisher's exact test). The allele frequencies in subjects with normal PC level were 0.22 and 0.78 for the G and A allele, respectively. *Conclusions.* In the present study, we found no association between PC levels and the NQO1 polymorphism previosly described in the GAIT Project as the most influencing genetic determinant of plasma PC level. This discrepancy could be only partially attributed to the different allele frequencies found in the GAIT. *Project.* Given the low prevalence of mutation in PROC in our population and the described high heritability of PC levels in the general population, factors related to its variation remain still elusive.

P079

PREVALENCE OF AT DEFICENCY IN THE GENERAL POPULATION: PRELIMINARY RESULTS FROM THE VITA PROJECT

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Background. Antithrombin (AT) deficiency is associated with a high risk of venous thromboembolism, but few data are available regarding its prevalence in the general population, with an estimated prevalence of 1:600 in blood donors (Tait, 1994). Aim of the study. To provide additional data on the prevalence of AT deficiency in the general population and its association with thromboembolic risk. Methods. We screened 59 subjects, identified as having the lowest AT amidolytic activity (range 39 to 67 U/mL) among the 15055 subject enrolled in the Vicenza Thrombophilia and Atherosclerosis (VITA) *Project.* DHPLC analysis of the AT gene was carried out after PCR amplification of all 7 exons. In a pilot study, DHPLC analysis revealed a 90% sensitivity for mutation of the AT gene. *Results.* Among the 59 subjects evaluated, we identified 4 subjects (two related), all showing the same point mutation in exon 6 (Ala384Ser). This mutation has been already identified as causing type II deficiency with a reactive site defect (Perry, 1991). No history of venous thromboembolism was however reported in the probands or in their first degree-relatives. *Conclusion.* Among subjects enrolled in the VITA Project, the prevalence of molecular AT deficiency appears to be low (approx 1:3800), with a possible strong founder effect and not clearly related to venous thromboembolism. It is therefore likely that symptomatic AT deficiency is a very rare disorder, with a prevalence actually lower that the number of subjects enrolled in the VITA Project (hence, below 1:15000 subjects).

P080

ROLE OF C677T AND A1298C MTHFR, A2756G MTR AND -786 C/T ENOS GENE POLYMORPHISMS IN ATRIAL FIBRILLATION SUSCEPTIBILITY

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Hyperhomocysteinemia has been suggested to play a role in the Non-Valvular Atrial Fibrillation (NVAF) pathogenesis. Polymorphisms in genes coding for homocysteine (Hcy) metabolism enzymes may be associated with hyperhomocysteinemia and NVAF. 456 NVAF patients and 912 matched controls were genotyped by an electronic microchip technology for C677T and A1298C MTHFR, A2756G MTR, and -786C/T eNOS gene polymorphisms. Hcy was determined by an immunoassay method (FPIA assay). The genotype distribution of the 4 polymorphisms as well as genotype combinations did not differ in patients and controls. Hcy was higher in patients than in controls (15.2, 95%CI 14.7-15.7 vs 11.3, 95%CI 11.0-11.6 µmol/L; p<0.0001). In both populations a genotype-phenotype association (p<0.0001) between Hcy and C677T MTHFR polymorphism was observed; in controls a significant (p=0.029) association between tHcy and -786C/T eNOS polymorphism was also observed. Hcy was higher in 677TT/1298AA combination with respect to all the other combinations in both patients and controls (p<0.001 and p<0.0001). MTR and eNOS polymorphisms did not interact with the C677T MTHFR polymorphism in influencing tHcy levels. At the multivariate analysis the NVAF risk significantly increased in the upper quartiles of Hcy compared to the lowest: OR from 2.8 (1.68-4.54 95%CI) in Q2 to 12.9 (7.96-21.06 95%CI) in Q4. Our data demonstrated the 4 polymorphisms, although able, at least in part, to affect Hcy were not associated with an increased risk of NVAF per se or in combination.

P081

HEREDITARY THROMBOPHILIA IN CHILDREN WITH MIGRAINE

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Introduction. Growing evidence suggests a possible relationship between migraine and stroke in young patients. Few studies have investigated the prevalence of prothrombotic genetic risk factors (factor V Leiden, MTHFR C677T and prothrombin G20210A) in patients with migraine, and conflicting results have been reported. During migraine

attacks both increased platelet aggregability and plasma coagulability have been described; polymorphisms C807 of the glycoprotein (GP) Ia gene is associated with high platelet GPIa-IIa density and consequently with higher platelet adhesion to type I collagen. *Material and methods.* Aim of the present study was to assess the prevalence of several genetic prothrombotic risk factors in a cohort of pediatric patients (number 95), aged 5 to 18 years, suffering from headache (36 migraine with aura, 35 migraine without aura and 24 tension type headache). A previous study on platelet aggregability function performed in patients followed in one center confirmed increased aggregability in patients with migraine (J Headache Pain, 2002). DNA was extracted from peripheral blood mononuclear cells in all patients by standard methods; polymorphisms were analyzed by PCR amplification, digestion by restriction enzymes and agarose gel electrophoresis. C807T, factor V Leiden, MTHFR C677T and prothrombin G20210A polymorphisms were analyzed. The allelic frequencies were compared within the three groups of patients with headache and with those expected in Caucasian population (Dinauer, Br J Hem, 1999; Bauduer, Mol Genet Metab, 2005). Statistical analysis was performed with the chi square or Fisher's exact test. *Results.* frequency obtained are reported in table number I and II. No difference were detected in allelic frequencies among the different populations ($p<0.05$). *Discussion.* our data seem not to confirm a role of the prothrombotic genetic factors analyzed (C807T, factor V Leiden, MTHFR C677T and prothrombin G20210A) in the pathogenesis of headache and migraine in children.

Table 1.

	GP Ia C807T			*Factor V Leiden G1691A*			*MTHFR C677T*			*Prothrombin G20210A*		
	C/C	*C/T*	*T/T*	*G/G*	*G/A*	*A/A*	*C/C*	*C/T*	*T/T*	*G/G*	*G/A*	*A/A*
MIGRAINE WITH AURA (N=36 C807T, N= 17 others)	16 (44%)	17 (48%)	3 (8%)	17 (100%)	0	0	5 (29%)	8 (47%)	4 (24%)	17 (100%)	0	0
MIGRAINE WITHOUT AURA (N=35)	14 (40%)	18 (51%)	3 (8%)	33 (94%)	2 (6%)	0 (37%)	13 (46%)	16 (1%)	6 (97%)	34 (3%)	1	0
TENSION TYPE HEADACHE(N=24)	8 (33%)	13 (54%)	3 (12%)	21 (88%)	3 (12%)	0	8 (33%)	12 (50%)	4 (17%)	23 (96%)	1 (4%)	0
ALL	38 (40%)	48 (50%)	9 (10%)	71 (93%)	5 (7%)	0	26 (35%)	36 (47%)	14 (18%)	74 (97%)	2 (3%)	0

Table 2.

	MIGRAINE WITH AURA	*MIGRAINE WITHOUT AURA*	*TENSION TYPE HEDACHE*	*ALL*	*CAUCASIAN POPULATION*
807 T	32%	34%	39%	34,3%	39%
1691A	0	3%	6,5%	3,2%	1,4%
677T	47%	40%	42%	42%	41-43%
20210A	0	1,4%	2%	2,6%	1,5%

P082

PROTEIN Z GENE POLYMORPHISMS (INTRON F 79 G>A; -13 A>G) ARE NOT ASSOCIATED WITH ACUTE CORONARY SYNDROMES

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Introduction. Protein Z is a vitamin K-dependent glycoprotein found to play a relevant role in coagulation. Actually, a series of variants naturally occurring within the protein Z gene locus have been reported to significantly influence protein Z plasma levels in healthy controls and in ischemic stroke patients. However, no data are available on the association between protein Z gene polymorphisms and protein Z plasma levels in patients with acute coronary syndromes (ACS). Aim of this study was to evaluate the possible relationship between protein Z intron F 79 G>A and -13 A>G gene polymorphisms, protein Z plasma concentrations and ACS. *Methods.* Protein Z gene polymorphisms (79 G>A; -13 A>G) and protein Z plasma levels were investigated in 244 unrelated patients (188 M; 56 F) with ACS and 352 (271 M; 81 F) age- and sex-comparable healthy subjects. Protein Z levels were measured by using a commercial enzyme-linked immunoadsorbent assay (Asserachrom Protein Z; Diagnostica Stago, Asnieres, France) and protein Z gene polymorphisms were analysed using polymerase chain reaction and digestion with restriction enzymes. *Results.* Protein Z levels were found to be significantly lower according to the different genotypes for both polymorphisms (p for trend <0.001). However, neither genotype distribution (p=0.3, and p=0.6 for -13 A>G and 79G>A polymorphisms, respectively) nor allele frequency for both polymorphisms was significantly different between patients and controls. Logistic regression analysis found no association between rare alleles and ACS (-13AG+GG: OR 0.8, 95%CI 0.5-1.2, p=0.3; 79GA+AA: OR 1.2, 95%CI 0.8-1.6, p=0.3). *Conclusions.* The present study shows a significant influence of protein Z gene polymorphisms on protein Z plasma levels in ACS patients and healthy controls, whereas no association for rare alleles of polymorphisms encoding protein Z gene (Intron F 79 G>A and -13 A>G) and the occurrence of ACS was found.

P083

RISK OF OCCURRENCE OF DEEP VENOUS THROMBOSIS AND PROTEIN Z DEFICIENCY

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Protein Z (PZ) serves as a cofactor for FX inhibition by protein Z-dependent protease inhibitor. *In vivo* and *in vitro* studies aimed at investigating the role of PZ levels in venous thrombosis produced conflicting results. We have investigated whether reduced PZ levels and PZ gene common variants are associated with deep venous thrombosis. In 197 patients with deep vein thrombosis and in 197 age- and sex-matched controls, PZ plasma levels and gene polymorphisms were evaluated by means of an enzyme-linked immunosorbant assay and direct cycle sequence analysis. Similar PZ levels were found in controls (1.44+0.63 μg/ml) and in patients (1.44±0.96 μg/mL). The prevalence of PZ levels below the 5.0 (0.52 μg/mL) or the 2.5 percentile of controls (0.47 μg/mL) was higher in patients (10.2% and 8.7%, respectively) than in controls (4.1%; OR: 2.7 [95% C.I.: 1.2-7.3] and 2.0%; OR: 4.6 [95% C.I.: 1.5-13.9], respectively). This relationship was independent of the effect of age, sex, and FV Leiden and FII A20210 allele (OR: 2.8, 95% C.I.: 1.1-7.3 and 4.9, 95% C.I.: 1.4-17.3). A significant association with PZ levels was shown for the intron C g42a and the intron F g79a polymorphisms in cases (R2:0.129) and in controls (R2:0.140). However, allele and genotype frequencies of the PZ gene polymorphisms investigated were similar in the two groups. Present data suggest an association between very low PZ plasma levels and the occurrence of deep venous thrombosis, PZ gene polymorphisms little contributing to this relationship.

P084

THE G20210A PROTHROMBIN VARIANT AND THE RISK OF THROMBOEMBOLISM AND FETAL LOSS IN PREGNANT WOMEN: DATA OF A RETROSPECTIVE FAMILY COHORT STUDY

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Background and Objectives. Women with thrombophilia may present with vascular complications during pregnancy or in the postpartum period. In this retrospective, single center,cohort study our objective was to assess the risk of thromboembolic or obstetric complications in women belonging to a large number of families identified because of a symptomatic proband with single identified G20210A protrombin mutation. *Design and Methods.* Female family members who had been pregnant at least once were enrolled in the study. One hundred and thirty pregnancies occurred in 57 heterozygous carriers and 108 pregnancies in 47 non-carriers of the prothrombin mutation. *Results.* The number of thromboembolic and obstetric complications was calculated and compared in

carriers and in non carriers women. No thromboembolic events occurred, during pregnancy and the postpartum period, in the two groups. In the 57 women carriers of the G20210A mutation 24 of the 130 pregnancies (18.4% per pregnancy) resulted in an unexplained fetal loss, as compared to 18 of the 108 pregnancies in the non-carriers (16.6% per pregnancy). This was not statistically different in the two groups (RR 1.06, 95% CI 0.6 to 1.86). Also when we considered separately the trimester-loss the difference did not reach statistical significance. When we evaluated women with more than one fetal loss, the G20210 prothrombin carriers tended to have an higher risk as compared to normal relatives but this difference did not reach statistical significance (RR 1.38, 95% CI 0.34 to 5.6). *Conclusions*. Women, heterozygous carriers of the G20210A mutation seems to be not at increased risk for vascular complications during pregnancy as compared to their relatives without the mutation. Studies addressing the cost-benefit ratio of a thromboprophylaxis during pregnancy and postpartum period are strongly needed.

P085

THROMBOPROPHILAXIS DURING PREGNANCY IN FACTOR V LEIDEN CARRIERS: AN ONGOING PROSPECTIVE FAMILY EVALUATION

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Background. Factor V Leiden is the most common thrombophilic polymorphism associated with thrombotic as well as with pregnancy complications. At present in clinical practice there are distinct opinions on the use of antithrombotic prophylaxis in these women during pregnancy because of the absence of randomized clinical trials that, however, are very difficult to be performed. We present here the data of an ongoing prospective observation on pregnant women who are carriers of the mutation and were followed in our Centre because family members of symptomatic probands previously identified. *Methods and results.* Out of 203 fertile women carriers of factor V Leiden mutation (age 18 to 45 years), 25 had at least one pregnancy during seven years of follow-up and an overall number of 28 pregnancies. Seven of these women had previous thrombotic or obstetric complications: three women (one homozygous carrier) had a previous deep vein thrombosis (two during pregnancy), two had recurrent superficial vein thrombosis (one during pregnancy), one women had a first-trimester loss and one had two first-trimester losses. No adverse events were present at the base-line in the remainder 21 women (two homozygous carriers). Thromboprophylaxis with low-molecular-weight heparin (enoxaparin or nadroparin 40 mg daily) started few hours after the delivery and for 6 weeks at the postpartum period to women without previous thrombotic or obstetric complications. Women with previous events started prophylaxis (enoxaparin or nadroparin 40 mg/daily) early after pregnancy was diagnosed. All women wore elastic stockings, applied 24 mmHg of pressure at the ankle, starting after pregnancy was diagnosed and for 6 weeks in the postpartum period. One first-trimester loss occurred in the follow-up in a previous heterozygous asymptomatic woman. A successful outcome occurred in the other 27 gestations, also in women with previous events. *Conclusions.* This limited observation is clearly not conclusive but suggests that thromboprophylaxis may be indicated during pregnancy only in symptomatic family carriers of factor V Leiden mutation. In asymptomatic carriers, prophylaxis can be considered only in the postpartum period.

Atherothrombosis and cardioembolism

P086

VENOUS AND ARTERIAL THROMBOSIS IN PATIENTS WITH HIV INFECTION: A CASE-CONTROL STUDY

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Background. An increased rate of thromboembolic events has been reported in HIV-positive patients. However, available information is mostly based on the results of case reports or retrospective, non controlled cohort studies. *Methods.* The charts of 169 HIV-infected patients followed at the local ambulatory for the management of HIV infections and of 180 randomly selected blood donors, both active and former donors, were reviewed. Patients were asked to fill a specific questionnaire and were subsequently interviewed. Information on family and personal history of cardiovascular disorders and on the presence of personal risk factors for venous and arterial thrombosis was collected. All reported events were adjudicated if adequate documentation of objective tests was available. *Results*. Mean age and sex were similar in the two groups. A vascular event was documented in 6 HIV-infected patients (3.55%) and in none of controls (p=0.0108). These were acute myocardial infarction (2 patients), deep vein thrombosis (2 patients), ischemic stroke (1 patients), and acute arterial thrombosis of a lower limb (1 patients). Some risk factors were more prevalent in HIV-infected patients than in controls: family history of cardiovascular disorders (36.1% and 25.0%, p=0.02), cigarette smoking (60.3% and 23.3%, p<0.0001), and hypertriglyceridemia (43.8% and 15.0%, p<0.0001). Other risk factors were more prevalent in controls: obesity (9.4% and 3.6%, p=0.03) and use of oral contraceptives (33.3% and 2%, p=0.0005). In multivariate analysis, neither cardiovascular risk factors, nor HIV infection were independently associated with the presence of thromboembolic events. *Discussion.* Our study confirmed the hypothesis that HIV positive patients have an increased risk of thromboembolic complications, especially arterial thrombosis. Smoking and hypertriglyceridemia are important modifiable risk factors that clinicians should aggressively address in the management of HIV-positive patients. However, other risk factors may play an important role and should be further investigated.

P087

METFORMIN IMPROVES OXIDATIVE STRESS AND DECREASES PLATELET ACTIVATION IN NEWLY DIAGNOSED TYPE 2 DIABETIC SUBJECTS

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Objectives. In type 2 diabetes metformin treatment reduces cardiovascular (CV) risk beyond the effect of glycemic control. Since oxidative stress and the consequent enhanced platelet activation greatly contribute to accelerated atherosclerosis in diabetes, we hypothesized that metformin could concur in reducing oxidative stress in type 2 diabetic patients. *Methods*. We randomized 26 newly diagnosed type 2 diabetic subjects to assume either metformin (M, n=13) or gliclazide (G, n=13) for 12 weeks. Drugs were titrated as needed on the bases of blood glucose profiles and HbA1c levels so to achieve good glycemic control. Before and after treatment, blood and urinary samples were collected to determine blood glucose, insulin, HbA1c, Vit A and Vit E levels and 8-iso-PGF2-alfa and 11-dehydro-tromboxane B2 (TXM) urinary excretion, an *in vivo* oxidative stress and a thromboxane dependent platelet activation marker, respectively. *Results.* Notwithstanding a comparable improvement in metabolic control (HbA1c < 7 after both M and G), 8-iso-PGF2-α (M =942±92 vs 811±75 pg/mg cr, p<.01; G=746±80 vs 765±82, p=n.s) and TXM (M=2190±196 vs 1865±103 pg/mg cr, p<.01; G=1586±223 vs 1440±270, p=n.s) urinary excretion decreased after metformin but not after gliclazide treatment. After meftormin, antioxidant vitamins A and E levels significantly increased while they remained unchanged or decreased after gliclazide. *Conclusions.* These data suggest that metformin could improve oxidative stress, preserve antioxidant function and restrain platelet activation in type 2 diabetes. This could contribute to the observed CV risk reduction following metformin treatment.

P088

CHOLESTEROL ENHANCES PLATELET RECRUITMENT VIA GP91PHOX- MEDIATED CD40L UPREGULATION

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An interplay between cholesterol and platelet function has been suggested to favour arterial thrombosis but the underlying mechanism has not been fully elucidated. Platelet recruitment, that mimics platelet accumulation at the site of vascular injury, was enhanced in hypercholesterolemic patients compared to controls and significantly correlated with platelet glycoprotein IIb/IIIa glycoprotein expression. Pre-treatment of platelets with a competitive peptide for CD40L (sc-1593 P)significantly inhibited platelet recruitment and down-regulated platelet glycoprotein IIb/IIIa. *In vitro* study performed by pre-treating platelets with LDL demonstrated an increase of platelet recruitment, that was paralleled by CD40L and glycoprotein IIb/IIIa over-expression; platelet recruitment and IIb/IIIa expression were inhibited by pretreating platelets with the CD40L competitive peptide sc-1593 P. LDL-treated platelets showed an increase of oxidant species production, that was dependent upon NADPH oxidase activation. Incubation of platelets with a NADPH oxidase inhibitor resulted in a significant decrease of platelet recruitment, and platelet CD40L and IIb/IIIa expression. In patients with hereditary deficiency of gp91phox, the central core of NADPH oxidase, platelets showed low production of oxidant species, down-regulation of platelet CD40L and glycoprotein IIb/IIIa and inhibition of platelet recruitment compared to platelets from controls. These data suggest that cholesterol enhances platelet function by upregulating platelet expression of CD40L and IIb/IIIa glycoprotein via NADPH oxidase-dependent oxidative stress generation.

P089

LIPID PEROXIDATION AS A DETERMINANT OF PLATELET ACTIVATION IN HYPERTENSIVES WITH MICROALBUMINURIA

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Objective. We evaluated the extent of *in vivo* platelet activation in hypertensives with or without microalbuminuria, and its association with oxidative stress and endothelial activation. *Methods.* Urinary 11-dehydro-thromboxane (TX)B2 and 8-iso-prostaglandin (PG)F2α (*in vivo* markers of platelet activation and oxidative stress) were measured in 30 patients with essential hypertension and microalbuminuria (MH), 30 patients with essential hypertension (EH), and 30 controls (HS). Endothelial function was assessed by dosing plasma nitric oxide (NO), ICAM-1 and asymmetric dimethylarginine (ADMA). *Results.* Urinary 11-dehydro-TXB2 was higher in MH (median 805 pg/mg creatinine) compared to either HS or EH (291 and 414 pg/mg; $p<0.0001$). Urinary 8-iso-PGF2α was also enhanced in MH (median 279 pg/mg creatinine) compared to either HS or EH (146 and 157 pg/mg; $p<0.0001$).

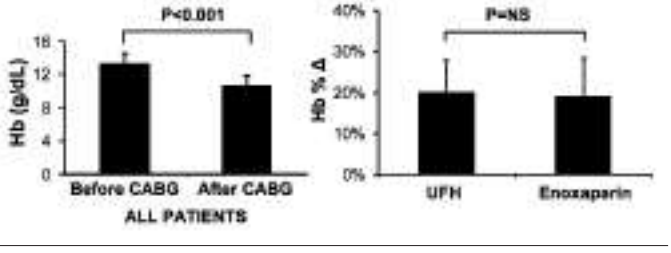

Figure 1.

A significant correlation was found between 11-dehydro-TXB2 and 8-iso-PGF2α in hypertensives (Rho=0.73, $p<0.0001$). An impairment in endothelial function was found in MH, with decreased NO ($p<0.0001$) and increased ICAM-1 ($p<0.0001$) and ADMA ($p<0.0001$) levels compared to either HS or EH. On multiple linear regression analysis both urinary 8-iso-PGF2α (β=0.49; $p<0.0001$) and microalbuminuria (β=0.36; $p<0.001$) were independently related to 11-dehydro-TXB2 in hypertensives. To elucidate the role of oxidative stress as a determinant of platelet activation, we evaluated the effects of vitamin E (900 mg/d for 1 month) in 10 MH patients. Treatment led to normalization in median 11-dehydro-TXB2 ($p<0.01$) and 8-iso-PGF2α ($p<0.01$). The change in 8-iso-PGF2α directly correlated with the change in 11-dehydro-TXB2 (Rho=0.69, $p=0.03$). *Conclusions.* We conclude that lipid peroxidation is a major determinant of persistent platelet activation in MH.

P090

INSULIN RESISTANCE AS A DETERMINANT OF PLATELET ACTIVATION IN OBESE WOMEN

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We previously reported that android obesity is associated with persistent platelet activation in otherwise healthy women. Although insulin resistance is frequent in android obesity, it is also often present in gynoid obesity. We tested the hypothesis that insulin resistance per se contributes to increased platelet activation in this setting, independently of underlying inflammation. A cross-sectional study including 40 obese (BMI≥30 kg/m^2, aged 24-63 years) and 20 non-obese (BMI<25 kg/m^2) healthy women, was performed. As index of insulin sensitivity, SI, i.e. the effect of insulin on glucose uptake was calculated and plasma adiponectin, C-reactive protein (CRP) and CD40 ligand (CD40L) levels were measured as potential determinants of increased platelet activation. Obese women had markedly higher 11-dehydro-thromboxane (TX)B2 excretion rate (median 718 vs. 211 pg/mg/creatinine), CRP (1.13 vs. 0.48 mg/L) and CD40L levels (4.45 vs. 0.90 ng/mL), ($p<0.0001$) than controls. Obese women had lower SI (median 2.51 vs. 5.0 10-4 min-1/(mU/mL), $p<0.002$) and adiponectin (6.3 vs. 10 mg/mL, $p<0.01$) than controls. We also found a significant inverse correlation between 11-dehydro-TXB2 and SI (Rho=-0.72, $p<0.0001$) or adiponectin (Rho=-0.56, $p<0.0002$), and a direct correlation with waist-to-hip ratio (WHR) (Rho=0.32, $p<0.043$), CD40L (Rho=0.66, $p>0.0001$) and CRP (Rho=0.67, $p<0.0001$). On multiple regression analysis WHR>0.86 (r=0.27, $p<0.04$) and SI (r=-0.72, $p<0.032$) predicted the rate of 11-dehydro-TXB2 excretion, independently of adiponectin, inflammatory and lipid patterns. In order to investigate the cause-effect relationship of these associations, we examined the effects of a 12-week weight loss program on urinary 11-dehydro-TXB2 in 10 obese women with impaired SI. Successful weight loss (0.6 kg loss/week) achieved in 5 subjects was associated with an increase in SI (+92%) and a decrease in CD40L (-27%), CRP (-37%) and 11-dehydro-TXB2 (-53%), ($p<0.05$). Our findings demonstrate that insulin resistance is a major determinant of platelet activation in female obesity.

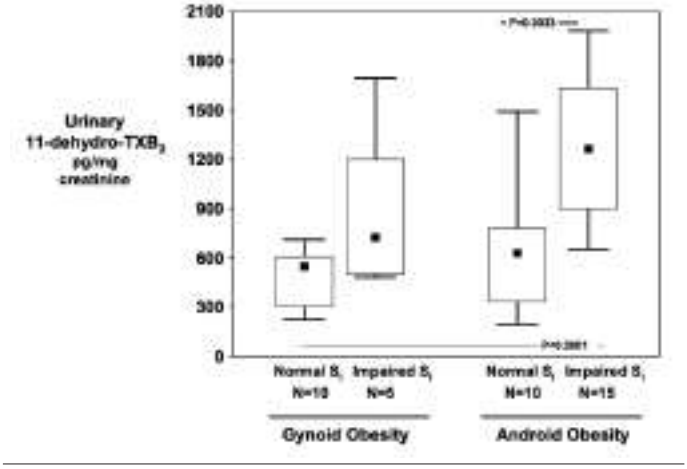

Figure 1.

P091

C-REACTIVE PROTEIN AND ITS DETERMINANTS IN EUROPEAN POPULATIONS AT DIFFERENT RISK OF MYOCARDIAL INFARCTION: RESULTS FROM THE IMMIDIET PROJECT

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Introduction. C-Reactive Protein (CRP), mainly produced by the hepatocytes in response to infection, tissue injury and under chronic inflammatory conditions, was positively associated with the risk of cardiovascular disease. We studied the differences in CRP and its determinants in three European populations at different risk of myocardial infarction: Italy, Belgium and England. *Methods.* CRP levels were measured by immunoturbidimetric method (IL, Milan, Italy). Subjects were all healthy people randomly selected from the general population through the list of GPs. Subjects with CRP > 10 mg/L were excluded. *Results.* Women had higher CRP levels than men in each country (Table) and Italian males and females had higher CRP levels than Belgian and English persons (p <0.0001).

Table. CRP blood levels in mg/L (SD).

	Males	*Females*	*P for gender*
ITALY (348)	1.83 (1.46)	2.25 (2.08)	0.02
BELGIUM (444)	1.53 (1.60)	1.89 (1.79)	0.002
ENGLAND (454)	1.53 (1.42)	1.73 (1.67)	0.04

After multivariate analysis, in females, the most important determinants of CRP were higher BMI (p<0.0001), triglycerides (p 0.002) and Factor VII Ag (p<0.0001), hypertension (p 0,004) and younger age (p 0.04) while in men CRP determinants were smoking habits (p<0.0001), country (p 0.001) and higher BMI (p 0.004) and age (p 0.002). This model explained 7.35% and 23.31% of total CRP variability in males and females respectively. Interaction analysis by gender was statistically significant: indeed the relation between CRP and age, smoking, hypertension and TG was significantly different in males and females (p<0.0003, 0.0006, 0.04, 0.0007, respectively). *Discussion.* CRP levels are highly influenced by obesity, lipid profile and FVII levels, all features of the metabolic syndrome. However, when the metabolic syndrome was included in the model, it explained only in females 4% of total CRP variability. Genetic and dietary factors should be considered.

P092

PREVALENCE OF HYPERTENSION, USE AND EFFECTIVENESS OF ANTIHYPERTENSIVE TREATMENT IN THREE EUROPEAN COUNTRIES. THE IMMIDIET STUDY

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Introduction. Optimal blood pressure (BP) control is of paramount importance in ensuring prevention of cardiovascular events. In hypertensive non-diabetic subjects the most favourable BP treatment goals are systolic BP <140 mmHg and diastolic BP <85 mmHg. To determinate the prevalence of hypertension and the use and the effectiveness of antihypertensive treatment in three European Countries in the frame of the IMMIDIET study. *Methods.* the IMMIDIET Project was a cross-sectional study conducted in Italy, Belgium and England, to investigate differences in the distributions of genetic and dietary cardiovascular risk factors. The subjects (n=2011), were randomly recruited from General Practices and were all apparently healthy, non-diabetic and aged 25-60 years. Blood pressure was measured using an automatic device (OMRON-HEM-705CP). Hypertension was defined when a subject had SBP ≥140 mmHg and/or DBP ≥90 mmHg and/or he was on anti-hypertensive treatment. *Results.* 24% of the European population was hypertensive; among them only 35% was treated. Males were more hypertensive than females (31% vs 17%, p<0.0001), but females were more treated than males (45% vs 29%, p=0.0003). The prevalence of hypertension was higher in Italy (30%) in comparison with Belgium (22%, p<0.0001), and UK (23%, p<0.0001). The frequency of the antihypertensive treatment was 36%, 37%, 29%, in Italian, Belgian and English hypertensive subjects, respectively. Out of those treated for hypertension, only 26%, 33% and 43% in Italy, Belgium, United Kingdom, respectively, reached the Gold Standard. After exclusion of subjects who started the antihypertensive therapy in the year of recruitment (26%), the percentage of subjects under control was similar. *Conclusion.* Two thirds of the hypertensive European population is still out of control. Antihypertensive drug therapy appears under-used and poorly successful. Targeted interventions are necessary to improve use and effectiveness of antihypertensive drug therapy.

P093

SUBOPTIMAL ANTITHROMBOTIC THERAPY IN PATIENTS WITH NON VALVULAR ATRIAL FIBRILLATION

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Atrial fibrillation (AF) is a strong, independent risk factor for stroke because it is associated with formation of left atrial thrombi. Although warfarin is significantly more effective than aspirin in treating AF in patients at high risk of stroke, a recent study showed some aspects of the under-use (50-60%) of warfarin in these subjects. To verify in the Italian population of AF patients the appropriate use of the antithrombotic therapy, we stratified the risk of stroke among AF patients and followed-up them after therapy adjustment. The study was carried out on 255 (males 120, females 135, age 61-83 years) consecutive patients affected by non-valvular atrial fibrillation. Of the 255 patients studied, 230 (90%) were stratified to high risk, 16 (6%) to moderate risk and 9 (4%) to low risk. Among all patients, 60% were treated with anticoagulants, while 30% were treated with aspirin and 10% were untreated. It is to underlyne that in the high risk cohort only 63% were treated with anticoagulants. 203 patients were followed-up for 27 (range 6-36 months). At the start of the follow-up, all but 15 patients stratified to high risk (n=163) were treated with anticoagulants. The remaining 15 patients, who had poor compliance for anticoagulants, togheter with 16 patients with moderate risk and 9 patients with low risk were treated with aspirin. During the follow-up, among 163 patients anticoagulated, 14 (8.5%) experienced a cerebrovascular accident (12 stroke, 2 TIA), while among 40 patients treated with aspirin 4 (10%) had a cerebrovascular accident (1 stroke, 3 TIA). So the treatment with anticoagulants reduced the risk of ischemic stroke by about 75%, while the treatment with aspirin didn't seem modify the usual rate of ischemic events. The study shows a suboptimal use of antithrombotic therapy in AF patients and strongly recomend to extend the correct anticoagulation to all high risk AF patients.

P094

ARTERIAL ISCHAEMIC STROKE IN CHILDREN: PRELIMINARY DATA FROM A RETROSPECTIVE STUDY ON 62 CASES

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Between January 1995 and August 2005, sixty-two cases of paediatric

arterial (ischemic) ischemic stroke were admitted to the Giannina Gaslini Children's Hospital in Genova and to the OIRM-S.ANNA in Turin. There were 12 newborns (5 males, 7 females, median age 3 days, range 1-20 days) and fifty children (22 males, 28 females, median age 3.7 years, range 2 months- 14.8 years). As far as underlying disease and risk factors are concerned, congenital heart disease only accounted for 18% of children. There was a relevant incidence of moya moya disease (12%) and varicella infection (10%). Sixteen children (32%) had 6 different prothrombotic conditions (2 heterozygous FVG506Q, 2 FV H1299R, 5 Lupus Anticoagulant, 2 post methionine load hyperhomocysteinemia, 3 reduced PC and 2 reduced PS). Among the newborns, two resulted heterozygous for FV G506Q and 2 were heterozygous for PRT G20210A. Fourteen children and eight newborns did not receive any antithrombotic treatment; 25 children received only aspirin, 2 were treated with enoxaparine alone, five received both, 3 were treated with dipyridamole and 1 with unfractioned heparin. Aspirin was given to one newborn, heparin to two and both drugs were given to one. At discharge, 10 (28%) of the treated children showed no neurological impairment, while 24 (67%) had focal alterations. Three out of 11 (27%) patients in the untreated group showed residual focal damage at discharge. Three of the 4 treated newborns (75%) had neurological deficit. The same incidence of neurological damage was found in untreated newborns (6/8, 75%).

Our study confirms that AIS in children is a rare disease with relevant incidence of severe neurological involvement. Prothrombotic alterations appear to play a minor role in the aetiology. On the basis of our retrospective study, the administration and type of antithrombotic treatment would seem to have no effect on neurological outcome.

P095

STATINS ENHANCE CIRCULATING VITAMIN E

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Statins are suggested to possess pleiotropic actions including anti-inflammatory, antithrombotic and antioxidant property, that could also contribute to the statin's antiatherosclerotic effect. As far as the antioxidant effect is concerned, statins are able to reduce markers of oxidative stress while it is unclear if they can also influence the antioxidant status. *Methods.* Vitamin E plasma levels, a reliable marker of antioxidant status, were analyzed in 192 patients with suspected metabolic disorders. Metabolic syndrome (MS) was diagnosed in 112 out of 192 according the criteria of the International Diabetes Federation definition. Among patients with MS 30 were treated with statins for at least 6 months and 82 were statin-free. 80 statin-free patients, who did not meet the above criteria for the diagnosis of MS, were considered as controls. *Results.* Compared to controls, MS patients had lower levels of vitamin E (4.78±1.31 vs 5.22±0.89 µmol/mmol cholesterol, p=0.01). Dividing MS patients according to statin treatment showed statin-treated patients had values similar to controls (5.24±1.17 vs 5.22±0.89 µmol/mmol cholesterol, p>0.05) and higher than statin-free patients (5.24±1.17 vs 4.61±1.33, p=0.02). *Conclusions.* These data show that MS patients have a low antioxidant status, that is mitigated by concomitant use of statins. This field suggest that statins could enhance vitamin E plasma levels, ameliorating the balance between oxidative stress and antioxidant status in a population at high risk for cardiovascular disease.

P096

HYPERVISCOSITY AS A POSSIBLE RISK FACTOR FOR CEREBRAL ISCHEMIC COMPLICATIONS IN NONVALVULAR ATRIAL FIBRILLATION PATIENTS

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Recent studies have suggested that hyperviscosity is frequent in nonvalvular atrial fibrillation (NVAF) patients. Aims of this study were to evaluate if hemorheological alterations play a role in the occurrence of cerebral ischemic events in NVAF patients and to explore a possible association between inflammation and hyperviscosity in these patients. We studied 62 NVAF patients with history of at least one cerebral ischemic event and 94 NVAF patients without. A control population included 130 age and sex matched healthy volunteers. Hemorheological variables [whole blood viscosity (WBV), plasma viscosity (PLV), erythrocyte deformability index (DI) and hematocrit], fibrinogen and high-sensitivity C-reactive protein (hs-CRP) levels were assayed. An alteration in WBV at 94.5 s-1 and DI was found more frequently in patients with a previous ischemic event at univariate and multivariate analysis (OR: 3.19, p=0.023 and OR: 4.26, p=0.002, respectively) adjusted for age, sex, hypertension, diabetes, history of coronary artery disease, left ventricular dysfunction, smoking habitus, dyslipidemia, hematocrit, fibrinogen, hs-CRP and hemorheological parameters. These results stimulate prospective studies on the role of hemorheological alterations in the occurrence of cerebral ischemic complications in NVAF patients.

P097

PROTEIN Z LEVELS AND PROGNOSIS IN PATIENTS WITH ACUTE CORONARY SYNDROMES

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Introduction. Protein Z, a vitamin K-dependent glycoprotein, serves as a cofactor for the inhibition of the activated coagulation factor X. During the last years, a role for low levels of protein Z in prothrombotic disorders such as ischemic stroke and acute coronary syndromes (ACS) has been reported. Aims of this study were to test the changes of protein Z and the association with outcome at 1 year of follow-up in patients with ACS who underwent percutaneous coronary intervention with stent implantation. *Methods.* A total of 193 patients (150M; 43F) with ACS were included. Protein Z plasma levels were evaluated at admission, and after 1 year of follow-up. All patients were followed for major adverse cardiac events (MACE). *Results.* Protein Z plasma levels were found to decrease significantly (p< 0.0001) at 1 year (1529.1±670.9 ng/mL) with respect to the baseline (1723.1±816.9 ng/mL). MACE occurred in 41 (21.2%) patients during follow-up. Logistic regression analysis showed a significant association between protein Z below the 5th percentile of our control group and subsequent MACE (OR: 3.3; 95%CI 1.04-10.7; p=0.04). Moreover, Cox regression analysis showed that low protein Z levels at the admission were significant predictors of MACE at 1 year (HR: 2.5; 95%CI 1.02-6.5, p=0.04). *Conclusions.* In conclusion, our results show that in patients with ACS 1) protein Z decreases moving from the acute to the convalescent phase, and 2) low levels of protein Z are significantly associated with adverse outcome at 1 year of follow-up.

P098

STROKE IN RENAL TRANSPLANT RECIPIENTS

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Cardiovascular diseases are the most important causes of increased morbidity and mortality in patients receiving renal transplantation (RT). An increased rate of stroke has been widely reported in patients on chronic dialysis, but in only one study there is information about RT patients. Aim of this study was to evaluate the rate of stroke in our RT patients. A series of 538 consecutive RT recipients were followed up, 20 patients were lost at follow-up. Our RT patients (M 334, F 184) had a median age at transplantation of 47 (18-70) years. Four patients (0.8%) developed an ischemic cerebral event (1 TIA and 3 stroke, 2 fatal) (median age 62 years, range 57-77 yrs); the events occurred a median time of 60 (3-96) months after RT. Events occurred in patients aged >57 year. Among the population observed, 102 patients were older than 57 years and the incidence of stroke/TIA in this group was 3.9%. Two patients were affected by arterial hypertension and 2 by diabetes mellitus. No other known risk factor for stroke was present. Renal function was normal in 3 cases (GFR >60 mL/min/1.73 m^2) and reduced in one case (GFR=35 mL/min/1.73 m^2). *Conclusion.* Our data indicate that RT patients have a low incidence of cerebral ischemic events and they occur in elderly patients. The lower incidence of stroke/TIA reported in our study in comparison to the previous study (2.8%), is probably due to low-dose aspirin treatment used in our RT patients as routine thromboprophylaxis

P099

G20210A PROTHROMBIN MUTATION IS MORE FREQUENT IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE (PAD), ESPECIALLY IN THOSE WITH CRITICAL LIMB ISCHEMIA

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Few data are available on thrombophilic alterations and risk of PAD. We evaluated the presence of inherited thrombophilic alterations [G20210A prothrombin (PM) and R506Q FV Leiden (FVLM) mutations, Antithrombin, Protein C and S deficiencies] in 212 pts [132 males; median age: 72 y (27-94 y)] with PAD [Fontaine's stage II: n=135, 86 males, median age: 70 y (27-94 y); Fontaine's stage III/IV: n=77, 46 males, median age: 74 y (44-94 y)] consecutively referred to our Dept. As control group we studied 210 apparently healthy subjects (median age: 65 y (50-89 y). The prevalence of PM was slightly higher in PAD pts than in controls (6.1% vs 3.8%, p=0.389) but it was significantly increased (13.0% vs 3.8%, p=0.01) in those pts with critical limb ischemia (Fontaine's stage III/IV). The prevalence of natural anticoagulant deficiencies (Antithrombin, Protein C and S) and of the FVLM was not different among PAD pts and controls, and no differences were observed in relation to the Fontaine's classification. The unadjusted relative risk (OR) of PAD associated with carriership of the PM was 1.65 (95%CI: 0.67-4.07). The OR was 1.42 (95%CI: 0.53-3.81) after adjustment by logistic regression for possible confounding factors [age, gender and traditional risk factor for PAD (smoking, diabetes, hypertension, dislipidemia, BMI]. The risk was increased about 4-fold in those pts with critical limb ischemia carrying the PM (OR: 3.76, 95%CI: 1.43-9.94). After adjustment for possible confounding factors the estimated OR increased to 4.56 (95% CI: 1.42-14.6). In conclusion, the PM seems to be a risk factor for severe stages of PAD. In contrast, the FVLM and deficiency of natural anticoagulants are not associated with PAD and its severity. If this can be of importance to select pts at higher worsening risk and to choose different treatments in these subjects should be addressed by specifically designed clinical studies.

Venous thromboembolism: epidemiology, risk factors, diagnosis

P100

MANAGEMENT OF PRIMARY CARE PATIENTS WITH SUSPECTED DEEP VEIN THROMBOSIS: USE OF A THERAPEUTIC DOSE OF LOW-MOLECULAR-WEIGHT HEPARIN TO AVOID URGENT ULTRASONOGRAPHIC EVALUATION

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Background. Out of hospital management of patients with suspected deep vein thrombosis (DVT) can be problematic. The accuracy of clinical prediction rules in the primary care setting may be inadequate, D-dimer testing may not be available, and the cost-effectiveness of urgent ultrasonographic evaluation is uncertain. *Objective.* The purpose of this study was to determine the efficacy and safety of an empiric single therapeutic dose of low-molecular weight heparin (LMWH) in the time interval preceding ultrasound investigation in patients presenting to primary care physicians (PCPs) for suspicion of DVT. *Methods.* Consecutive patients with suspected DVT who presented to the office of a PCP outside regular Thrombosis Center working hours were enrolled. All eligible patients received a single therapeutic dose of LMWH (100 anti-Xa IU/kg weight) and were scheduled to undergo clinical and instrumental evaluation at the Thrombosis Center the morning after. Clinical events were documented after a 3-month follow up. *Results.* 534 consecutive patients with suspected DVT were included in this study. A total of 102 patients had subsequent diagnosis of DVT. We detected no episodes of pulmonary embolism, major bleeding, or death during the 18 hour window between the administration of LMWH and objective evaluation. Of the 432 patients in whom diagnosis of DVT was subsequently excluded, only 3 (0.7%; CI: 0.2 to 2.0%) developed venous thromboembolic events during the 3-month follow-up period. *Conclusions.* Empiric treatment with a single therapeutic dose of LMWH is effective and safe for outpatients with suspected DVT initially managed in a primary care setting. This strategy has the potential to reduce the need for urgent diagnostic imaging.

P101

OBJECTIVE ASSESSMENT OF PULMONARY EMBOLISM CAN BE DEFERRED WITHOUT INCREASED RISK

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Background. Management of patients with suspected Pulmonary Embolism (PE) is problematic if diagnostic imaging is not available. Pretest Clinical Probability (PCP) and D-dimer (D-d) assessment were shown to be useful to identify those high risk patients for whom empirical, protective anticoagulation is indicated. Objective of the study. To evaluate whether PCP and D-d assessment, together with the use of low molecular weight heparins (LMWHs), allow objective appraisal of PE to be deferred for up to 72 hours, 336 consecutive patients with suspected PE were prospectively investigated. Methods. In case of deferment of diagnostic imaging for PE, patients identified at high-risk (those with high PCP or those with moderate PCP and a positive D-d), received a protective full-dose treatment of LMWH; the remaining patients were discharged without anticoagulant. All patients were scheduled to undergo objective tests for PE (ventilation/perfusion lung scanning or computed tomography lung scan) within 72 hours. Standard antithrombotic therapy was then administered when diagnostic tests confirmed Venous ThromboEmbolism (VTE)(Figure 1). *Results.* In total, 336 patients with suspected PE were included in this study. Of 336 patients, 211 (62.7%) were classified as low-risk group and 125 (37.2%) as high-risk group.

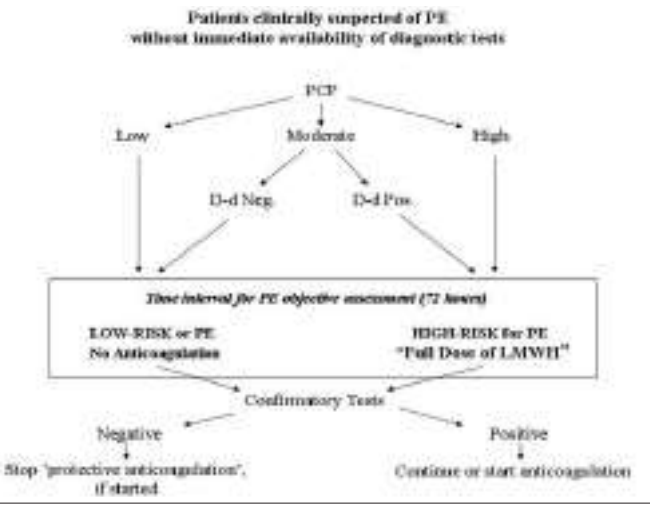

Figure 1.

The prevalence of VTE was 6.1% (95% CI 2.7-9.3) in the low-risk group and 50.4% (95% CI 41.7-59.1) in the high-risk group. In total, VTE was confirmed in 76 (22.6%) of 336 patients (95% CI 18.2-27). The median time of deferred tests was 49 hours for patients considered as being at low-risk and 42.5 hours for those categorised as high-risk: this difference was not statistically significant. Concerning the LMWH treatment, the median duration was 35.5 hours (Table 1).

Table 1. Patients' characteristics.

Baseline features	*Low-risk group (n=211)*	*High-risk group (n=125)*	*p value*
Age in years	59.3 (22-91)	60.3 (23-91)	n.s.
Sex (F/M)	98/113	59/66	n.s.
Time since onset of symptoms (days)	1.7	1.5	n.s.
Co-morbidity and cancer (%)	16 (7.5)	25 (19.2)	0.03
Median time of deferred test (hours)	49.5	42.5	n.s.
Median time of protective anticoagulation (hours)	NA	35.5	-

n.s.: not significant.

At the short-term follow-up (72 hours), a single thromboembolic event (0.8%, upper 95% CI 2.3%) occurred; at the 3-month follow-up, 3 events (1.1%, upper 95% CI 2.3%) occurred in the group of patients in whom diagnosis of PE had previously been ruled out (n= 260). None of the patients had major bleeding events during the follow-ups. Two patients (0.7%) died of malignancies diagnosed before and after the study entry, respectively. None of these patients showed symptoms of VTE or bleeding. One patient was lost to follow-up (Table 2).

Table 2. Outcome of Short-and Long-term FU.

Categories of patients (n)	*Prevalence of VTE at the time of disgnostic imaging*	*Events at the short-term FU n/N (%)*	*Events at the long-term FU* n/N (%)*
Low-risk group (211)	13/211 (6.1) [95% CI 2.7-9.3]	0/211 (0) [upper 95% CI, 0.3]	0/198 (0) [upper 95% CI, 0.3]
High-risk group (125)	63/125 (50.4) [95% CI 41.7-59.1]	1/25 (0.8) [upper 95% CI, 1.2]	3/62 (4.8) [upper 95% CI, 6.2]
All patients*	76/336 (22.6) [95% CI18.2-27]	0/211 (0) [upper 95% CI, 0.8]	0/198 (0) [upper 95% CI, 2.3]

*FU: Follow-up; CI: Confidence Intervals; *: all patients, excluded those who had a disgnosis of VTE at the time of imaging.*

Conclusions. Objective diagnostic assessment of PE is important and usually requires multiple approaches specially in case of oligosymptomatic patients. When this is not immediately possible, management can prove highly unsatisfactory and physicians tend to hospitalise and/or treat patients with empirical anticoagulation irrespective of the actual risk of VTE; this situation is quite common during night or week-end's referral. Our study demonstrates that our simple and reproducible approach allows a safe deferral of diagnostic imaging for PE for up to 72 hours.

P102

HOSPITAL BURDEN OF PULMONARY EMBOLISM

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Background and aim. Pulmonary embolism (PE) represents the third cardiovascular emergency after acute coronary syndrome and stroke and the main cause of acute death in hospitalised patients. The aim of the present study was to analyse retrospectively the impact of PE in terms of discharge diagnoses and in-hospital mortality in an Italian teaching hospital. *Materials and Methods.* Hospital discharge schedules (HDS) and Diagnosis Related Groups (DRGs) of patients admitted and discharged with diagnosis of PE from the Policlinico Le Scotte of Siena, Italy, during a period ten years long (1994-2003) were reviewed. *Results.* In the considered period 761 patients (382 males and 379 females with mean age±SD 72.66±13.31 years) were discharged with diagnosis of PE. In 562 patients, 277 males and 285 females with mean age ± SD 74.05±13.25 years, the diagnosis was performed in medical wards, whereas in 199 patients, 105 males and 94 females with mean age ± SD 68.67±12.69 years, the diagnosis was made in surgical wards. Figure 1 shows the age distribution of PE patients.

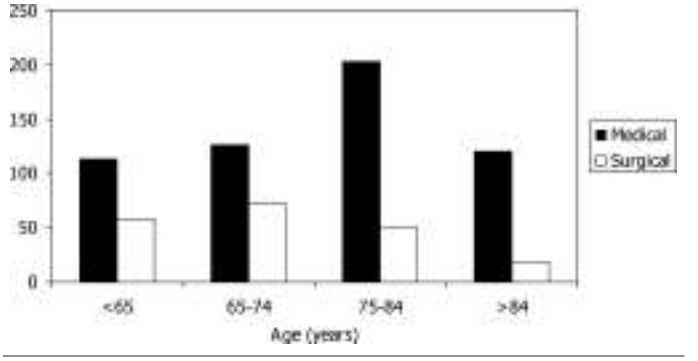

Figure 1.

Peak of surgical patients was in the age 65-74 years, while in medical patients it was in the age 75-84. Mortality in medical patients was 23.3% while in surgical patients was 21.8%. Table 1 shows main findings of our study.

Table 1.

Age	*hospital admissions N (%)*	*hospital deaths N (%)*	*Diagnoses of PE N (% PE on hospital admissionsi)*	*Mortality for PE*	*Mortality% for PE on total hospital deaths*
≤64 years	195.993 (60)	2.014 (1.02)	171 (0.08)	16 (9.35%)	0.79
≥65 ≤74 years	63.915 (19.5)	2.455 (3.84)	198 (0.30)	39 (19.6%)	1.58
≥75 ≤ 84 years	47.758 (14.6)	3.468 (7.26)	252 (0.52)	72 (28.5%)	2.76
≥85 years	18.890 (5.9)	2.790 (14.76)	140 (0.74)	50 (35.7%)	1.79
Total	326.556 (100)	10.727 (3.28)	761 (0.23)	177 (22.6%)	1.65

Discharge diagnoses of PE and mortality for PE increased with age (from 0.08% in patients under 65 years to 0.74% in patients 85 years old and older for diagnosis, from 9.35% in patients under 65 years to 35.7% in patients 85 years old and older for mortality). Mortality for

PE respect total mortality had a peak in the group of age 75-84 years (2.76%). Conclusion. PE represents a frequent but often under looked hospital emergency. Our study, nevertheless limitations due to retrospective analysis, absence of standardized diagnostic algorithm and subjectivity of physicians in compilation of HDS, could contribute to the knowledge of burden of PE in hospitalised patients.

P103

LUPUS ANTICOAGULANT: HOW MANY TESTS AND WHICH TESTS?

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The presence of lupus anticoagulant is one of the laboratory criteria for the diagnosis of antiphospholipid syndrome (APS). According to guidelines lupus anticoagulant (LA) is detected with the use of two or more tests with different assay principles. False negative results may hamper the diagnosis of APS and clinical laboratories must choose how many and which tests to perform in order to provide the best sensitivity. To evaluate the diagnostic performance of LA tests we analyzed 1702 consecutive patients referred to our laboratory for lupus anticoagulant (LA) from November 2004 until February 2006. the assays used were STACLOT LA (Stago Roche), DRVVT (IL), KCT (home made) and SCT (IL). According to the criteria of the SSCISTH, 240 samples (14.1%) from 201 patients had positive results for one or more tests. 76 samples (31.7%) had 4 positive tests; 49 samples (20.4%) had 3 positive tests, 68 samples (28.3%) had 2 positive tests and 47 (19.6%) samples had 1 positive test. In conclusion, if only two tests are done for LA, the maximal sensitivity is obtained with STACLOT LA and SCT (92.5%). If three tests are used the maximal sensitivity is obtained with STACLOT LA, SCT and DRVVT (99.6%). In our experience the use of three tests, although time expensive, increases the diagnostic sensitivity, and provides a more reliable result.

P104

FIBRINOLYTIC AND INHIBIN A VARIATIONS IN PREGNANCY-RELATED HYPERTENSIVE DISORDERS

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Mechanisms leading to pregnancy-related hypertensive disorders, in particular pregnancy-induced hypertension (PIH) and pre-eclampsia (PE), are still unclear. Diagnostic criteria are clinical, since specific markers of the disease are lacking. An involvement of the fibrinolytic system in pregnancy-related hypertensive disorders has been suggested. We aimed to evaluate the behaviour of t-PA, PAI-1, PAI-2, which is produced by the placenta, and the placental hormone inhibin A in women with a normal (68), and a PIH (21) or PE (35) complicated pregnancy. Blood samples for the fibrinolytic and inhibin A assays were drawn before the 20th gestational week (gw), between the 20th and 30th gw, and after the 30th gw; routine coagulation, metabolic, renal and liver function tests, US umbilical artery pulsatility index (UAPI), placental and newborn weight were measured. In normal pregnancies, PAI-2 and inhibin A levels progressively increased. In complicated pregnancies, both t-PA and PAI-1 levels were significantly higher than in controls; in particular, PAI-1 significantly increased after the 20th gw especially in PE patients, and a similar behaviour was seen for inhibin A. PAI-2 levels were significantly lower after the 30th gw in patients with both PIH and PE. The PAI-1/PAI-2 ratio was significantly higher in PE than in control patients since the 20th gw, and only after the 30th gw in PIH. Inhibin A significantly correlated with t-PA, PAI-1 and PAI-1/PAI-2 ratio, and inversely with newborn weight. PAI-2 correlated with newborn and placental weight, and inversely with UAPI. Between the 20th and 30th gw, levels of inhibin A >525 pg/L and PAI-1 >25.5 ng/ml disclosed high sensitivity and specificity values in distinguishing PE from both PIH and normal pregnancies. Fibrinolytic test, especially PAI-1, and inhibin A monitoring during pregnancy may help the early diagnosis of pregnancy-related hypertensive disorders; in addition, PAI-2 appears a promising predictor for foetus outcome.

P105

CLINICAL, INSTRUMENTAL, AND LABORATORY PRESENTATION OF PULMONARY EMBOLISM IN THE ELDERLY: ANALYSIS OF LITERATURE STUDIES

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Background and aim. Diagnosis of pulmonary embolism (PE) in the elderly is difficult and frequently missed because of non-specificity and often atypical presentation. The aim of the present study was to review the clinical, instrumental and laboratory studies reporting features of PE in patients 65-years old and older. *Materials and Methods.* Prospective and retrospective english language studies dealing with the clinical, instrumental and/or laboratory presentation of in and out medical and/or post-surgical patients aged more than 65 years with confirmed PE published in the last twenty years (1986-2005) and indexed in MEDLINE were reviewed. On 264 articles and/or abstracts analysed, ten met our criteria for clinical, six for ECG and chest x-ray, three for echocardiographic, seven for arterial gas analysis and five for D-dimer presentation. *Results.* A total of 650 patients were analysed in the studies considering clinical aspects (246 males/404 females). Mortality ranged from 6 to 32%. Dyspnoea (range 59-91.5%), tachypnea (46-74%), tachycardia (29-76%) and chest pain (26-57%) were the most common clinical symptoms and signs. Immobility was the most frequent risk factor (15-67%) whereas DVT was detected in 15-50%. Sinus tachycardia, right bundle branch block and ST-T abnormalities were the most frequent ECG findings, while chest x-ray was found abnormal in <50% in one-half of the studies and >70% in the other one-half. More than 50% of patients had signs of right heart overload at echocardiography. Arterial gas analysis revealed severe hypoxemia and mild hypocapnia as the main finding. D-Dimer resulted higher than cut-off in four of the five considered studies. *Conclusion.* As shown in our study, presentation of PE in the elderly is highly non-specific; this may explain why, in presence of multiple cardio-pulmonary comorbidities typical of geriatric patients, the diagnosis is difficult. Our study could contribute to the knowledge of PE in the elderly.

P106

INCREASED PLASMA LEVELS OF P-SELECTIN IN SPONTANEOUS DEEP VENOUS THROMBOSIS

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Background. P-selectin may play a role in the pathogenesis of venous thromboembolism. High P-selectin levels have been found in patients with deep-venous thrombosis (DVT) as compared to controls without a history of DVT. It is unknown whether P-selectin distribution differ between spontaneous and secondary DVT, and whether atherosclerosis may affect the link between P-selectin and DVT. *Methods.* The study population consisted of 39 patients with a history of spontaneous DVT, and 25 patients with a previous secondary DVT. Thirty-eight patients without a history of venous thromboembolism attending the outpatient clinic for routine medical visits served as controls. Carotid atherosclerosis was assessed at inclusion by carotid artery ultrasonography. *Results.*Plasma levels P-selectin were significantly higher in the whole DVT group relative to controls without DVT (142.2 pg/mL versus 80.0 pg/mL, p=0.0001). Patients with spontaneous DVT had increased P-selectin values (178,0 pg/mL) as compared to both patients with secondary DVT (127.0 pg/mL; p=0.001), and controls without DVT (p=0.0001). After adjusting for possible confounders in multivariable regression analysis, the association between spontaneous DVT and P-selectin remained statistically significant (p=0.002). Patients with spon-

taneous DVT and at least one carotid plaque had double P-selectin levels (287 pg/mL) than patients with spontaneous DVT without any plaque (139.0 pg/mL, p=0.01). However, circulating P-selectin in this latter group was still significantly higher than in control patients with (83.0 pg/mL, p=0.008) or without carotid atherosclerosis (73,0 pg/mL, p=0.002). *Conclusions.* We found higher circulating levels of P-selectin in patients with spontaneous DVT as compared to secondary DVT or controls without a history of DVT. Spontaneous DVT was associated with high P-selectin levels independently from the presence of atherosclerosis. Within patients with spontaneous DVT, however, the presence of carotid atherosclerosis seemed to correlate with higher P-selectin values.

P107

LABORATORY DIAGNOSIS OF LUPUS ANTICOAGULANT COMPARISON OF TWO COMMERCIAL KITS

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Aim of this study was to evaluate the diagnostic efficacy of an automated SCT assays (Silica Clotting Time HemosIL, IL) and a modified APTT based assay (Lupus Anticoagulant Test, Technoclone GmbH) performed at low and high phospholipid concentrations, to diagnose Lupus Anticoagulant (LA). Forty patients who met the criteria for antiphospholipid syndrome (APS), 15 patients on OAC therapy, 10 patients treated with low molecular weight heparin (LMWH), 5 patients with coagulation factors deficiency (3 factor VIII:C and 2 von Willebrand factor deificiencies) and 40 blood donors were investigated. The following panel of coagulation tests was performed in each patient and control plasma, using ACL 9000 automated coagulometer [Instrumentation Laboratory, (IL) Milano, Italy]: aPTT (APTT-SP, IL), DRVVT (LAC Screen, IL) Silica Clottin Time (SCT, IL) and modified APTT (Technoclone). The normal ranges for all the coagulative screening and confirmatory tests were obtained from 40 healthy donors (20 males and 20 females). Intra and inter assay coefficients of variation (CV) of SCT and APTT modified were obtained by replicative analysis of normal pool (NP) and an LA sample used as a positive control (LA+), 10 times within the same run and 10 times in different runs. Excellent intra and inter-assay CVs were obtained with both SCT and modified APTT tests. A strong significant correlation was found between SCT and modified APTT assays (r= 0,98; p<0,0001). Our data indicate that these commercial assays are both specific tests for LA because of the 70 subjects investigated with known coagulation abnormalities all non LA patients were correctly identified as LA negative and all LA patients were confirmed to be LA positive by confirm test. Performances make both these commercial assays useful tests for screening patients suspected of having a LA on photo optical coagulometers.

P108

ANTIPHOSPHOLIPID ANTIBODY TESTING IN PIEMONTE AND VALLE D'AOSTA: A MULTILABORATORY EXTERNAL QUALITY ASSURANCE PROGRAM

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Screenings for antiphospholipid antibodies (aPL) are frequently assayes in the workup of thombophilic patients or women with recurrent fetal loss. Recently the updated classification criteria for aPL syndrome (APS) has been published. Both lupus anticoagulant (LA) and anticardiolipin (aCL) IgG and IgM are maintained as laboratory criteria, and IgG and IgM antiβ2-glycoprotein-I (aβ2GpI) assays are added in the updated criteria. The sensitivity and specificity of LA, (IgG and IgM) aCL and I(gG and IgM) aβ2GpI detection varies depending on the choice of tests, cutoff values, and results interpretation. This variation is detrimental because persistent laboratory aPL positivity in patients with history of thrombosis is an indication for long-term or high intensity anticoagulant therapy. Aim of this study is to investigate differences in LA, aCL and aβ2GpI testing and reporting practices among diagnostic laboratories of Piemonte and Val d'Aosta. A survey of 5 plasmas to screen for LA and 5 sera to screen for aCL and aβ2GpI was sent to all 15 laboratories enrolled for aCL and aβ2GpI testing and to all 16 laboratories enrolled for LA testing. Program requesting the following information: manufacturer/type of assay/s; isotype tested; values used to define negative/positive and semi/quantitative cut offs and how they were determined; whether interpretative comments were provided and their content. Moreover, as suggested by current guide lines, to optimize standardization of aCL and aβ2GpI two reference samples (monoclonal Sapporo Standard Antibodies HCAL and EY2C9) are introduced as positive internal quality controls for aCL and aβ2GpI testing. Registrants are required to report their antibody results value in term of related Sapporo Standard value and also in local or kit units routinely reported. Finally, in order to evaluate whether or not the use of the SapporoÃ¢â‚¬â„¢s antibodies (to express the results as related standards values) is useful in the standardization of aCL and aβ2GpI ELISA assays, we are going to screen 75 samples with 5 different aCL and 5 different aβ2GpI commercial kits. Elaboration of data is still ongoing.

P109

BRAIN NATRIURETIC PEPTIDE AS A PRECLINICAL MARKER OF CHRONIC PULMONARY HYPERTENSION IN PATIENTS WITH PULMONARY EMBOLISM

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Background. About 4% of patients with previous pulmonary embolism (PE) will suffer from chronic pulmonary hypertension (CPH) within 2 years of follow up. Brain natriuretic peptitde (BNP) is used as a prognostic marker in acute PE. However, there are no data on the potential utility of BNP as a pre-clinic marker of CPH secondary to acute PE. *Objectives.* to explore the relationship between pulmonary artery systolic pressure (PAPs) obtained with Doppler echocardiography and the N-terminal (Nt)-proBNP. *Methods.* Patients with objectively confirmed PE were evaluated with Doppler transthoracic echocardiography after at least six months from the index event. CPH was defined as a PAPs > 40 mmHg at rest and was calculated adding transtricuspidal gradient to the mean right atrial pressure. Plasma levels of Nt-proBNP <100 pg/mL in men and <150 pg/mL in women were considered normal. Pulmonary arteries reperfusion was evaluated with pulmonary perfusion scan. *Results.* 49 patients (men age 64.5 + 13.1 years; 22 men) were enrolled. Mean time from index event and follow up evaluation was 18.5 months (range 6 to 46). Seven patients had CPH, 2 were symptomatic. Nt-proBNP plasma levels were elevated in 6 of 7 patients (sensitivity: 85.7%; 95% CI: 48.7, 97.4%). Nt-proBNP plasma levels were normal in 35 of 42 patients without CPH (specificity: 76.2%; 95% CI: 61.5, 86.5%). 1 of 33 patients with normal Nt-proBNP plasma levels had CPH (predictive value of negative test: 97.0%; 95%CI: 84.7, 99.5%). Mean Nt-proBNP plasma levels were statistically different in patients with CPH and in patients without CPH (592.7 vs 146.9; p=0.004).This difference remained significative after adjustment for confounding factors (p<0.05). There was a good positive correlation between pulmonary artery systolic pressures and Nt-proBNP plasma levels (r: 0.64; p=0.00003). *Conclusions*.: Our data suggest that Nt-proBNP may be used to exclude asymptomatic CPH in patients with previous PE.

P110

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) RESULTS OF A CASE-CONTROL STUDY EVALUATING SIX COMMERCIAL REAGENTS IN ASSESSING THE RISK OF VENOUS THROMBOEMBOLISM

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Background. Case-control and prospective studies have shown that shortened activated partial thromboplastin time (APTT) is associated with the occurrence and recurrence of venous thromboembolism (VTE). However, studies carried out so far investigated only single reagents, thus preventing generalization of conclusions. In this case-control study six commercial APTT reagents were comparatively evaluated for their capacity to assess the relative risk of VTE. *Methods.* APTTs of 164 patients who had had an episode of objectively-confirmed VTE were compared to those of 167 control subjects. Results were expressed as APTT-ratios (test- to-reference coagulation times). By definition, the shorter the test coagulation times the smaller the APTT-ratios. Odds ratios and 95% confidence intervals (95%CI) were calculated as a measure of the relative risk of VTE in the presence (relative to the absence)

of the risk factor. We considered as risk factor an APTT-ratio smaller than the 5th percentile of the distribution of the control population. *Results.* Median APTT-ratios for patients were significantly smaller than those for the control subjects ($p<0.001$) in all instances. Odds ratio (95%CI) for VTE obtained with each reagent ranged from 4.7 (2.0-11.1) to 1.9 (0.7-5.4); 95%CI were relatively narrow and the null value was included in only two of the six reagents. *Conclusions.* This comparative case-control study shows that the majority of the investigated APTT reagents are able to detect hypercoagulability associated with an increased risk of VTE. This simple and inexpensive test can be conveniently used to assess the risk in thrombophilic patients.

P111

ARE LUPUS ANTICOAGULANT POSITIVE PATIENTS AT RISK OF DEVELOPING HEPARIN INDUCED THROMBOCYTOPENIA ANTIBODIES?

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Background. Lupus anticoagulant (LAC) belongs to the family of the antiphospholipids antibodies (aPL) directed towards complexes of phospholipids and proteins. Antibodies-mediating thrombosis of Heparin-induced Thrombocytopenia (HIPA) bind Platelet Factor 4(PF4)-heparin complexes. HIPA and aPL have remarkable similarity: both their target proteins undergo conformational changes upon interacting with phospholipids or heparin giving rise to neoepitopes responsible for the immune response. *Aim of the study.* To assess if LAC positive patients affected by thrombosis are at an increased risk of developing HIPA. *Methods.* We enrolled 78 LAC positive patients affected by thrombosis an treated with heparin. Seventy-eight matched patients treated with heparin but LAC negative were taken as controls. All patients were tested for HIPA. The Odd Ratio (OR) was measured to estimate the risk. *Results.* Results are shown in Table 1. Eight patients developed HIPA (Table 2): 5 had deep vein thrombosis (DVT) and 3 an arterial thrombosis; one showed severe thrombocytopenia. Six of them were LAC positive and two were negative: a woman with recurrent idiopathic DVT and a man affected by acute myocardial infarction. *Conclusions.* This case-control study doesn't show a significant association between LAC exposure and the development of HIPA. Yet, our data suggest that patients with LAC could be at risk of generating HIPA (OR=3.2), as if platelet activation induced by LAC might give rise to other anti-platelet antibodies. As a matter of fact, Jouhikainen et al. found an anti-platelet antibody weighing 65 kDa in LAC positive patients: this is the weight of PF4 when bound to heparin. We might speculate that this complex could be the target of LAC: the interaction might generate neoepitopes on the PF4-heparin complex which could be responsible for the induction of HIPA.

Table 1.

	HIPA+	*HIPA -*	*TOT*
LAC +	6	72	78
LAC -	2	76	78
TOT	8	148	156

OR=3.2 p=0.71

Table 2.

LAC +	LAC -	TOT	DVT
4	1	5	
Arterial thrombosis	2	1	3
TOT	6	2	8

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P112

ANTIPHOSPHOLIPID SYNDROME AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

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The Antiphospholipid Syndrome (APS) is a trombophilic disorder characterised by the recurrent venous and/or arterial thrombosis and/or pregnancy morbidity. The pathogenesis is still unknown. Accumulating evidence suggests the existence of modulatory interaction and bi-directional communications between the neuroendocrine and immune systems; alterations in either of these systems may be functionally felt in the other and could contribute to the pathogenesis of autoimmune diseases. The hypothalamic-pituitary-adreal (HPA) axis plays a pivotal role in this network. Normal basal levels of cortisol were described in APS patients; adrenal failure was reported as complication of this syndrome. However, it is not still known if in APS patients without hypoadrenalism there are alterations of the HPA axis. A careful investigation of this aspect could evaluate a role of HPA axis in the clinical evolution of this disease. In our study we assessed the adrenal function (cortisol basal levels and cortisol levels after adrenocoticotrophic hormone, ACTH, stimulation test by 1 microg i.v. and by 250 microg i.v. at time +30' and +60') in 15 subjects of both sexes with primitive APS (diagnosis according to the Sapporo Criteria) and in 11 age-, sex-matched healthy subjects. The cortisol basal levels resulted significantly higher in patients affected by APS ($p<0,01$) than the controls. After infusion of ACTH i.v., the cortisol levels were higher in the patients with APS than in the healthy subjects ($p<0.01$ at time +30' after ACTH 250 microg i.v.; $p<0.01$ at time +30' and +60' after ACTH 1 microg i.v.). The anti-21-hydroxylase antibodies resulted positive of low title in 3 of 15 patients. These data are certainly unespected and of not easy interpretation. They indicate that the adrenal function is not reduced in APS patients. Increased response of the tests could express a reduction of the ability to control the autoimmune antibody mediate process.

P113

ANTITHROMBIN FUNCTIONAL DETERMINATION. METHOD-DEPENDENT RESULTS

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Background. Antithrombin (AT) assays based on FXa inhibition have been reported to better discriminate between carriers and non carriers of AT deficiency than methods based on thrombin inhibition. However, in some AT deficiencies (e.g. Cambridge AT variant), the anti-FXa-based method may miss identification of heterozygous individuals. We report on our results obtained in normal and AT deficient subjects studied with different AT methods. *Subjects.* Twenty (20) normal individuals (N), 15 AT deficient patients (Pts). *Methods.* Functional tests: anti-FXa activity (AT-Xa); anti-bovine thrombin activity (AT-IIa); progressive AT activity (AT-pr). Antigenic determination (AT-Ag): rocket immunoelectrophoresis (Laurell). *Results.* AT mean values and ranges obtained in different AT assays are reported in the Table.

Table.

Subjects (n)	*AT-Xa%*	*AT-IIa%*	*AT-pr%*	*AT-Ag%*
N 19)	104 (91-129)	101 (90-125)	101 (82-132)	105 (84-142)
(1)	64	86	90	97
Pts 13)	45 (22-71)	57 (31-74)	50 (30-75)	48 26-81)
(1)	57	88	67	78
(1)	92	55	124	103
Reference Interval	84-125	83-119	76-126	75-134

In 19 normal subjects no significant difference is evident in values obtained with all AT assays. One normal asymptomatic subject presented low AT-Xa (64%) vs. AT-IIa (86%) and normal levels for the other assays. In 13 AT deficient patients AT-Xa is significantly lower ($p<0.0001$)

than AT-IIa. Discrepant results were obtained in two patients that presented normal AT levels in one of the two AT functional assays (AT-Xa or AT-IIa). *Comments.* Few discrepancies between anti-Xa and anti-IIa-based methods have been reported in the literature. For two of our patients diagnosis would be missed on the basis of normal results obtained in one of the two functional assays. Different molecular defects may influence the Antithrombin deficiency detection by functional methods. Genetic analysis may contribute to the defect characterization.

P114

CLINICAL FEATURES ARE POORLY PREDICTIVE CRITERIA FOR DIAGNOSIS OF INHERITED THROMBOPHILIA

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Background. Laboratory screening for inherited thrombophilia is warranted in young patients, especially those with severe venous thromboembolism (VTE) occurred spontaneously or recurrently. The opportunity to carry out the laboratory investigation in older patients is debated and sometimes discouraged, especially in the case of mild clinical manifestations or provoked events. However this policy could miss a number of carriers of thrombophilia, leaving undiagnosed their kindreds. *Aims.* To investigate whether clinical features are predictive for diagnosis of inherited thrombophilia in order to establish rational criteria for admission of patients with VTE to laboratory screening. *Methods.* We analyzed the clinical records of 1,165 patients (M/F 492/673) with previous VTE of the legs. Inherited thrombophilia was defined as the presence of deficiency of antithrombin, protein C, and protein S, factor V Leiden, prothrombin G20210A. Thrombophilia was defined as severe (deficiency of natural anticoagulants or multiple defects) or mild (factor V Leiden or prothrombin G20210A alone). Multiple regression was carried out labeling as dependent variable the presence of inherited thrombophilia or alternatively the presence of severe or mild thrombophilia: the independent variables considered in the multivariate analysis were the age of first VTE (< or > 45 years), the type of clinical manifestation (defined as severe in the case of proximal DVT and/or pulmonary embolism and mild in the case of distal DVT or superficial vein thrombosis), the circumstances of the first VTE (defined as spontaneous or provoked by exposure to transient risk circumstances such as surgery, trauma, bed rest, pregnancy and puerperium, oral contraceptive intake), and the presence or the absence of recurrent VTE. *Results*. The overall prevalence of inherited thrombophilia was 34.8% (severe in 6.7% and mild in 28.1%). Diagnosis of inherited thrombophilia was not associated with the age of first VTE, type of first VTE, and circumstances of first VTE; a significant association (p=0.04) was found with a history of recurrent VTE. Severe thrombophilia was associated with young age (p<0.0001), severe first VTE (p=0.0009) and spontaneous first VTE (p=0.0531) but not with a history of recurrent VTE (p=0.2029). On the opposite, mild thrombophilia was associated only with a history of recurrent VTE (p=0.0554). In the subgroup of 50 individuals with the putative higher probability of dagnosis (age < 45 years, severe clinical presentation, spontaneous first event, history of recurrences), thrombophilia was found in 52% of the cases, 18% severe and 34% mild. In the subgroup of 29 individuals with the putative lower probability of diagnosis (age > 45 years, mild clinical presentation, provoked first event, no history of recurrences), mild thrombophilia was found in 41% of the cases (p=0.48). *Conclusions.* Clinical features seem only partially predictive of the presence of inherited thrombophilia in patients with VTE and adoption of stringent selection criteria to admit patients to laboratory screening could miss a substantial number of individuals with mild thrombophilia.

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ANTIPHOSPHOLIPID SYNDROME IS NOT A RISK FACTOR FOR CHRONIC PULMONARY HYPERTENSION IN PATIENTS WITH A PREVIOUS EPISODE OF PULMONARY EMBOLISM

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Background. With the development of non-invasive diagnostic techniques such as Doppler echocardiography, chronic pulmonary thromboembolic hypertension (CPTH) has been diagnosed more frequently in patients with a previous episode of pulmonary embolism (PE). Prospective trials have clearly showed that without an appropriate therapy, patients with CPTH have an unfavourable prognosis. *Objectives*. to evaluate the role of potential risk factors for CPTH in patients with a previous episode of PE. *Methods.* Patients with objectively confirmed PE were evaluated with Doppler transthoracic echocardiography after at least six months from the index event. CPTH was defined as a PAPs > 40 mmHg at rest and was calculated adding transtricuspidal gradient to the mean right atrial pressure. The following potential risk factors for CPTH were considered: antiphospholipid syndrome (either anticardiolipin or lupus anticoagulant antibodies positive), age, gender, sex, type of initial treatment, pulmonary hypertension at the presentation, recurrence of PE, concomitant deep vein thrombosis (DVT), history of venous thromboembolism and reperfusion of the pulmonary arteries (evaluated with pulmonary perfusion scan) at follow up. *Results.* 49 patients (men age 64.5+13.1 years; 22 men) were enrolled. Mean time from index event and follow up evaluation was 18.5 months (range 6 to 46). Seven patients had CPH, 2 were symptomatic. Presence of antiphospholipid antibodies did not result significantly associated with an increased risk of CPTH (1/7 patients with CPTH and 1/42 patients without CPTH; p=0.26). Of the other potential risk factors evaluated, only pulmonary hypertension at the presentation was marginally significant associated with an increased risk of CPTH (5/6 patients with CPTH and 12/32 patients without CPTH; p=0.07). However, after multivariate analysis, none of the considered risk factors resulted independently associated with an increased risk of CPTH. *Conclusions.* Patients with previous episode of PE with antiphospholipid antibodies have not an increased risk of CPTH.

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INVESTIGATION FOR ATHEROSCLEROTIC ALTERATIONS IN CARRIERS OF INHERITED THROMBOPHILIA WITH OR WITHOUT PREVIOUS VENOUS THROMBOSIS

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Background. A significant association of increased signs of atherosclerotic disease in subjects with spontaneous venous thrombosis has recently been demonstrated. Our study aimed at assessing whether subjects with inherited thrombophilia, with or without previous venous thromboembolism (VTE), had increased signs of atherosclerotic alterations as represented by carotid plaques and/or higher values of the intima-media thickness (IMT) of the common carotid artery (CCA), and/or by reduced (<0.90) ankle/brachial index (ABI). *Material and Methods.* Ultrasonography (US) of carotid arteries and ABI measurement were performed in: A) 98 subjects (36 males; 51 with a previous VTE), aged 65 y or less, median age 48 y (27-65), with inherited thrombophilic alterations (deficiency of antithrombin= 1, protein C= 9, protein S= 8; G20210A prothrombin mutation= 27, F. V Leiden mutation= 53); and B) in 95 healthy controls (34 males), matched for sex and age, median age 49 y (26-65). All US examinations and ABI measurements were performed by one experienced operator who was unaware of the nature (case or control) of the examined subject. *Results.* Increased IMT values [> 90th percentile of the control group= >0.71 mm and > 0.73 at right (R) and left (L) CCA, respectively] were recorded in 10 (10.2%) and 11 (11.2%) cases in the R and L arteries, respectively, and in 9 (9.5%) in R and L CCAs in controls. Two cases (2%) had one plaque each (both with £ 30% of stenosis), in the internal L carotid; 2 controls had one plaque each in either arteries (both with £ 30% of stenosis). The ABI values were always normal in cases and controls. *Conclusions.* These findings indicate that subject with inherited thrombophilia, with or without previous VTE episodes, do not have an increased risk of atherosclerotic diseases.

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EXTERNAL QUALITY ASSESSMENT (EQA) PROGRAM OF THE AZIENDA OSPEDALIERO UNIVERSITARIA S. ORSOLA-MALPIGHI (BOLOGNA): RESULTS OF D-DIMER TESTS

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The strategy to diagnose venous thromboembolism (VTE) includes three different diagnostic tools: a) the degree of clinical probability categorizing the magnitude of the risk of VTE presence; b) measuring D-dimer levels; c) execution of non-invasive instrumental tests. A wide number of D-dimer methods are now available, and most of them are fully automated and available 24 hours a day. Generally, the sample turnaround time is very short (mainly less than 10 minutes) and the results are observer-independent. However, it is well known that D-dimer methods are poorly standardized. The upshot is that comparing

results obtained with different methods is impossible and every result is method-specific. Starting from 2005, evaluation of D-dimer assays has been added to our EQA program for blood coagulation. Currently, 106 labs are participating in the D-dimer evaluation. Six different samples of frozen plasmas (with normal, abnormal and borderline D-dimer levels) have been prepared and sent to participants in 5 different exercises; when the same sample was sent in more than one exercises it was coded differently. The mean D-dimer levels obtained by the participating labs using various systems (combinations of different methods on different apparatus) in three different samples are shown in the figure. Samples C052, C051 and C054 were prepared to obtain normal, borderline and abnormal D-dimer levels, respectively. As expected, the mean values recorded with the various systems were highly different. Labs were also asked to interpret their results (as normal, abnormal or borderline). 90% of labs interpreted correctly as normal the C052 sample, 79% and 66% as abnormal the C054 and C051samples, respectively. In conclusion, D-dimer values measured by using different systems are highly variable as absolute levels; the interpretation of results is acceptable for samples with low and high D-dimer levels, while it is more problematic for samples with values close to the cut-off used for VTE exclusion (borderline levels).

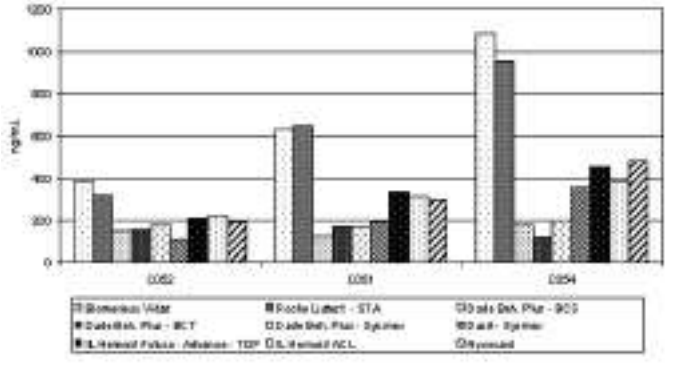

Figure 1.

P117bis

THE EXTERNAL QUALITY ASSESSMENT (EQA) PROGRAM OF THE AZIENDA OSPEDALIERO UNIVERSITARIA S. ORSOLA-MALPIGHI (BOLOGNA): RESULTS OF TWO PILOT EXERCISES FOR THROMBOPHILIA TESTING

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Testing for thrombophilic alterations is presently performed in many coagulation laboratories, from the most specialized to the smallest clinics. In 2005, two pilot exercises for the evaluation of assays for thrombophilia screening have been performed within our EQA program for blood coagulation. Two different pools of frozen plasmas (with normal and abnormal values) have been prepared, differently coded, and sent to the participants in 2 exercises. 24 labs participated to this pilot study and were asked to measured Protein C activity, Protein S (clotting and free fraction), activated Protein C resistance (APCR) and homocysteine. Two DNA samples were also sent for assessment of the G20210A prothrombin, R506Q FV Leiden and C677T MTHFR mutations. The mean values obtained in the two different samples using various systems (combinations of different methods on different apparatus) are shown in the Table below. Only 4 labs performed DNA analysis for G20210A prothrombin, R506Q FV Leiden and C677T MTHFR mutations and the results were correctly interpreted in all but one case [1 incorrect interpretation among a total of 42 (2.4%)]. Thrombophilia testing is widely performed in Italian coagulation labs but few of them are currently participating to an EQA program for these assays. However, many researchers have documented the benefits of participating to inter-laboratory comparison programs and proficiency testing to improve the lab performance and reduce the rate of unacceptable results. Starting from 2006, thrombophilia tests have been included in the EQA program for blood coagulation of the Azienda Ospedaliero Universitaria S. Orsola-Malpighi (Bologna) and, to our knowledge, it is the only one currently organized in Italy.

	Normal sample		*Abnormal sample*	
	Mean	*CV%*	*Mean*	*CV%*
Protein C, chromogenic assay (%)	85.4	9.2	45.7	17.0
Protein S, clotting assay (%)	74.5	8.6	47.9	18.7
Free Protein S (%)	83.5	12.3	56.7	9.7
APCR (ratio)	3.10	16.0	2.80	13.5
Homocysteine (µmol/L)	5.9	6.3	20.4	5.9

P118

DEEP VENOUS THROMBOSIS (DVT) IN INTENSIVE REHABILITATION: PREVALENCE AND ENTITY IN PATIENTS UNDERGOING MAJOR ORTHOPEDIC SURGERY

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Introduction. In major orthopedic surgery thromboembolism is the first cause of death. Over the last years more and more patients, after surgery, are transferred to intensive rehabilitation units, where physicians have to face the problem of treating patients with high probability of recent DVT and with indication to gymnastics. *Purpose.* to determine prevalence and characteristics of DVT in these patients. *Materials and methods.* we performed a retrospective study on 241 consecutive patients admitted to an intensive rehabilitation setting after elective total hip replacement (THR), total knee replacement (TKR) or hip fracture surgery (HFS) between 2004 and 2005. All patients received prophylaxis with low molecular weight heparin (LMWH) or warfarin starting from the day of surgery and were screened with duplex ultrasonography (DUS) at admission in rehabilitation unit. In case of proximal DVT the rehabilitation program was stopped at least for one week or up to the demonstration of thrombus adhesion. *Results.* DUS resulted non diagnostic for calf veins in 10 pts (4,2%). Among other 231 patients, 54 (23,3%) had DVT: 14 (6%) proximal and 40 (17,3%) distal. Proximal DVT resulted less frequent in TKR and more frequent in HFS, as shown in Table.

Table 1.

	DVT	*Proximal*	*Distal*
TKR	28/72 (38.8%)	3 (10.8%)	25 (89.2%)
THR	14/83 (16.8%)	5 (35.8%)	9 (64.2%)
HFS	12/76 (15.7%)	6 (50.0%)	6 (50.0%)
Total	54/231 (23.3%)	14 (6.0%)	40 (17.3%)

During hospitalization no clinically relevant pulmonary embolism nor bleeding were observed. *Conclusions.* in patients admitted to a rehabilitation program after major orthopedic surgery DVT is frequent and its prevalence and clinical relevance differs in the various subgroups of patients. The DUS screening allowed us to discover patients (almost 1 in 4) to treat with anticoagulant therapy, and for whom rehabilitation must be temporarily postponed. The absence of embolic complications in our practice supports us in going on with this diagnostic and therapeutic strategy.

P119

PHARMACOKINETIC PROPERTIES OF ANTITHROMBIN CONCENTRATE (AT III KEDRION(®)) SUPPLEMENTATION IN PATIENTS WITH CONGENITAL ANTITHROMBIN DEFICIENCY

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In human beings, the fractions of total-body AT in plasma-,vascular-associated, and extravascular-pool are about 40%, 10% and 50% respectively. The daily catabolic rate of total-body AT averages 23%,with a fractional 46% contributed by vascular-associated AT.Thus, vascular-associated AT distributes between plasma and an endothelial receptor(heparan-sulfate),and its amount is a function of the AT concentration measured in plasma. As a result, upon infusion of AT concentrates, and depending on the amount of AT infused, most likely to affect the 50% elimination time, healthy subjects and congenital AT deficient patients may show differences in the vascular-associated pool of AT.A

single infusion of the concentrate AT III Kedrion (nanofiltered) was infused at a dosage of 40, 100 and 50 U/kg b.w.in 18 healthy volunteers (HV1, mean AT activity 93(±)10%,mean AT antigen 90(±)13%),12 HV (HV2, mean AT activity 93(±)9%, mean AT antigen 91(±)10%) and 12 patients with congenital AT deficiency(ATD)respectively. Among patients, 9 had typeI AT deficiency (mean AT activity 51(±)8%,mean AT antigen 47(±)7%),3 type II AT deficiency (mean AT activity 49(±)14%, mean AT antigen 92(±)11%), and 8 were on oral anticoagulant treatment. Net pharmacokinetic values were obtained by subtracting the baseline values from those relative to the post-infusion phase. By non-compartmental analysis, recoveries of AT activity and antigen were similar in HV1, HV2 and ATD. The 50% elimination time [= t1/2 β, but considering the time points until 24 hours only] for AT antigen was not significantly different in the three groups, but the 50% elimination time for AT activity was longer in the ATD group (30(±)11 hours) and more so in patients with type II deficiency (38(±)11 hours) than in HV (20(±)9 hours, $p<0.05$), suggesting competition of active and inactive AT species for the binding to heparansulfate. To rule out product specificity of these findings, we compared the affinity of Fondaparinux® and of Idraparinux® for different commercial antithrombin concentrates,as a function of the rate of factor Xa neutralization in purified systems.The Ki for Xa neutralization (7.5 and 15 nM) at 0.3U/mL AT(from Kedrion, Baxter, Grifols, Pharmacia, Aventis)with increasing penthasaccaride concentrations was similar with all AT preparations and was two-fold higher with Fondaparinux than with Idraparinux ($p<0.0001$).

P120

RELATION BETWEEN FVIII LEVELS AND ROTEM PARAMETERS IN PATIENTS WITH VTE

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Background. Thromboelastometry (ROTEM) represents an interesting tool for the study of thrombophilia. Both inherited and acquired thrombophilia may be explored by ROTEM. Among thrombophilic conditions, increased factor VIII levels has been associated with increased incidence of (recurrent) venous thromboembolism (VTE). *Patients.* Out of one hundred patients with first VTE, 10 presented with increased levels of plasma FVIII above the 95 percentile. No other associated thrombophilic conditions were present in these patients. Evaluation by thromboelastometry (using different activators, i.e., Natem, Extem, Intem and Fibtem) has been performed according with the standard protocols supplied by the manufacturers. Twenty VTE patients in whom neither elevated FVIII levels nor other congenital or acquired thrombophilic conditions nor different in platelets count were present, acted as controls and were evaluated with the same methods. *Methods.* Among parameters of ROTEM evaluated in each sample, clotting time (CT), clotting formation time (CFT), maximum clot firmness (MCF), maximum velocity (Vmax), the Area Under Curve (AUC) were chosen to compare the different assays in patients and controls. Coagulation tests including platelets count, PT, PTT, clotting inhibitors, common thrombophilic polymorphism, antiphospholipid antibodies were performed according to standard methods. *Results.* All 10 patients with elevated FVIII levels (FVIII+; 272±63%, mean values±SD) presented with a increased AUC values in Intem and Extem assays of 6260±535 and 6477±573, respectively, as compared to VTE patients with normal FVIII levels (FVIII-; 102±14%, mean values±SD) and no associated thrombophilia in whom levels of AUC were 5096±272 and 5137±285, respectively (Table 1).

Table 1. AUC mean values in the different assays.

	Natem	*Extem*	*Intem*	*Fibtem*
FVIII+ (n pzt 10) 272±63%	*5452±388 **	*6477±573 ***	*6260±535****	*2812±934*****
FVIII- (n pzt 20) 102±14%	*4592±266 **	*5137±285***	*5096±272 ****	*1198±293*****

**, **, *** p<0.005; **** p<0.05.*

Conclusions. In patients with VTE and no thrombophilic conditions, the presence of elevated levels of factor VIII is associated with significant increased of the AUC as compared to subjects with normal FVIII levels. Since an increase in AUC reflects an hypercoagulable state, these findings account for the contribution of increased FVIII levels. The clinical relevance of increased AUC levels obtained in different assays by ROTEM needs to be addressed in studies of prospective cohorts of VTE patients.

P121

VENOUS THROMBOEMBOLISM (VTE) IN PATIENTS WITH ANTIPHOSPHOLIPID ANTIBODIES AGAINST PROTEIN C AND/OR PROTEIN S: A CASE-CONTROL STUDY

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Background. The risk of VTE in subjects with antiphospholipid antibodies directed against the protein C (PC) and/or protein S (PS) is still unclear. *Methods.* We carried out a case-control study with cases and controls matched for sex and age. Cases were consecutive patients with objectively documented VTE. Controls were healthy subjects who had not experienced previous VTE. The following tests were performed in all subjects: a complete screening for inherited thrombophilia, Lupus anticoagulant (LAC) according to the ISTH guidelines, the ELISA for IgG and IgM anti-PC, anti-PS and anticardiolipin (ACA) antibodies. *Results.* We enrolled 179 cases, of whom 40 were excluded because carriers of inherited thrombophilia (factor V Leiden, PT G20210A, PC defects). Among the remaining 139, 30 resulted LAC positive (including mild or very mild LAC positive) and 5 ACA positive (>40 UI/mL) (2 out of the five were also LAC positive) and were excluded because of the known increased risk for VTE associated to these conditions. Of the remaining 106 cases, 5 exhibited anti-PS antibodies (>20 AU/mL); 4 anti-PC antibodies (> 20 AU/mL), and 1 both anti-PC and anti-PS antibodies. Among 179 controls, 9 were excluded because they were carriers of inherited thrombophilia, and 2 because of LAC positive. Of the remaining 168 controls, 4 presented with anti-PS antibodies and 1 anti-PC antibodies. The OR (odds ratio) for the development of VTE in subjects with anti-PS and/or anti-PC antibodies was 3.27 (95%CI, 1.085±9.857; p=0,035). *Conclusions.* Our data are consistent with the presence of an association between antiphospholipid antibodies directed against PC and/or PS and VTE. Further studies of a larger sample size are required to confirm this hypothesis and to evaluate the role of different types of anti-PC and/or PS antiphospholipid antibodies in predisposing to VTE.

P122

REAGENT-SPECIFICITY OF PARAMETERS OF WAVEFORM ANALYSIS IN PATIENTS WITH LUPUS ANTICOAGULANTS

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Transmittance waveforms are the data generated during clot formation on photo-optical coagulation analyzers and are used to define specific events of the clotting reactions. An abnormal deflection in the pre-coagulation-phase of the prothrombin time (PT), called slope 1, has been observed in a high percentage of patients with antiphospholipid antibodies. The abnormal slope 1 appears reagent-specific and is inversely correlated with the aCL IgG titer and with the IgG titers of autotantibodies directed against β2GPI and prothrombin. With the automated coagulometer MDA-180 (BioMerieux), we recorded waveform analysis parameters obtained with 2 APTT reagents (Platelin LS, BioMerieux; APTT-P, Dasit) and with 4 PT reagents (Simplastin L, BioMerieux; Thromboplastin S, Dasit; Neoplastin Plus, Roche; Recombiplastin, IL) in citrated plasma (3.9%) from healthy volunteers (n=60), from unselected outpatients referred to the central laboratory (n=200), from 200 patients on oral anticoagulant treatment free of lupus anticoagulants, from 153 patients with lupus anticoagulants not on oral anticoagulant treatment and from 39 lupus anticoagulant patients on oral anticoagulation. We calculated 99% confidence intervals for 11 waveform parameters with each reagent in healthy volunteers and compared the frequency of abnormalities observed in the remaining patient populations. With the 6 reagents, an abnormal slope 1 was observed in 0% to 18% of outpatients referred to the central laboratory, in 0% to 18% of patients on oral anticoagulant treatment free of lupus anticoagulants, in 4% to 46% of patients with lupus anticoagulants not on oral anticoagulant treatment, and in 5% to 46% of lupus anticoagulant patients on oral anticoagulation. The BioMerieux reagents showed the higher sensitivity to the presence of lupus anticoagulants in terms of the abnormal slope 1, and a correlation with antiβ2GPI IgG titers was only present for these reagents. The recombinant reagent (IL) was not sensitive at all. These results confirm reagent-specificity of parameters of waveform analysis in patients with lupus anticoagulants and suggest a similar frequency of abnormalities irrespective of the history of thrombosis.

P123

EVALUATION OF A NEW D-DIMER METHOD: INNOTRAC AIO!™

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Introduction. D-dimer is a cross-linked fibrin degradation product released into the circulation during the process of blood clot breakdown. It is used as early sensitive marker for thrombotic disorders to rule out venous thromboembolism (VTE) when its concentration is below a clinically validated cut-off. The results of different quantitative D-dimer tests can differ significantly because of the variety of fibrin degradation products in plasma, the specificity of D-dimer assays and the calibrators used in the test. *Aim of study.* evaluation of a new quantitative dry reagent immunoassay, Innotrac Aio D-dimer, compared with Vidas D-dimer bio-Merieux method. *Method*. assays were performed on 91 consecutive emergency room in-patients with symptoms that suggested VTE, 114 neoplastic patients and 51 healthy subjects (men=25, women=26). The blood venous specimens were drown into citrate coagulation tubes (0.109M), were centrifugated at 3000 r.p.m. for 10 min and then analyzed by automated Aio™ Immunoanalyzer (Innotrac Diagnostic) and Vidas analyzer. Aio D-dimer assay is based on dry chemistry and fluorometry detection; instead of Vidas assay is an automated quantitative enzyme-linked immunoassay based on fluorescent detection. Statistical analysis was performed for the three patient groups. We calculated Roc curve, correlation coefficient, linear regression equation and Bland Altman graphic to analyzed data. *Results.* ROC curve was calculated at Vidas value cut off of 500 ng/mL for excluding VTE. Area under the curve was 0.98 (Table 1). At this cut off value Aio method sensibility is 96.92 (95% CI= 89.32% to 99.63%) and specificity is 80.77%. However, the better cut-off for Aio method is 608 ng/mL, because of the specificity that rises to 88.46% without significant modifications of sensitivity (95.38%; 95% CI=87.10% to 99.04%). Linear regression analysis (Table 2) showed good agreement between the two methods as well as the Bland-Altman analysis despite an overestimation of results obtained with Aio test. *Conclusion.* our study showed a relatively good correlation between the new rapid Aio D-dimer assay and the Vidas D-dimer assay. Even if high variability among different D-dimer tests is well-known, this new method showed a good sensitivity and specificity for VTE. To improve specificity strongly bound to VTE, a recalculation of cut off values should be performed.

Table 1.

ROC curve Aio	*Results*
Area under the ROC curve	0,9817
Std. Error	0,01056
95% confidence interval	0.9610 to 1.002
p value	< 0.0001

Table 2.

PATIENTS	*N*	*R^2*	*REGRESSION LINEAR EQUATION*
Emergency room in-patients	91	0.89	y=0.7466x+126.99
Neoplastic patients	114	0.73	y=0.5021x + 198.65
Control patients	51	0.61	y=1.0632x -173.37

p<0.001

Venous thromboembolism: epidemiology, risk factors, recurrences

P124

THE RISK OF RECURRENT VENOUS THROMBOEMBOLISM AMONG HETEROZYGOUS CARRIERS OF FACTOR V LEIDEN OR PROTHROMBIN G20210A MUTATION A SYSTEMATIC REVIEW OF PROSPECTIVE STUDIES

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Background. Factor V Leiden (FVL) and prothrombin G20210A mutation (PTM) are the two commonest genetic polymorphisms known to predispose to a first episode of venous thromboembolism (VTE). However, it is unclear whether these inherited genetic conditions are also risk factors for recurrent VTE. *Objective.* To assess the risk of recurrent VTE associated with heterozygous carriage of each of these mutations. Methods. All randomized controlled trials and prospective cohort studies that reported the incidence of recurrent VTE in patients with and without FVL and PTM after discontinuation of anticoagulant treatment were sought by electronic and manual searches and analyzed. *Results.* Ten studies fulfilled the inclusion criteria. Recurrent VTE occurred in 113 of the 538 heterozygous carriers of FVL (21.0%) as compared to 363 of the 2499 non-carriers (14.5%), yielding to a statistically significant increased risk ratio for VTE recurrence of 1.42 (95% CI, 1.18 to 1.71; Cochran's Q for heterogeneity p=0.06; I2 test = 46.6%). Recurrent VTE occurred in 39 of the 201 heterozygous carriers of PTM (19.4%) as compared to 407 of the 2841 non-carriers (14.3%), yielding to a non-significant increased risk ratio for VTE recurrence of 1.28 (95% CI, 0.96 to 1.72; Cochran's Q for heterogeneity p=0.17; I2=30.9%). *Conclusions.* In symptomatic patients with VTE heterozygous carriage of FVL is associated with a statistically significant increased risk of recurrent thromboembolism. A milder and statistically non-significant increased risk of recurrence is conferred by heterozygous carriage of PTM.

P125

PREVALENCE OF THE METABOLIC SYNDROME IN PATIENTS WITH IDIOPATHIC DVT

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Background. Patients with idiopathic venous thromboembolism (VTE) are at increased risk of asymptomatic atherosclerosis and cardiovascular events. The metabolic syndrome is a cluster of risk factors for atherosclerosis. Its impact on VTE is unknown. *Methods.* In a case-control study, consecutive patients with objectively confirmed deep vein thrombosis (DVT) and control subjects with objectively excluded DVT underwent clinical assessment for the presence of the metabolic syndrome according to the National Cholesterol Education Program criteria. The presence of known risk factors for DVT was documented. Patients with DVT secondary to cancer were excluded. The prevalence of the metabolic syndrome was compared between patients with idiopathic DVT and controls. *Results.* We enrolled 112 patients with idiopathic DVT and 107 controls. The mean age was 64.5 years and 63.7 years, respectively. The metabolic syndrome was diagnosed in 50.9% of patients with idiopathic DVT and in 34.6% of controls (OR 1.96; 95% CI: 1.10, 3.51). After adjustment for age, gender, body mass index, and smoke, the metabolic syndrome remained independently associated with idiopathic DVT (p<0.01). When individual components of the metabolic syndrome were subsequently adjusted for potential confounders, we found that waist circumference (p<0.001) and triglycerides (p=0.017) were independently associated with idiopathic DVT. In patients with secondary DVT, the prevalence of the metabolic syndrome was 27%. *Conclusions.* The metabolic syndrome may play a role in the pathogenesis of idiopathic DVT and may act as link between venous thrombosis and atherosclerosis.

P126

DEEP VENOUS THROMBOSIS AND DYSFUNCTIONAL THYROID DISEASES: A PILOT STUDY

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Background. Several *in vivo* studies have shown that clinically overt hyperthyroidism and overt hypothyroidism are respectively associated with a prothrombotic state and a bleeding tendency. A hypercoagulability state is also hypothesized in subclinical hypothyroidism. To explore the hypothesis that some cases of apparently unprovoked deep venous thrombosis (DVT) are secondary to dysfunctional thyroid diseases, we planned a pilot case control study aimed to estimate the frequency of overt and subclinical thyroid dysfunction in patients with a previous episode of DVT. *Methods.* We here report the results of an interim analysis. All consenting patients with a DVT of the lower limbs, in the previous 2 years, were potentially eligible. DVT was diagnosed by compression ultrasound. A blood sample was drawn from each patient to measure free thyroxin (fT4), free triiodothyronin (fT3), thyroid-stimulating hormone (TSH), and thyroid antibodies: antithyroid peroxidase, antithyroglobulin and antiTSH-receptor antibodies (TPOAb, TgAb and TRAb). *Results.* Until March 2006 we enrolled 50 patients with unprovoked DVT, 46 patients with secondary DVT, and 29 controls. Mean age was 68.6, 59.3, and 61.0 years, respectively. Median level of TSH was 2.01, 2.14*, and 1.69* UI/mL, respectively (*p<0.05). A new subclinical hypothyroidism was diagnosed in 7° (14.0%), 2 (4.4%), and 0° patients, respectively (°p<0.05). Only 1 case (in the control group) with clinical overt hypothyroidism, 1 with subclinical hyperthyroidism (in the unprovoked DVT group) and 1 with clinical overt hyperthyroidism (in the control group) were observed. Elevated thyroid antibodies were newly detected respectively in 4 (8.0%), 7 (15.2%), and 8 (27.6%) patients. *Conclusion.* The preliminary data of our study show a significantly increased frequency of subclinical hypothyroidism in patients with unprovoked DVT. The clinical relevance of our preliminary findings needs to be addressed in larger prospective studies.

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VATHT (VENOUS AND ARTERIAL THROMBOSIS HISTORY AND THYROID) STUDY

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Background. Hyperthyroidism may increase the risk of venous thromboembolic (VTE) events. However, no studies with clinical end-points have ever been published. Myocardial infarction is a well-known complication of hypothyroidism, in particular if clinically overt, but few clinical data are available on the risk of VTE or cerebrovascular diseases in hypothyroid patients. We aimed to estimate the relative risk of venous and arterial thromboembolic events from a retrospective cohort of patients with dysfunctional thyroid disease. *Methods.* A predefined questionnaire was administered to consecutive outpatients attending the Department of Endocrinology for a follow-up visit. A detailed history of arterial and venous thromboembolism, venous and arterial risk factors, and rare cerebrovascular diaseses was collected. If available, an objective diagnosis of the referred thrombotic events was checked for confirmation. *Results.* We here report the results of an interim analysis performed on 301 patients, 87 and 8 with overt and subclinical hyperthyroidism, 34 and 122 with overt and subclinical hypothyroidism, and 50 with elevated thyroid antibodies and euthyroidism. Mean age was 45.8, 58.8, 53.9, 51.1, and 48.0 years, respectively. VTE was referred in 10° (10.5%) patients with hyperthyroidism (1 pulmonary embolism, 4 deep venous thrombosis [DVT], and 5 superficial vein thrombosis [SVT]), in 8° (5.13%) with hypothyroidism (2 DVT and 6 SVT), and in 4 (8.0%) euthyroid patients (4 SVT) (°OR: 2.2, 95% CI: 0.8-5.7). An ischemic cardiac or cerebrovascular event was detected in 3* (3.2%) patients with hyperthyroidism, in 14* (9.0%) with hypothyroidism (6 overt and 8 subclinical), and in 1 (2.0%) euthyroid patient (*OR: 3.0, 95% CI: 0.8-10.8). *Conclusion.* Our preliminary data suggest for the first time that hyperthyroidism, in particular clinically overt, may be associated with an increased incidence of VTE and confirm that hypothyroidism is associated with an increased risk of arterial thrombotic events.

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INCIDENCE OF ARTERIAL CARDIOVASCULAR EVENTS IN PATIENTS WITH IDIOPATHIC VENOUS THROMBOEMBOLISM. A CASE-CONTROL STUDY

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Recent studies have shown a higher incidence of arterial cardiovascular events in patients with venous thromboembolism (VTE) of unknown origin than in those with the secondary form of disease. Whether patients with idiopathic VTE are exposed to the risk of subsequent arterial cardiovascular events more frequently than the general population is unknown. In a case-control study we compared the rates of subsequent arterial cardiovascular events (i.e., acute myocardial infarction, ischemic stroke and peripheral arterial disease) in 151 consecutive patients with objectively confirmed spontaneous VTE and 151 sex and age-matched control subjects randomly selected from the database of two family-physicians. We collected information about cardiovascular risk-factors (hypertension, hypercholesterolemia, diabetes, obesity and smoke) in the study population at the time of VTE episode, or corresponding date for the controls, and considered the follow-up from this time. Patients and controls who had suffered from arterial cardiovascular events before the index date were excluded. No statistical differences were found in mean age (65.0 years for the VTE patients and 64.3 for the controls) and cardiovascular risk-factors between the two groups. During a mean follow-up of 43.1 (±21.7) months there were 16 arterial cardiovascular events in the VTE patients and 6 in the control group (HR, 2.84; 95% CI, 1.11 to 7.27; p=0.03). The difference remained significant after adjusting for age and other cardiovascular risk factors (HR 2.86, 95% CI, 1.07 to 7.62). Overall mortality was also higher in the VTE patients (12 vs 4 deaths, p=0.04). We conclude that arterial cardiovascular events are more common in patients with previous idiopathic VTE than in the general population. These findings may have practical implications.

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METABOLIC SYNDROME AND HYPERHOMOCYSTEINEMIA IN PATIENTS WITH DEEP VEIN THROMBOSIS: A CASE-CONTROL STUDY

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Objective. High plasma levels of total homocysteine are a risk factor for deep vein thrombosis (DVT). Presence of metabolic syndrome has been recently associated with an increased risk of DVT. High plasma levels of total homocysteine are frequently observed in patients with metabolic syndrome. However, there is no information on the relationship between hyperhomocysteinemia and metabolic syndrome in the pathogenesis of DVT. *Methods.* In a case-control study, consecutive patients with objectively confirmed idiopathic DVT and control subjects with objectively excluded DVT underwent clinical assessment for the presence of the metabolic syndrome according to the National Cholesterol Education Program criteria. Fasting plasma levels of total homocysteine were measured in patients and in controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were used as a measure of association between DVT and hyperhomocysteinemia and presence of metabolic syndrome. Interaction between hyperhomocysteinemia and metabolic syndrome on development of DVT was evaluated using a logistic regression model. *Results.* 101 patients with idiopathic DVT and 94 age-mathced controls (64.2 and 63.9 years) were enrolled. The metabolic syndrome was diagnosed in 53 patients with idiopathic DVT and in 32 controls (OR 2.14; 95% CI 1.15-3.98). Hyperhomocysteinemia was diagnosed in 43 patients with idiopathic DVT and in 27controls (OR 1.84; 95% CI 0.97-3.89). After adjustment for possible confounding factors such as age and gender, and considering the possible interaction between metabolic syndrome on plasma levels of total homocysteine, only metabolic syndrome was independently associated with DVT (OR 2.18; 95% CI 1,18-4,01; p=0.012). Coexistence of both risk factors was associated with DVT (OR 2.34; 95% CI 1.08-5.07; p=0.03). *Conclusions.* Metabolic syndrome may play a role in the pathogenesis of idiopathic DVT independently to the presence of hyperhomocysteinemia. Coexistence of both these risk factor did not further increase the risk of DVT.

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PRELIMINARY DATA OF THE EPIDEMIOLOGICAL STUDY ON CONGENITAL AND ACQUIRED RISK FACTORS RELATED TO THROMBOEMBOLIC DISEASES IN LIGURIA REGION (ITALY)

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The epidemiological study on congenital and acquired risk factors related to thromboembolic diseases in Liguria Region (Italy), promoted by the Liguria Region and sponsored by Italian Health Ministry, started in October 2004. Twenty public hospital centers, homogeneously spread in the region, collected data with the aim of evaluating the prevalence of thromboembolic diseases (myocardial infarction, stroke, peripheral artery disease, venous thromboembolism) and the role of risk factors, particularly of the genetic risk factors. In the first phase of the study a computerized data base for data collection was realized and laboratory methods for identification of thromboembolic risk factors were standardized. A cd-rom containing the complete data base software, realized using the MS Access program,© was supplied to all centers. The collected data have been periodically sent to the medical Statistic Unit for the elaboration. Standard laboratory methods for the study of coagulation factors (fibrinogen, factor VII and VIII, protein C, protein S, aPCR, lupus anticoagulant APA , ACA, PAI), usually performed in the laboratories of the public hospital centers, were obtimized and validated to obtain comparable data. The analysis of genetic polymorphisms for the Factor V R506Q, H1299R, Y1702C and for prothrombin G20210A was performed using validated methods in each laboratory. In the first six months of the study 653 patients were enrolled with the following thromboembolic diseases: Deep Vein Thrombosis (255 patients), Pulmonary Embolism (180), Retinal Vein Occlusion (120), Abdominal Vein Thrombosis (32), IMA/CAD aged < 55 years (24), Stroke/TIA aged < 55 years (18), Peripheral Arterial Disease (16), Cerebral Vein Thrombosis (8). Preliminary data on genetic risk factors performed in 400 of these patients showed 65 -R506Q- and 5 -H1299R- eterozigous polymorphisms of Factor V gene and 38 -G 20210A- eterozigous polymorphisms of prothrombin gene.

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NATURAL HISTORY OF CEREBRAL VEIN THROMBOSIS: A SYSTEMATIC REVIEW

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Background. Cerebral vein thrombosis (CVT) has been considered until a few years ago an uncommon disease with significant long-term morbidity and a high mortality rate. New non-invasive diagnostic techniques have increased the frequency with which this disease is diagnosed; despite this, there continues to be little data on its natural history. *Objectives.* To evaluate the mortality rate, the rate of disability at long-term follow-up and the incidence of recurrences after a first episode of CVT; to determine clinical and radiological predictors of death and dependence; and to identify possible risk factors for recurrence. *Data source.* MEDLINE and EMBASE databases (until October 2005), reference lists of selected articles and authors' libraries. *Results.* Nineteen studies and a total of 1488 patients were included in our analysis. Duration of follow-up varied widely between studies ranging from 12 to 145 months. Mortality rate during peri-hospitalization period is 5.6% (range 0 to 15.2%), and 9.4% (range 0 to 39%) at the end of follow-up period. Noteworthy, most of the deaths occurred during follow-up were related to an underlying condition such as cancer (24 of 38 evaluated deaths) and they were not direct consequence of CVT. Eighty-eight percent of surviving patients recover completely or have only a mild functional or cognitive deficit. Two third of patients with CVT recanalized within the first few months after presentation and 2.8% (range 0 to 11.7%) had objectively confirmed recurrence. *Conclusion.* Patients with CVT have a low risk of death and most patients have a good long-term prognosis, however, the risk of recurrence is not negligible.

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BEHAVIOUR OF THROMBOTIC MARKERS IN TURNER'S SYNDROME

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Turner Syndrome is a disease due to complete or partial absence of the second X-chromosome and conditioning, among others, gonadic dysfunction. A long-lasting oestro-progestinic (EP) therapy is necessary for a complete sexual development and to prevent consequences due to the lack of oestrogens (osteoporosis, cardiovascular disease). Related risk of venous thrombo-embolism during treatment with oral contraceptives doubles in general population and it is higher in people with hereditary thrombophilia. *Aim of the study.* To evaluate the prevalence of thrombotic factors in Turner syndrome, to prevent thrombosis during treatment. *Patients.* 75 patients (aged between 1 and 38 yrs) were enrolled: 45 karyotype 45X, 30 other karyotypes. At the beginning of the study 45 were treated with EP, 19 with Growth Hormone and 11 in other ways. No evidence of personal and familial history of thrombosis. Thrombotic factors studied: Homocysteine, AT, PC, PS, FII and FV Leiden mutations, FVIII, PT, PTT. *Results.* PT, PTT, FVIII and ATIII were normal in all patients. In 2 low levels of PC (in EP therapy) and in 9 low levels of PS (4 in EP therapy) were detected. Homocysteine was elevated in 3 patients (in EP therapy); the patients were successfully treated with Vitamin B6-B12 and Folate. FII mutation was carried in 2 pts (2.7%), FV mutation in 4 (3.5%); long term prophylaxis with ASA has been proposed in these subjects. There were no significant differences between different cariotypes. Actually none of the studied patients has developed thrombotic events. *Conclusions.* Risk of venous thrombo-embolism during treatment with oral contraceptives in Turner' syndrome is overlapping to that of general population in EP therapy. It is important to check the thrombophilic state in these patients before starting substitutive therapy necessary for their psycho-physical wellness, to minimize the risk of thrombotic complications.

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AGE AND PREVALENCE OF RISK FACTORS FOR VENOUS THROMBOEMBOLISM: RESULTS FROM THE MASTER REGISTRY

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Background. The incidence of first-time venous thromboembolism (VTE) rises exponentially with age. Approximately 50% to 75% of patients with VTE have a readily identifiable risk factor, either transient or permanent. Whether the prevalence of risk factors for VTE varies with age is currently unclear. *Methods.* MASTER is a multicenter registry aimed to prospectively collect information on the long-term clinical outcome in a large cohort of patients with acute VTE. Information on temporary and permanent risk factors (excluding congenital and acquired thrombofilia) and on the use of prophylaxis were captured by an electronic data network at the time of the index event in patients with objectively confirmed acute VTE. *Results.* We enrolled 2119 patients (49.8% males) of whom 424 (20%) <40 years, 529 (25%) between 41 and 60 years, 943 (44.5%) between 61 e 80 years, and 223 (10.5%) >80 years. Known risk factors were identified in 63.9%, 52.6%, 54.6%, and in 58.3% of patients, respectively; differences in the prevalence of known risk factors among groups were statistically significant (p=0.002). After logistic regression, cancer related thrombosis was less common in patients <40 years than in patients >80 years (OR 0.2, 0.14-0.40), newly diagnosed cancer after VTE was less frequent in patients <40 years (0.11,0.03-0.42), between 41 and 60 (0.36, 0.15-0.82), and between 61 and 80 (0.42,0.20-0.80) than in patients >80. Patients <40 were more likely than patients >80 to have a transient risk factor (1.76,1.27-2.46), whereas patients between 61 and 80 were less likely (0.73,0.54-0.98) than patients >80 years. Finally, VTE occurred despite prophylaxis more frequently in <40 than in >80 years (1.94,1.26-2.99). *Conclusions.* We observed significant differences in the prevalence of identifiable risk factors among age groups. Current prophylactic regimens may be less effective in younger patients.

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DELAY IN DIAGNOSIS OF VENOUS THROMBOEMBOLISM: RESULTS FROM THE MASTER REGISTRY

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Background. The non-specific signs and symptoms of venous thromboembolism (VTE) can make diagnosis difficult. A substantial delay in the diagnosis of deep vein thrombosis (DVT) and pulmonary embolism (PE) has been reported in more than 20% of patients. This study was aimed to identify individual and clinical predictors of the timing of diagnosis of VTE. *Methods.* MASTER is a multicenter registry aimed to prospectively collect information on the long-term clinical outcome in a large cohort of patients with acute VTE. Information on clinical presentation and diagnostic methods, temporary and permanent risk factors, and prophylaxis were captured by an electronic data network at the time of the index event in patients with objectively confirmed acute VTE. *Results.* Data on 2047 patients (1024 males), 1505 with DVT and 542 with PE, were analysed. Diagnosis of DVT was done in less than 5 days from onset of symptoms in 709 (47.1%) patients, between 5 and 10 days in 456 (30.3%), and in more than 10 days in 340 (22.6%). Diagnosis of PE was done in 347 (64.0%), 107 (19.7%), and 88 (16.2%), respectively (OR for delayed diagnosis PE vs DVT: 0.50; 95% CI 0.41-0.62). In both DVT and PE groups, age or gender were not associated with delayed diagnosis. In DVT patients, factors associated with earlier diagnosis were the concomitant presence of 3 signs or symptoms of DVT (pain, edema, and erythema) (p=0.014 vs 1 or 2 signs or symptoms), pain (p=0.049 vs edema or erythema), and history of VTE (p=0.016). Neither transient or other permanent risk factors nor ongoing VTE prophylaxis influenced the timing of diagnosis. In PE patients, only the number of signs or symptoms at presentation (p=0.014) and the presence of transient risk factors for VTE (trauma, surgery, immobilization)(p=0.001) were significantly associated with the timing of diagnosis. *Conclusions.* Substantial delays are common in the diagnosis of DVT or PE. The severity of presentation, history of VTE, and the presence of transient risk factors favor earlier diagnosis.

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RISK FACTORS FOR CVC-ASSOCIATED THROMBOSIS IN CANCER PATIENTS: ANALYSIS OF THE ETHIC STUDY

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Background. The clinical benefit of prophylaxis of venous thromboembolism (VTE) related to central venous catheter (CVC) in cancer patients is unclear. A recent randomised double-blind placebo-controlled trial (ETHICS) showed a non-significant 22% risk reduction of CVC-related thrombosis in patients receiving enoxaparin. The aim of this study was to assess the risk factors for CVC-related thrombosis to identify those patients who may actually benefit from prophylaxis. *Methods.* Database of the ETHIC study was used to perform a multivariate logistic regression. In the univariate analysis, the following covariates were analyzed: gender, age, cancer site and histology, metastasis, CVC diameter and type, duration of CVC insertion procedure, inadequate position of CVC tip (CVC tip in upper half of superior vena cava), site of CVC insertion and chemotherapy. Variables with a p-value <0.20 at univariate analysis were included in the multivariate model. *Results.*A CVC-related thrombosis was found in 50 of the 310 patients (16%) with adequate venography performed at 42 days from randomisation. In univariate analysis the following risk factors for thrombosis were identified: inadequate position of CVC tip (Odds Ratio (OR) 6.1, 95%CI 2.9 to 12.8), left side CVC insertion (OR 3.1, 95%CI 1.7 to 5.8) and age >60 years (OR 2.5, 95%CI 1.0 to 6.2). In the multiple logistic regression analysis, an inadequate position of CVC tip (OR 4.4, 95%CI 2.0 to 9.7) and left side CVC insertion (OR 2.5, 95%CI 1.3 to 5.0) were independent risk factor for VTE. *Conclusions.* An inadequate position of CVC tip and left side of CVC insertion are independent risk factors for CVC-related thrombosis in cancer patients. These findings may contribute to identify patients at increased risk of VTE in which prophylaxis may be of increased clinical benefit.

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A PROSPECTIVE REGISTRY ON THE LONG-TERM CLINICAL OUTCOME OF VENOUS THROMBOEMBOLISM (MULTICENTER ADVANCED STUDY FOR A THROMBOEMBOLISM REGISTRY - MASTER): DESCRIPTION OF THE STUDY COHORT

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Background. Available literature on the long-term clinical outcome of patients with venous thromboembolism (VTE) are not necessarily representative of the full spectrum of VTE population. The aim of this multicenter registry was to prospectively collect data on the long-term clinical outcome in a large cohort of patients with acute VTE. *Patients and methods.* Information about clinical presentation and diagnostic methods, temporary and permanent risk factors, prophylaxis and treatment were captured by an electronic data network at the time of the index event in patients with objectively confirmed acute VTE. A 24-month follow-up included clinical examination after 6 and 12 months and after 2 years. *Results.* From January 2002 to October 2004, 2119 patients (1056 males) were included in the registry in 29 Italian centers. At entry, the mean patient age was 59.2+18.4 years (range 13-99 years). 1541 (72.7%) patients were affected by deep-vein thrombosis (DVT), of which 124 of the upper limb, 206 (9.7%) by pulmonary embolism (PE) and 372 (17.5%) by both DVT and PE. 676 patients(31.9%) underwent home-treatment of VTE. 955 patients (45.1%) had one or more temporary risk factors. 381 patients (18.0%) had a known cancer at the time of the index event: 235 (73.9%) of these patients were on chemo- or hormonetherapy. In 50 patients (2.4%) cancer was discovered at the time of the index event. 311 patients (14.7%) had a previous VTE. Based on an estimated rate of recurrence ranging between 4 and 6% per year and of death ranging between 6 and 10% per year, we estimate that relative risks ranging from 1.7 up to 2.0 could be detected in our cohort with α=0.05 and β=0.20. *Conclusions.* The long-term follow-up will allow to improve our understanding on the long-term clinical course of VTE and to optimize its management.

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ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH SPONTANEOUS VENOUS THROMBOEMBOLISM

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A high incidence of atherosclerotic lesions has been found in patients treated for idiopathic venous thromboembolism (VTE). This finding suggests a correlation between venous thromboembolism and atherosclerosis. Endothelial dysfunction is a precocious marker of atherosclerosis and it has a predictive value on the incidence of ischemic cardiovascular events. *Aim of the study*. To evaluate the prevalence of endothelial dysfunction in patients with at least an episode of idiopathic VTE as compared with age- and sex-matched controls without idiopathic VTE. *Patients and Methods.* Patients treated for symptomatic, objectively confirmed, idiopathic, proximal deep vein thrombosis of the lower limbs with or without pulmonary embolism were included in a case-control study. Patients were excluded from the study if they presented any risk factors for cardiovascular diseases (smoking, familial history of early atherosclerosis), other conditions associated with endothelial dysfunction (arterial hypertension, heart failure, dyslipidemia, diabetes, chronic renal failure, hyperhomocysteinemia), estro-progestinic therapy or pregnancy. The control group was composed by age- (±5 years) and sex-matched controls with the same exclusion criteria and without previous episodes of VTE. All patients and controls underwent evaluation of endothelial function, by the non-invasive assessment of flow-mediated vasodilation (FMD) of the brachial artery, by B-mode ultrasonography,

using a standardized procedure, in the morning under fasting conditions. Plasmatic concentrations of sP-Sel, vWF and sCD40L, and urinary excretion of 11-dehydro-TxB2 excretion were also evaluated as supplementary markers of endothelial or platelet activation. *Results*. Overall, 28 patients (8 females, 20 males; mean age 59±15 years) and 28 matched controls (mean age 60±16,8 females) were included in the study. FMD was 3.5±0.6% in patients (95% CIs: 2.2 to 4.8) and 5.7±0.6% (4.2 to 6.8) in controls (p=0.015). Brachial blood flow (88±9.34 vs 93.8±12.8 ml/min, respectively, p=NS) and hyperemic blood flow (408.6±43.8 vs 448.2±52.9 ml/min, respectively, p=NS) did not differ between the two groups. sP-Sel (41.4±7.2 vs 24.1±2.9 ng/mL, p=0.03) as well as vWf:Activity (150.1±9.2 vs 117.7±9.7%, marker of endothelial activation, p=0.004) were higher in patients than in controls; on the contrary, urinary 11-dehydro-TxB2 and plasma sCD40L levels, marker of platelet activation, were not different between VTE cases and controls. *Conclusions*. Patients with previous, idiopathic VTE present an endothelial dysfunction as compared with age- and sex- matched controls. This result supports the hypothesis that idiopatic venous thromboembolism is a condition at enhanced risk of atherosclerosis.

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PORTO-MESENTERIC VENOUS THROMBOSIS IN A PATIENT WITH PROTHROMBIN G20210A MUTATION AND ACQUIRED THROMBOPHILIC STATE DUE TO SILENT COELIAC DISEASE

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Portal and splancnic abdominal veins are unusual sites for venous thrombosis. Underlying inherited thrombophilia (in particular FV Leiden and prothrombin G20210A mutations) and other acquired thrombophilic states (i.e. myeloproliferative disorders, antiphosopholipid syndrome, oral contraceptives, etc.) often interact in the pathogenesis of these events. We report the case of a 27 yr-old man, admitted to our Institution because of asymptomatic pancytopenia and spleen enlargement. Ultrasound and CT scans revealed portal hypertension, together with the presence of a large portal cavernoma and partial occlusion of the mesenteric vein. No previous personal or family history of venous thromboembolism was reported, nor significant risk factors were detectable. Screening for thrombophilic conditions showed heterozygous G20210A prothrombin mutation, moderate hyperhomocysteinemia (45 μmol/L), reduced (functional) protein C (53%) and low-normal (immunologic) protein S (66%) levels. The latter abnormalities and the presence of low plasma folate and ferritin led to search for intestinal disorders affecting nutrient absorption. Immunologic (positive anti-endomyseal and anti-tissue transglutaminase antibodies) and duodenum biopsy data confirmed the diagnosis of coeliac disease. Biochemical abnormalities were corrected by folate supplementation and gluten-free diet. Due to the late diagnosis of the porto-mesenteric venous thrombosis, with development of collateral circulation, anticoagulation was not started. This patient shows the uncommon history of a silent venous thrombosis at unusual site, in which previously undiagnosed both genetic (prothrombin G20210A mutation) and acquired (protein C/S deficiency, hyperhomocysteinemia due to celiac disease) thrombophilia was likely to play a role. Only few cases of thrombotic episodes have been reported in coeliac patients: due to the relatively high prevalence of the diaease and of inherited thrombophilia, these events are presumably underreported. However the role of other, still unidentified, factors involved in the pathogenesis of thrombotic complications in coeliac disease cannot be ruled out.

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THROMBOPHILIA AND BILATERAL UTERINE ARTERY NOTCHES

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Decreased utero-placental blood flow during pregnancy has been found associated with the carriership of prothrombotic mutations, but there are some discrepancies on this issue. We prospectively evaluated a group of women with bilateral notch of uterine arteries at 23 weeks of gestational age , in whom the presence of inherited causes of thrombophilia was investigated. FV Leiden, FIIA20210 mutations were evaluated during pregnancy, while levels of natural inhibitors (Protein C, Protein S, antithrombin) three monts after the delivery. 41 women (mean age 29.1±4.9) were enrolled, feto-maternal outcomes were recorded. Six (14.6%) out 41 women showed an inherited thrombophilia (2 FV Leiden heterozygotes, 3 FII A20210 heterozygotes and 1 protein S deficiency). Among them, 4 (66.6%) showed an adverse obstetric outcome (1 intrauterine fetal death, 2 Small- for -gestational age below 5th percentile, 1 severe preeclampsia). Among the remaining non-thrombophilic women (n=35), 9 (25.7%) had an adverse outcome (6 Small-for-gestational age newborns , 3 intra-uterine fetal death), with an OR 5.8 (95% C.I. 0.9-37.4). We believe that, on the basis of previous case-control studies showing an association between thrombophilia and adverse outcomes, many clinicians translated data into promiscuous testing of patients; nevertheless, we strongly believe that our role is to suggest screening in well selected cases, instead of denying the clinical role of these markers.

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CARDIOVASCULAR RISK FACTORS AND THE RISK OF VENOUS THROMBOEMBOLISM

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The association between established cardiovascular risk factors (arterial hypertension, hypercholesterolemia, cigarette smoking, overweight, and diabetes mellitus) and the risk of venous thromboembolism (VTE) is not entirely understood. We have screened for arterial hypertension, hypercholesterolemia, cigarette smoking, overweight, and diabetes mellitus, 190 consecutive patients (99 men and 91 women; mean age 45.68 ±14.56 yrs) who had been referred to Our centre for a first episode of VTE: 188 DVT of lower extremities, of whom 35 associated with pulmonary embolism (PE), and 2 isolated PE. DVT was documented by ultrasonography or venography, and PE was diagnosed using ventilation/perfusion scans and/or pulmonary angiography. As many as 284 age- and sex-matched apparently healthy subjects (122 men and 162 women; mean age 40.33±14.37yrs), from the same ethnic background, served as controls. Arterial hypertension was found in 48/190 (25.3%) of VTE patients and in 39/284 (13.7%) of controls (p=0.001; OR:2.12; 95%CI 1.32-3.39; Chi-squared test). Hypercholesterolemia (total cholesterol levels >5.2 mmol/L in repeated evaluations over a 3-yr period) was more common in VTE patients than in controls (109/190, 57.4% vs. 115/284, 40.5%; p<0.001; OR: 1.98, 95%CI 1.36-2.87, Chi-squared test), as was BMI >25 (118/172, 68.6% vs. 120/272, 44.1%; p=<0.001, OR: 2.76; 95% CI 1.85-4.13; Chi-squared test). The prevalence of cigarette smoking was 106/190, 55.8% vs. 100/284, 35.2%; p<0.001, OR:2.32; 95% CI 1.59-3.38; Chi-squared test). The prevalence of diabetes mellitus was not different between patients and controls (4.8% of VTE patients and 4.3% of controls). The relevant association between some established cardiovascular risk factor (arterial hypertension, hypercholesterolemia, cigarette smoking, overweight) and VTE implies that adequate treatment/prophylaxis of them should be seriously considered in primary and secondary prevention of VTE.

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HIGH LEVELS OF FACTOR VIII ARE AN INDEPENDENT RISK FACTOR FOR VTE RECURRENCE. A PROSPECTIVE STUDY

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Background. Venous thromboembolism (VTE), term which includes deep vein thrombosis (DVT) and pulmonary embolism (PE), is a major cause of morbidity and death. Recurrence rate after a VTE episode is high and the role of thrombophilic abnormalities in increasing this risk is still controversial. *Aim.* To estimate the recurrence rate after a first episode of VTE and some of its determinants, including thrombophilic abnormalities. *Study design and patients.* Prospective follow-up study of 365 consecutive patients aged 18 to 92 years without a known malignancy treated for a first objectively confirmed thrombotic event and referred to the Thrombosis Center of Florence. *Main outcome Measures*. Recurrent thrombotic events based on thrombophilic risk factors, sex, type of initial thrombotic event (idiopathic or secondary to a transient risk factor such as oral contraceptive use, pregnancy and puerperium, immobilization, trauma, surgery). Thrombophilic risk factors evaluated were:

antithrombin, protein C, free protein S, APC-resistance, factor V Leiden, prothrombin G20210A variant, fasting homocysteine (95th percentile), lupus anticoagulant, anticardiolipin antibodies, factor VIII activity (>150 U/dL), lipoprotein (a) (>300 mg/L). *Results.* A total of 365 patients with a first VTE episode (age 57±16 yrs; males 182, females 183; idiopathic VTE 180, secondary VTE 185) were followed up for 60±74 months (range 1-666). In this period 29 recurrences were observed (7.9%). Factor VIII levels were 176.6±52.5 and 152.2±55.7 U/dL in patients with and without recurrence respectively. Factor VIII levels >150 U/dL were associated to recurrence [univariate analysis: OR 2.48 (95%CI 1.03-5.94), *p*=.042; multivariate analysis adjusted for age, sex and thrombophilic risk factors: OR 2.92 (95%CI 1.08-7.90), *p*=.035]. A significant interaction between elevated factor VIII and homocysteine levels in increasing the risk of recurrence was also found [OR 5.13 (95%CI 1.51-17.44), *p*=.009]. Among the 180 patients with a first idiopathic VTE episode (age 59±16 yrs), 20 had a recurrence (11.11%) (males 13;females 7) after a follow-up of 46±67 months (range 1-666). Factor VIII levels >150 U/dL were associated to recurrence at univariate analysis [OR 2.97 (95%CI 1.00-8.77), *p*=.049] but not after adjustment for age, sex and thrombophilic risk factors. On the contrary, the association of increased factor VIII levels and hyperhomocysteinemia was confirmed to be independently related to recurrence also in this group [OR 3.84 (95%CI 1.29-11.41), *p*=.016]. None of the other thrombophilic risk factors was independently associated to recurrence. *Conclusions.* These results: 1) confirm that increased factor VIII levels are a risk factor for VTE recurrence; 2) underline the relevant role of the combination of high factor VIII levels with hyperhomocysteinemia in enhancing the risk of recurrence after a first either idiopathic or secondary VTE episode; 3) question the role of an extensive thrombophilic screening to stratify the risk of VTE recurrence in the single patient.

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I AM HEREDITARY: THE PATIENT'S VIEW OF HAVING THROMBOPHILIA SCREENING

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Appropriateness of thrombophilia screening is debated for the unclear clinical and economic implications. Patients' psychological effects are also questioned. The aim of this study was to explore the subjective implication of having a thrombophilia screening through the patient's eyes. Because of the heuristic goal, a qualitative method was used through semi-structured tape-recorded interviews and transcripts analysis. Differently from quantitative research, in the qualitative approach the researcher's task is to make sense of the narrative experience of a small, diversified sample observed in-depth. The analysis is performed by developing thematic categories from the text, testing and refining them against hypotheses (BMJ 311:182-4, 1995). Fourteen patients out of 40 previously referred for thrombophilia screening accepted to be interviewed (4 men, 10 women, mean age 59 years, range 24-76;10 negative, 4 positive; interviews' mean length 28.9', range 12.47'-44.08'). Two main themes were identified: 1. *I am hereditary*. A complex frame seems to be built up around the test, perceived as a generic one for 'genetic inheritance'. The test seems to link together all the patient's experience of illness, the 'obviously' tiring therapy, and the family context, in particular the children. 2. *It is much better to know* because, patients suggested, to know is to care. The thrombophilia screening results seem to have a different destiny: often they may be forgotten; they may stimulate further knowledge, or naïve ideas about the disease. The communication about results seems to cause a deep contradiction: have I to face a troubling or a trivial disease? Results suggested that our patients did not perceive anxiety after the tests, but mainly a sense of confusion. Because the test is connected by patients to many life aspects, confusion gets pervasive and important to consider. Further studies could focus better this issue through analysing videoed doctor-patient encounters while communication of the test results occurs.

Venous thromboembolism: prophylaxis and therapy

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MANAGEMENT OF PATIENTS WITH ACUTE VENOUS THROMBOEMBOLISM FINDINGS FROM THE RIETE REGISTRY

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Background. Guidelines for the treatment of venous thromboembolism (VTE) are mainly based on randomized controlled trials, but a number of patients with VTE are excluded from trials due to co-morbidities. Thus, treatment regimens derived from clinical studies may not be suitable for all patient groups. *Objectives.* The Registro Informatizado de la Enfermedad TromboEmbolica (RIETE) was initiated in March 2001 to record current clinical management of VTE. It is a multicenter, observational registry designed to gather and analyze data on treatment patterns and clinical outcomes in consecutive patients with symptomatic, objectively confirmed, acute venous thrombosis or pulmonary embolism. Its aim is to provide information on the Internet to help physicians to evaluate treatment options in different patient groups, aiding treatment selection. *Methods*. Centers enroll all consecutive patients with symptomatic, objectively confirmed VTE. Treatment and outcome data are recorded. A minimum 3-month follow-up period is required. *Results.* 14,416 patients have been so far enrolled in RIETE. 3,303 of them (23%) had at least one reason to be excluded from clinical trials: recent bleeding (2.5%); renal insufficiency (14%); thrombocytopenia (1.1%); abnormal prothrombin time (8.7%) or pregnancy (0.6%). During the 3-month follow-up period the rates of fatal pulmonary embolism (PE) (odds ratio: 4.6; 95% CI: 3.5-6.0); and fatal bleeding (odds ratio: 3.7; 95% CI: 2.4-5.8) were significantly higher in these patients than in the remaining 11,113 patients. *Conclusions.* The expanding RIETE database provides data on the clinical outcome and prevention of PE in a real-world situation with an unselected patient population, in contrast to the rigorously controlled conditions of randomized clinical studies. It can, therefore, provide insights into the natural history of PE, and it can help to identify practices for providing prophylaxis to at-risk patients, and improved treatment of VTE, especially for those patients not usually included in randomized clinical trials.

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INDOBUFEN PREVENTS TISSUE FACTOR EXPRESSION IN HUMAN MONOCYTES THROUGH A THROMBOXANE-DEPENDENT MECHANISM

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Background. Indobufen is a reversible inhibitor of platelet cyclooxygenase (Cox) activity, thereby suppressing thromboxane (Tx) synthesis. It is effective in a broad spectrum of prothrombotic conditions ranging from graft occlusion after CABG surgery, to restenosis after carotid endarterectomy, to thromboembolic events in patients with heart disease, and to intermittent claudication. More recently the ability of indobufen to suppress the enhanced Tx biosynthesis in a subset of episodes of platelet activation during the acute phase of unstable angina has been highlighted. This effect, which is not shared by aspirin, has been attributed to the inhibition of the inducible Cox isoform (Cox-2), which is expressed by monocytes in response to a local inflammatory milieu. *Aim.* To assess whether indobufen affects tissue factor (TF), the main initiator of thrombogenesis *in vivo*, and to investigate the relationship between Cox-derived products and TF. *Methods.* Human monocytes were obtained from peripheral blood of healthy donors. TF was evaluated as procoagulant activity (PCA) in monocyte lysates. TF protein and mRNA levels were determined by Western blot and RT-PCR analysis, respectively. Thromboxane B2 (TxB2) and prostaglandin E2 (PGE2) formation was measured in monocyte supernatant by immunoenzymatic technique. Cox-1 and Cox-2 protein level, tyrosine phosphorylation and mitogen activated protein kinase (MAP-kinase) activation were determined by Western blot analysis. *Results.* Indobufen prevents TF expression in human adherent monocytes exposed to LPS through alteration of TxB2/PGE2 ratio that occurs through reduction of Tx but not of PGE2

synthesis. Prevention of LPS-induced ERK1/2 phosphorylation is highlighted as the mechanism responsible for the anti-TF effect of indobufen. Conclusions: Indobufen down-regulates TF in monocytes. This novel activity, coupled with the antiplatelet effect, may add benefit for the use of indobufen in the management of atherothrombosis.

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HOME THERAPY FOR DEEP VEIN THROMBOSIS AND PULMONARY EMBOLISM IN CANCER PATIENTS

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Background. Outpatient treatment of deep vein thrombosis (DVT) has become a common practice in uncomplicated patients. Scanty data are presents in patients with comorbidity (such as cancer) or concomitant symptomatic pulmonary embolism (PE). Cancer patients with DVT are often excluded from home treatment because of high risk of bleeding and recurrent DVT. We tested the feasibility and safety of the Home Treatment (HT) program in cancer patients with acute Venous Thromboembolism (VTE). *Material and Methods.* Consecutive cancer patients having a confirmed episode of DVT or PE were treated as outpatients unless they required admission for other medical problems, were actively bleeding or had pain requiring parenteral narcotics. Anticoagulant treatment for VTE was based on Low Molecular Weight Heparin (LMWH) followed by warfarin or LMWH alone at therapeutic dosages. An educational program for patients was implemented during the index visit. *Results.* Over a period of 3 years, 207 patients with cancer and acute VTE (139 with DVT and 68 with PE) were evaluated; 36 (17.4%) of them had metastatic disease. Treatment with LMWH and warfarin was prescribed to 106 (51.2%) while LMWH alone to 102 (48.8%). One hundred and twenty-seven patients (61.3%) (91 with DVT and 36 with PE) were entirely treated at home. Reasons for hospital admission in the remaining patients (n. 80) were poor compliance [22, (27.5%)], concomitant serious illness [52 (65%)] and refusal of home-treatment [6 (7.5%)]. There were no differences between patients treated at home and those hospitalized with regard to gender, mean age, site of cancer, presence of metastases and choice of anticoagulants (Table). After 6 months, recurrent DVT, PE and major bleeding occurred in 6.5%, 5.5.% and 1.5% of patients treated at home, and 8.3%, 9.3% and 2% of those hospitalised. These differences were not statistically significant (p=0.58). Twenty-seven patients (33%) in the hospitalized group and 33 (26%) in the home-treatment group died as a consequence of neoplasm. *Conclusions.* These results indicate that, regarding cancer patients with acute DVT and/or PE, there is no difference between hospitalised and home-treated patients in terms of major outcomes.

Table 1.

	Standard in-hospital		*Home Therapy*		*p-value*
Number of patients	*48 DVT*	*32 PE*	*91 DVT*	*36 PE*	*n.s.*
Mean age (range)	68.6 (37-92)		61,5 (32-90)		n.s.
Males	45 (56.2%)		67 (52.7%)		n.s.
Proximal DVT	43 (89.5%)	6 (18.7%)	82 (90.1%)	7 (19.4%)	n.s.
Distal isolated DVT	5 (10.4%)	2 (6.2%)	9 (9.8%)	2 (5.5%)	n.s.
Symptoms of PE*	7 (12.5%)	-	8 (8.7%)	-	n.s.
Metastatic cancer	10 (20.8%)	7 (21.8%)	13 (14.2%)	6 (16.6%)	n.s.
Site of cancer, n (%)					
Gastrointestinal	33 (41.2)		56 (44.1)		
Genitourinary	21 (26.2)		26 (20.4)	n.s.	
Breast	26 (32,5)		24 (18.9)		
Lung	6 (7.5)		14 (11)		
Haematologic	4 (5)		7 (5.5)		
Mean time from cancer to VTE diagnosis	30.8 months		28,9 months		n.s.
Ongoing chemo-, or radio or hormone therapy, n (%)	32 (40)		43 (33.8)		n.s.
Co-morbidity	21 (43.7)	21 (65,6%)	55 (60.4%)	20 (55.5%)	n.s.
In-hospital stay	8±2 days		3.1 hours		<0.0004

** In DVT patients only*

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PREANALYTICAL VARIABLES IN ORALLY ANTICOAGULATED PATIENTS MONITORING: PLASTIC TUBES VERSUS GLASS TUBES

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Clinical laboratories are gradually replacing glass tubes with plastic tubes in order to reduce health hazards to the personnel. Differences between plastic and glass tubes have been reported for coagulation tests and clinically significant differences in INR values may alter the management of orally anticoagulated (OA) patients. We compared INR obtained in glass tubes and in plastic tubes from the same manufacturer in 172 patients treated with oral anticoagulants and in 26 control subjects. INR was measured 30 minutes and 120 minutes after venipuncture. In control subjects no differences were observed in INR values between plastic and glass tubes. In patients treated with oral anticoagulants a statistically significant difference was observed at 30 minutes (plastic INR 2.62± 1.02; glass INR 2.64±1.07 p=0.0016 paired t test). At 120 minutes INR value in OA patients slightly but significantly increased both in plastic tubes (2.66 vs 2.62) and glass tubes (2.68 vs 2.64). In conclusion INR is statistically lower in plastic tubes when compared to glass tubes in OA patients. This difference is not clinically relevant since it is not higher than 10%, a value established as a benchmark for clinically relevant INR differences. In our experience plastic tubes can be used for monitioring INR in OA patients.

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STATINS EFFECT ON PLASMA D-DIMER LEVELS

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Background. D-dimer is a marker of procoagulant state. In particular, elevated levels of D-dimer were identified as a marker of an increased risk of recurrent venous thromboembolism (VTE). Statins have been claimed to prevent VTE. To assess the effect of statins on plasma D-dimer levels, we performed a systematic review of the published literature. *Methods.* We systematically searched the MEDLINE and EMBASE databases up to December 2005 with the following search terms (MeSH and textwords): D-dimer, Statins, Hydroxymethylglutaryl-CoA Reductase Inhibitors, Fibrin Fibrinogen Degradation Products. *Results.* Of 40 articles identified with the initial search strategy, a total of 15 studies were finally included for the analysis. Seven studies demonstrated a significant plasma D-dimer level reduction on statins therapy; 4 studies, a non statistically significant reduction, mainly in dyslipidemic patients with no anamnestic thrombotic events and no clearly elevated D-dimer levels; and 4 others, a lack of effect: studies were all, but one, conducted on patients with normal D-dimer levels at baseline. Overall, a trend toward an effective reduction was mainly observed in patients with increased mean D-dimer levels at baseline. No study has been published on the effect of statins therapy on plasma D-dimer levels in patients with previous VTE. *Conclusions.* There is some evidence to support an effect of statins on D-dimer levels. Although the results of the available studies are conflicting, no effect was observed when mean D-dimer levels were initially normal. An antithrombotic effect of statins might be particularly evident when a procoagulant state is occurring, such as following acute vascular events.

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GRADUATED COMPRESSION STOCKINGS OR LOW-MOLECULAR-WEIGHT HEPARIN FOR PREVENTION OF DEEP VEIN THROMBOSIS AFTER KNEE ARTHROSCOPY. A RANDOMIZED CLINICAL TRIAL

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Knee arthroscopy (KA) is considered a procedure at low risk of venous thromboembolic complications. However, in the absence of prophylaxis, the incidence of venographically proven deep vein thrombosis (DVT)

after KA has been reported to be as high as 20%. We prospectively randomized 1976 consecutive patients undergoing KA to wear graduated compression stockings (GCS) for 7 days, to receive LMWH (nadroparin, 0.4 ml subcutaneously o.i.d) for 7 days or LMWH for 14 days. Patients belonging to the first two groups underwent colour-coded-Doppler ultrasonography (CCD) after one week, while patients belonging to the third group after two weeks, or earlier in case of suspected DVT. Patients with normal CCD test did not receive further prophylaxis and were followed-up clinically for 3 months. Four patients with superficial thrombophlebitis were excluded from statistical analysis and from follow-up because they were administered therapeutical doses of LMWH. DVT, as shown by CCD, developed in 32 of the 660 patients (4.8%; 95% CI, 3.4 to 6.7) randomized to GCS group, in 12 of the 658 (1.8%; 95%CI: 1.0 to 3.1) randomized to 7 days of LMWH, and in 15 of the 658 (2.3%; 95%CI: 1.3 to 3.6) randomized to 14 days of LMWH. The difference between the GCS group and each of the two groups randomized to LMWH prophylaxis was statistically significant. Major bleedings were experienced by 1, 2, and 1 patients, respectively. None of 1913 patients with a normal CCD died or experienced symptomatic thromboembolic events during the 3-month follow-up period. We conclude that one week of fixed-dose LMWH is more effective than GCS for prevention of DVT arising after KA, without increasing the bleeding risk. Prolonging prophylaxis beyond the first week does not improve patients outcome.

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EFFECT OF BLOOD SALVAGE AND RE-INFUSION ON VTE COMPLICATIONS IN PATIENTS UNDERGOING ELECTIVE MAJOR ORTHOPEDIC SURGERY

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Introduction. In patients undergoing TKR, salvage and re-infusion of unwashed blood induces an activation of blood coagulation. The aim of this study was to assess whether blood salvage and/or blood transfusion were associated with an increase of VTE risk in patients undergoing major orthopedic surgery. *Methods.* Patients undergoing elective TKR and THR between January 2002 and September 2004 were enrolled in the GLORY registry, a large prospective clinical outcome-based study. Data on clinically overt VTE were collected at discharge and at 3 months thereafter, in 68 hospitals located in 11 countries. *Results.* Of 8952 patients enrolled, 4797 (53.6%) had TKR and 4155 (46.4%) THR. Blood salvage was used in 2227 patients (24.9%), either alone (n=694, 7.8%) or in association with blood transfusion (n=1533, 17.1%). Blood salvage alone was used in 572 TKR (11.9%) and in 122 THR patients (2.9%). Unwashed blood was re-infused in 1763 (82.6%) and washed blood in 371 patients (17.4%). LWMW prophylaxis was given in 75% of major orthopedic surgery patients. A higher incidence of in-hospital VTE was found in patients receiving blood salvage alone or blood transfusion alone than in patients receiving neither (2.5% and 2.2% respectively, versus 1.1%; $p<0.01$). A higher cumulative incidence of in-hospital and post-discharge VTE was found in patients undergoing blood salvage alone than in patients receiving neither (5.7% versus 1.7%, $p<0.001$). This was mainly accounted for by TKR patients. A higher incidence of in-hospital VTE was found in patients receiving re-infusion of washed blood than in patients receiving unwashed blood (TKR&THR: 5.1% versus 0.6%, $p<0.001$; TKR: 8.8% versus 0.8%, $p<0.001$). No difference in bleeding was observed among the groups. *Conclusion.* Re-infusion procedures are used in approximately 25% of patients undergoing elective major orthopedic surgery. Blood salvage and blood transfusion are associated with VTE in these patients.

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CEREBRAL SINOVENOUS THROMBOSIS IN CHILDREN: A SINGLE CENTRE PROSPECTIVE STUDY

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Introduction. Cerebral sinovenous thrombosis (SVT) in children is a serious disorder, and information is needed about its prevention and treatment; etiologies are many and multiple risk factors coexist. Aim of the present study was to evaluate genetic and acquired prothrombotic factors, efficacy and safety of anticoagulant treatment and outcome in

Table 1 (P150). Clinical characteristics, thrombophilia, etiology, treatment and outcome of children with cerebral sinovenous thrombosis.

Sex	Age (years)	Site	Simptoms	Prothrombotic factors		Underlying disease	Therapy	Outcome
				Number	Type			
M	9	right transverse sinus, longitudinal sinus, jugular vein	headache, focal signs, lethargy, vomiting	>2	C677T, G20210A, antiphospholipid antibodies, Protein S deficiency	appendicitis	LMW, oral anticoagulants +6 month	scomplete recanalization, no sequelae
M	75 days	right sigmoid sinus	seizures	0		otomastoiditis, patent forame ovale	LMWH +2 months	complete recanalization, no sequelae
M	9	right sigmoid sinus	headache, lethargy	1	Lp(a)	otomastoiditis	ASA +6 months	partial recanalization, no sequelae
F	4,5	right sigmoid sinus	headache, vomiting	0		otomastoiditis	ASA +6 months	complete recanalization, no sequelae
M	6,8	right sigmoid sinus	headache	1	antiphospholipid antibodies	otomastoiditis	ASA +6 months	partial recanalization, no sequelae
M	3	left sigmoid sinus, jugular vein	headache, lethargy, vomiting	0		otomastoiditis	ASA +3 months	complete recanalization, no sequelae
F	11	left transverse sinus	headache, focal signs, lethargy, vomiting	0		none	ASA +3 months	complete recanalization, no sequelae
F	4	left transverse sinus	headache, focal signs, lethargy	1	antiphospholipid antibodies	none	ASA +3 months	complete recanalization, no sequelae
M	3	left transverse sinus	focal signs, vomiting	1	antiphospholipid antibodies	otomastoiditis	ASA +3 months	complete recanalization, no sequelae
M	13	left transverse sinus, left internal jugular vein	headache, lethargy, vomiting	2	Lp(a), Protein S deficiency	policytemia vera	LMWH, continuous oral anticoagulants	partial recanalization, neurologic sequelae
M	5	left transverse/sigmoid sinus	headache, vomiting, seizures	0		ALL, chemotherapy,	LMWH +6 months central venous line	partial recanalization, no sequelae
F	5	transverse sinus	headache	0		none	ASA +6 months	complete recanalization, no sequelae
M	4	sigmoid sinus	headache, vomiting	0		otomastoiditis	ASA +6 months	complete recanalization, no sequelae
M	3	left transverse/ sigmoid sinus, longitudinal sinus, internal jugular vein	headache, vomiting	0		otomastoiditis	LMWH +2 months	complete recanalization, no sequelae
M	70 days	rectus sinus	seizures	2	C677T, G20210A	sepsi	LMWH +3 months	complete recanalization, no sequelae

children with SVT. *Patients and Results.* we prospectively studied all children with a clinical and radiological (angioTC) diagnosis of SVT observed at a single Center between January 1st 2000 and December 31st 2005. Overall patients were 15 (11 M, 4 F) with median age 4,5 years (range 70 days - 13 years) . In 10/15 (66,6%) SVT occurred after acute infective disease (local otomastoiditis in 8 and systemic in 2, respectively); in 2 cases (13,3%) underlying disease was respectively policytemia vera, and acute lymphoblastic leukemia on L-asparaginase and central catheter); no underlying disease in the remaining 3 (20%). Thrombophilia screening performed in all patients showed hereditary combined prothrombotic factors in 4/15 (26,6%) and acquired factors in 4/15 (26,6%; 1 associated with congenital thrombophilia). *Treatment.* 2/8 (25%) SVT post-otomastoiditis received short-term anticoagulation and 6/8 (75%) acetylsalicilic acid. Among the remainig patients 4/7 (57%) received long-term anticoagulation. One patient experienced nasal bleeding requiring transfusion. Complete recanalization was observed in 11/15 (73,3%; 6/8 post-otomastoiditis); among patients with partial recanalization thrombophilia was present in 3/4 (75%). Only 1/15 (6,6%) had neurological sequelae, and no recurrence was observed (median follow-up 39 months, range 12-75). (Table 1). *Discussion.* cerebral SVT in children is mainly secondary to underlying diseases, although associated hereditary thrombophilia is frequent. Thus a complete screening is recommended in all patients. Outcome is usually good with low recurrence rate. Long term anticoagulant therapy is highly recommended in patients with multiple prothrombotic factors. Only a short term course might be suggested for SVT following otomastoiditis and without thrombophilia, in order to avoid bleeding risk.

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THE ACUTE PHASE OF DEEP VENOUS THROMBOSIS OF THE LEGS: THE COURSE OF D-DIMER, FACTOR VIII AND THROMBOTIC BURDEN IN THE FOLLOW-UP (THE FUTA STUDY)

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Background. Few data are available on the natural course of D-dimer in relation to the thrombotic burden and levels of FVIII in the first months of treatment of proximal deep vein thrombosis (DVT) of the lower limbs. The aim of the study was to evaluate the course of D-dimer (D-d) in relation to the thrombotic burden and FVIII in patients with a first episode of proximal DVT of the lower limbs. Experimental design: This is a prospective single center study in which a cohort of subjects with a proximal DVT of the lower limbs were enrolled from the day of diagnosis with a follow-up of 180 days. A complete echo-doppler examination of the deep vein system of the lower limbs was conducted on the day of diagnosis (D0) and 7 (D7), 30 (D30), 90 (D90) and 180 days (D180) afterwards to establish the thrombotic burden. The thrombotic burden was defined according to a score which considered the number of districts with thrombi and the degree of occlusion. On the same days blood samples were taken for measuring D-d (Vidas D-dimer, BioMerieux,France, cut-off value: 500 ng/mL) and FVIII (chromogenic assay). *Results.* 27 patients have been enrolled so far (M/F: 30/18; mean age: 63,2; range: 21-81) with a complete follow-up in 12, a 90 day follow-up in 21 and a 30 day follow-up in 23. The mean levels of D-d decreased over time from 3.66 ug/mL at D0 to 2.86 ug/mL at D7, 0.59 ug/mL at D30, 0.23 ug/mL at D90 and 0.6 ug/mL at D180 and they were normal in 20/22 of patients at D90. The thrombotic burden also decreased over time and it remained significant in 6/21 (28%) of patients at D90. The levels of D-d were abnormal in only 1 of 6 patients with a significant thrombotic burden at D90. FVIII levels remained unchaged over time.

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EFFECTS OF DIFFERENT CUT-OFF VALUES OF TWO QUANTITATIVE D-DIMER (DD) METHODS TO ESTABLISH DURATION OF ORAL ANTICOAGULATION TREATMENT (OAT) AFTER A FIRST IDIOPATHIC EPISODE OF VENOUS THROMBOEMBOLISM (VTE)

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The PROLONG study has shown that continuing OAT in patients with abnormal D-dimers results in a significant reduction of VTE recurrence. However, in the PROLONG study a qualitative DD assay was used; quantitative assays might lead to some advantage by using different cut-off values. We measured DD by the VIDAS D-dimer (Biomerieux) and Hemosil D-dimer HS (Instrumentation Laboratory) in frozen plasma aliquots sampled 30±10 days after OAT cessation in 321 patients [173 males, median age: 67 Y (20-84y)] enrolled in the PROLONG study; patients who resumed OAT were excluded. The primary outcome was the composite of recurrent venous thromboembolism and major bleeding. During follow-up (542 y), 25 patients had recurrent VTE (7.8%, 4.6% person-years). The results obtained are similar for the two tests evaluated (Table). By increasing the cut-off levels the number of patients with altered DD (to be anticoagulated) decreases, the absolute number of recurrences decreases though their percentage increases, the NNT value decreases. Though the absolute number of unpreventable recurrences raises, their percentage in the population with normal DD does not change. In conclusion, the use of quantitative DD assays poses the problem of how to choose the the optimal cut-off value.

Table.

	N° of pts with altered DD altered DD	*N° of recurrence in pts with*	*Number needed to treat pts normal DD*	*N° of recurrence in pts with*
VIDAS N= 317				
300 ng/mL	241 (76.0%)	23 (9.5%)	10.5	2 (2.6%)
500 ng/mL*	160 (50.5%)	17 (10.6%)	9.4	8 (5.1%)
1000 ng/mL	72 (22.7%)	13 (18.0%)	5.5	12 (4.9%)
Hemosil HS N= 304				
150 ng/mL	184 (60.5%)	20 (10.9%)	9.2	5 (4.2%)
250 ng/mL *	99 (32.6%)	13 (13.1%)	7.6	12 (5.9%)
350 ng/mL	61 (20.1%)	12 (19.7%)	5.1	13 (5.3%)

**cut-off value recommended for VTE exclusion*

P153

PHARMACODYNAMIC OF LOW MOLECULAR WEIGHT HEPARIN (LMWH) IN OBESE SUBJECTS UNDERGOING BARIATRIC SURGERY: A PROSPECTIVE RANDOMISED STUDY COMPARING TWO DIFFERENT DOSES OF PARNAPARIN

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The optimal LMWH dose to prevent venous thromboembolism (VTE) complications after surgery in obese subjects remains controversial. Aim of this study was to evaluate the pharmacodynamic parameters of two doses of parnaparin administered to severe obese subjects (BMI >36) undergoing bariatric surgery to prevent VTE complications. Patients were enrolled in a multicentre, randomised, open, pilot study and were randomised to receive 4250 IU/day [n=20; 17 females; median age: 40 y (26-56); median BMI: 46.7 Kg/m^2 (36.5-56.7)] or 6400 IU/day [n=11; 11 females; median age: 48 y (27-63); median BMI: 43.1 Kg/m^2 (35.1-49.7)] of parnaparin s.c. for 9±2 days. The pharmacodynamic effects of parnaparin were analysed by measuring the anti-Factor Xa activity (anti-Xa; Instrumentation Laboratory) on day 1 (12 h after the first parnaparin injection), day 4 and day 6 after surgery [before (T0) and 4 h (T4) after parnaparin injection]. The anti-Xa levels (IU/mL) are reported in the Table (median and range).

Table.

	Day 1	*Day 4*		*Day 6*	
		(T0)	*(T4)*	*(T0)*	*(T4)*
4250 IU/day	0.041 (0.000-0.281)	0.014 (0.000-0.082)	0.198 (0.086-0.448)	0.000 (0.000-0.059)	0.231 (0.106-0.453)
6400 IU/day	0.071 (0.000-0.557)	0.008 (0.000-0.123)	0.428 (0.284-0.542)	0.026 (0.000-0.057)	0.523 (0.361-0.639)

In 91.4% of subjects receiving 4250 IU/day the anti-Xa levels were in the range 0.1-0.4 IU/ml at peak. Higher anti-Xa levels were observed in subjects receiving 6400 IU/day; indeed, in 83.3% of these subjects the

anti-Xa levels were greater than 0.4 IU/ml at peak. The anti-Xa levels measured 4 hours after injection at day 4 and 6 were not statistically correlated with BMI for both parnaparin dosages [Spearman correlation coefficients: –0.148 (p=0.396) and –0.392 (p=0.107) for 4250 or 6400 IU/day dose, respectively]. The dose of 4250 IU/day seems adequate to achieve prophylactic anti-Xa levels in obese subjects with a BMI of up to 50 Kg/m^2. On the other hand, most of the patients receiving 6.400 IU/day show anti-Xa levels higher than the recommended prophylactic values.

P154

SAFETY OF DALTEPARIN FOR VENOUS THROMBOEMBOLISM PROPHYLAXIS IN ELDERLY MEDICAL PATIENTS WITH RENAL INSUFFICIENCY: A PILOT STUDY

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Objectives. Prospective, cohort study to verify the incidence of dalteparin bioaccumulation (measured using anti-Xa levels), and bleeding during thromboprophylaxis with dalteparin in elderly patients with renal failure and acute medical illness. *Materials and Methods.* 115 consecutive patients (59 males, 56 females, mean age 83 8 y) admitted with acute medical illness were considered for the study. Eligible patients had none of the following: hepatic failure (INR >1.7), platelet count <100,000/mL, chronic use of warfarin, or an ongoing requirement for anticoagulation. Ninety-three patients judged to be at high thromboembolic risk (patients older than 75 y, with active cancer or previous venous thromboembolism) received dalteparin 5,000 IU daily; the other 22, considered at low risk received 2,500 IU daily. Dalteparin was given for 6 days. Anti-Xa activity was determined on day 1, before the first dalteparin dose, and on day 6, 4 hours after its administration. A complete compression ultrasound examination of leg veins was performed at admission and at discharge. The primary study end point was the anti-Xa activity levels at day 6. Secondary end points were: the occurrence of hemorrhage during the in-hospital stay, objectively confirmed symptomatic (limb pain and swelling) DVT, and objectively confirmed asymptomatic DVT. *Results*. There were no major bleeding events; no symptomatic thromboembolic events and no asymptomatic DVT were recorded (95% confidence interval 0 to 2.5%). Of the one hundred and fifteen patients, three (2.7%) had minor hemorrhage (95% confidence interval 0.6 to 6.7%). In all three cases anti-Xa activity was undetectable. There were no cases of venous thromboembolism. There was also no relationship between the degree of renal impairment and the peak anti-Xa heparin level at day 6: mild renal impairment, 0.030±0.087 IU/mL vs moderate renal impairment, 0.033±0.076 IU/ml vs severe renal impairment, 0.048±0.084 IU/mL, p=0.72. Anti-Xa activity never reached the therapeutic level of 0.5 IU/mL. *Conclusions.* Dalteparin thromboprophylaxis in elderly patients admitted with an acute medical illness who have renal impairment is associated with a low risk of both bioaccumulation and bleeding. Larger studies are required to validate our observations.

P155

CEREBRAL SINOVENOUS THROMBOSIS IN CHILDREN

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Background. Cerebral sinovenous thrombosis (CSVT) is not well understood in children. It has been considered a rare disorder in children with a reported incidence of 0.67×100.000/year. However it is now increasingly recognized since more sensitive non-invasive neuroimaging techniques as Magnetic Resonance Angiography (MRA) became available. Aim: we report clinical features, neuroimaging results, treatment and outcome of the cases observed at the Children's Hospital of Padua University. *Results.* Nine cases of cerebral sinovenous thrombosis were studied (8 male, 1 female). Clinical onset was in the neonatal age in three and between 2 and 6,3 years in the others. All neonates presented with seizures, while the other cases had signs of increased intracranial pressure. Diagnosis was based on MRA; only in two patients CT was abnormal. All neonates presented one or more prothrombotic abnormalities (1 Leiden V Factor, 1 Protein C and S reduction, 1 Protein C reduction). In post-neonatal age risk factors were present in all 6 patients (ATIII, PC, PS deficit and LAC in 1; otitis media/mastoiditis in 3; nephrotic syndrome in 1, dehydratation in 1). Six out of nine patients received anticoagulant therapy (LMWH), while two patients (treated before 2001) had aspirin. No bleeding was observed. Neurological sequelae were observed in 3 out of 8 cases (3/3 neonates), 1 patient, belonging the post-neonatal age of onset group, died; all these patients presented prothrombotic disorder. Conclusion: SVT is a potentially serious disorder in pediatrics, particularly in the neonatal age. We observed neurological sequelae in all cases with prothrombotic abnormalities; such an unfavourable outcome was more frequent in neonatal patients. Further studies are needed to assess the role of both the early insult to immature brain and thrombophilia in the outcome of CSVT. Antithrombotic therapy with LMWH seems to be free of risks in paediatric age.

Cell biology of hemostasis

P156

RAS ASSOCIATED WITH DIABETES (RAD) MODULATES INDUCIBLE NITRIC OXIDE SINTHASE (INOS) ACTIVITY IN VASCULAR SMOOTH MUSCLE CELLS (VSMC) FROM DIABETIC RATS

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Objectives. In diabetes, inflammation and increased oxidative stress could induce iNOS expression contributing to vascular damage. Rad, a G protein overexpressed in diabetic animals, binds calmodulin (CaM) in a Ca2+-dependent manner. Since Ca2+-CaM can modulate iNOS activity, aim of this study was to investigate the relationships between iNOS activity and Rad expression in vSMC from DR. *Methods and Results.* We measured Rad mRNA levels (Real Time PCR), iNOS expression (RT-PCR, Western Blot) and activity (radiometric technique) and nitrotyrosine levels (immunofluorescence) in cultures of aortic vSMC from 10 diabetic (90% pancreatectomy, DR) and 10 control (sham surgery, CR) rats, after 24 hrs incubation with 20 μg/mL LPS. LPS increased iNOS mRNA and protein levels to the same extent in CR and DR, while iNOS activity was about 7 folds greater in DR. Notwithstanding increased iNOS activity, cGMP levels were not different between DR and CR cells: DR cells, however, exhibited increased nitrotyrosine levels. As to the effect of LPS on RAD expression, exposure to LPS was followed in CR by a rapid increase in RAD, whereas in DR RAD mRNA levels decreased significantly. *Conclusions.* In DR increased CaM availability, possibly due to inhibition of RAD expression, may increase iNOS activity. However, the lack of i.c. cGMP increase and the enhanced nitrotyrosine generation in DR suggest that most of the NO produced in pro-oxidant milieu gets converted into peroxinytrite in these cells. Rad potential role in modulating iNOS activity in diabetes might provide new insight in the mechanisms linking diabetes and atherosclerosis.

P157

PROTEOME OF ENDOTHELIAL CELL-DERIVED PROCOAGULANT MICROPARTICLES

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Microparticles are small membrane vesicles that are released from cells upon activation or during apoptosis. Cellular microparticles in body fluids constitute a heterogeneous population, differing in cellular origin, numbers, size, antigenic composition and functional properties. Microparticles support coagulation by exposure of tissue factor (TF), the initiator of coagulation *in vivo*. Moreover, microparticles may transfer bioactive molecules to other cell, thereby stimulating them to produce cytokines, cell-adhesion molecules, growth factors and tissue factor, and modulate endothelial functions. However, the characterization of protein composition of microparticles have been poorly defined. This study describes the protein composition of endothelial cell-derived microparticles (EMPs) using a proteomic approach. Mass spectrometric analysis indicated the presence of newly described protein such as metabolic enzymes, proteins involved in adhesion and fusion processes, members of protein folding event, cytoskeleton associated proteins and nucleosome. In conclusion circulating EMPs behave as an actual storage pool, able to disseminate blood-borne TF activity and other bioactive effectors, as confirmed by our experiments showing an increased procoagulant activity of endothelial cells exposed to EMPs.

P158

PAR-1 AND PAR-2 ACTIVATION MEDIATES TISSUE FACTOR INDUCTION IN ENDOTHELIAL CELLS THROUGH A REDOX-SENSITIVE SIGNALING PATHWAY

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Objective. To elucidate the mechanisms whereby activation of protease-activated receptor 1 and 2 (PAR1 and PAR2) stimulates tissue factor (TF) induction in human endothelial cells (HUVEC). Methods: TF was evaluated as procoagulant activity in HUVEC lysates. PAR1, PAR2 and TF mRNA levels were determined by RT-PCR. Intracellular reactive oxygen species (ROS) generation was detected by flow cytometry. Tyrosine phosphorylation, MAPK activation, Cox-2 and eNOS protein levels was assessed by Western analysis. *Results.* HUVEC express both PAR1 and PAR2 mRNA. Receptor occupancy by PAR1 and PAR2 agonist peptides resulted in TF expression and ROS overproduction. Increased TF activity was prevented by antioxidants through a mechanism that involves tyrosine phosphorylation. TF induction by agonists for both PAR1 and PAR2 is mediated by ERK1/2, p38 MAPK and PI-3K activation. PAR1 and PAR2 agonists also up-regulated eNOS and Cox-2 enzymes, whose metabolites do not participate in PAR-induced TF expression. A divergence in the signaling pathway downstream PAR1 and PAR2 was found in the coupling of PAR activation to the G protein subunit Gαi/o. *Conclusions.* Results from this study entail common and divergent signaling pathways emanating from PAR1 and PAR2 activation that lead to TF induction and underline a role of ROS as key mediators in the appearance of an immediate procoagulant response by endothelial cells.

P159

GENE EXPRESSION PROFILE INDUCED BY PHYSICAL EXERCISE IN RATS

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Physical exercise training is a known protective factor against cardiovascular morbidity and mortality. Nevertheless, the underlying specific molecular mechanisms still remain unexplored. In this study we aimed to investigate the gene expression profile induced by moderate exercise training on left ventricle (LV) obtained from heart of exercise-trained (n=10) and sedentary control (n=10) rats. Rats in the training group exercised on a treadmill running at 20 m/min and 0-degree gradient for 1 h/day, 7 days/week, for 11 weeks. They were sacrificed 24 h after exercise. We used Affymetrix microarray technology to identify LV gene expression profile in response to exercise. RNA extracted from left ventricles was hybridized to Affymetrix 230 2.0 GeneChip rat genome array. Of the 28,000 genes represented on the GeneChip, a total of 120 genes displayed an altered expression associated with the exercise training. 101 out of 120 genes were up-regulated and 19 out of 120 genes were down-regulated in LV from exercise-trained rats with respect to sedentary control rats. Gene ontology analysis showed an alteration of genes involved in the following biological processes: response to stress, muscle development, cytoskeleton organization and biogenesis, ion and protein transport, regulation of transcription, modulation of apoptosis, cell proliferation and adhesion and cell-cell signalling. Our data may contribute to the comprehension of the molecular mechanisms responsible for the cardiovascular protective role of moderate physical exercise training. Further validation and functional studies are required in order to better evaluate the role of the genes emerged from the microarray analysis.

P160

MUTATION SCREENING ANALYSIS OF TGFBR1, TGFBR2 AND FBN1 IN PATIENTS WITH MARFAN SYNDROME, FAMILIAL TAA AND AAA.

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Marfan syndrome is a dominantly inherited connective tissue disorder with an incidence of 1 in 5,000-10,000. Thoracic and rarely abdominal aortic aneurysms represent the main cause of morbidity. Mutations in FBN1 gene are the predominant cause of classic Marfan syndrome and are detected in 90% of cases. TGFBR1 and TGFBR2 are genes codifying for receptors of TGF-β. Mutations in TGFBR2 were identified in MFS, TAA and Loeys-Dietz-syndrome patients; the last disorder displays also mutations in TGFBR1. Aim of the study was to search for mutations in TGFBR2, TGFBR1 and FBN1 in aortic diseases. We performed a genetic study using heteroduplex analysis of gDNA by DHPLC and direct sequencing. 46 patients (43 MFS, 2 TAA and 1 AAA) were investigated. We detected FBN1 mutations in 27 MFS and 1 TAA patients. A novel mutation (20nt-duplication) in TGFBR1-Ex1 was identified in 1 MFS patient, who carried also a FBN1 mutation. A known polymorphism in

TGFBR1-Ex1 was investigated. TGFBR1-6A-allele consists in a deletion of three GCG triplets coding for 3 alanine within a polyalanine tract of TGFBRI. The 6A-allele causes TGF-β signaling perturbation in cancer patients. The 6A-allele was identified in 7 MFS and 1 TAA patients. The variations were screened in 230 controls. TGFBR1-6A-allele seems to associate preferentially to patients respect to controls, although the difference is not yet statistically significant. A higher number of cases will be required to confirm this association. The 20nt-duplication in TGFBR1-Ex1 suggests a pathogenetic function as modifier gene, since a FBN1 mutation is also present in our MFS patient. Until now, no pathogenetic mutations were detected in TGFBR2 gene in our cases. From literature, the percentage of TGFBR2 mutations related to MFS and TAA patients is 5%; for this reason a higher number of patients need to be analyzed to confirm the data in our population.

P161

MRNA EXPRESSION OF GENES INVOLVED IN ATHEROSCLEROTIC DISEASE

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Abdominal aortic aneurysm (AAA) and carotid artery disease (CAS) are two manifestations of atherosclerosis, but the molecular bases responsible for the different localization are not completely understood. Aims of the study were to identify, at systemic level by microarray technology, altered genes involved in pathophysiology of atherosclerosis and expression profiles that are highly correlated with these two atherosclerotic phenotypes. At this purpose we analyzed expression profiles in total RNA from whole blood of 10 patients affected by AAA, 10 patients with CAS and 20 controls comparable for age and sex. We determined the expression of 14,000 genes by two colours microarray technology. 93 genes showed altered expression levels between AAA patients and controls: 77 up-regulated and 16 down-regulated genes. As concerns CAS patients, 62 genes showed altered expression: 40 up-regulated and 22 down-regulated genes. 26 genes were similarly altered in both AAA and CAS patients. Gene ontology analysis showed an alteration of the following biological processes: oxygen transport, protein synthesis, cytoskeleton organization and lipidic metabolism. Our results may contribute to a better understanding of the genes and biological processes involved in the pathophysiology of atherosclerosis and to the identification of a disease profile able to characterize affected subjects.

Cancer and thrombosis

P162

THROMBELASTOGRAPHIC PROFILES IN ABDOMINAL SOLID TUMORS

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Quantification of the magnitude of thrombotic risk associated with malignancy and with anti-cancer therapy is indispensable to use anticoagulant drugs which selectively interfere with hemostatic mechanisms protecting patients from VTE. However, none of activation coagulation markers has any predictive value for the occurrence of the thrombotic events in one individual patients. Current clotting methods can't reveal the overall dynamic clot formation; in contrast thrombelastographic methods specifically assess overall coagulation kinetics. The objective of the study was to evaluate wether an activation of coagulation as eventually revealed by ROTEM thromboelastometry may be correlated to higher risk for VTE in surgical neoplastic patients. Fifty patients with carcinoma of the digestive tract in preoperative period (23 M, 27 F) aging 62±12years and 130 healthy subjects (65 M,65 F) were studied. A thromboelastometric method based on thrombelastography after Hartert was employed. Measurements were performed on ROTEM Coagulation Analyzer (Penthapharm Ltd, Munich, Germany,distributed in Italy by Dasit, Milan). The continuous coagulation data from 50 min course were transformed into dynamic velocity profiles of WB clot formation. Among females there were higher MaxVel and AUC; t-MaxVel were shorter compared to males. The MaxVel was increased (cancer patients: females 20.7±6.6 mm 100 s, males 19.6±6 mm 100 s vs. 15.0±3 mm 100 s / 13±2 mm 100 s in controls (p=0.0001, respectively). The t-MaxVel was shortened (cancer patients : females 68.9± 14 s, males 81.9±28 s vs 117.5±15 s / 127.3±29 s in controls (p= 0.0001). The AUC was increased (cancer patients : females 6604±898 mm 100, males 6108±569 mm 100 vs 5722±479mm 100 / 5651±463mm 100 (p=0.0001). Unlike other assays, measuring variations in a single component during coagulation, thrombelastographic method records a profile of real-time continuous WB clot formation. Changes in dynamic clot formation measured by ROTEM, in neoplastic patients before surgery, as an indirect measure of thrombin generation, may provide extensive informations on hemostasis for clinical practice where tests have to be simple and results need to be available rapidly.

P163

A PROSPECTIVE EPIDEMIOLOGICAL STUDY ON THE PREVALENCE AND ROLE OF ANTIPHOSPHOLIPID ANTIBODIES IN CANCER PATIENTS

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Aims of the study. 1)To define the prevalence of aPL in a cohort of consecutive patients with cancer at diagnosis, asymptomatic for venous or arterial thromboembolism. 2)To evaluate a possible role of aPL as risk factor for thromboembolic disease and overall survival. Three years of follow-up have been scheduled. *Patients and Methods.* Patients were enrolled at diagnosis: a) solid tumors : breast, colon-rectal, head-neck, lung or b) onco-haematological diseases : lymphoprolipherative diseases, myelodisplastic disease and acute leukaemia. All patients were screened for Lupus Anticoagulant; anticardiolipin antibodies (ACA) and anti β2 Glycoprotein I antibodies (IgG and IgM) were assayed by ELISA commercial kits. Laboratory tests were repeated after thrombotic events. Patients were visited or phoned every three months. If signs or symptoms suggested a venous occlusive disease, CUS or venous US, or Spiral TC were performed to objectively diagnose a vascular event. *Results.* Enrollement was started in february 2004 and was stopped in february 2005. 137 patients were enrolled, 100 female and 37 male, median age 61 (range 29-83). 15 patients had haematological diseases, 21 colon - rectal tumors, 77 breast cancers, 16 head-neck tumors and 8 lung cancers. In February 2006 mean follow up was 15 months/patient. aPLs

were found positive in 19,7% of patients. Patients with colon-rectal disease had the higher prevalence of aPL: 26,25%. During the follow up 9 patients had a thromboembolic event , and 16 patients died (1 for pulmonary embolism). Among patients with a thrombotic event, 1 out of 9 was aPL positive; 4 out of 16 deaths was aPL positive. *Conclusions.* High prevalence of aPL in patients with cancer was found. After a mean of 15 months of follow up no relationship was found between thromboembolic events and aPL. Thrombotic events was secondary to a triggering factor in 70% of case. Coumulative events (thrombosis + death) were similar in the two groups. Follow up is still ongoing for one more year.

P164

ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS WITH GASTRIC CANCER

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Aims. The antiphospholipid antibodies have been found in a large variety of malignancies but data about their association with gastric cancer are lacking. This study was addressed to investigate, in a group of gastric cancer patients, the plasma levels and the prevalence of the most common antiphospholipid antibodies to clarify their role in gastric cancer thrombophilia. *Methods.* 40 cancer patients with non-metastatic gastric adenocarcinoma (TNM stages: T1-3, N0-2, M0) and 56 control subjects were tested for the presence of the anti-cardiolipin (anti-CL) and anti-β2-glicoprotein I (anti-β2GPI) IgG/IgM/IgA antibodies. No individual had a history of previous thrombotic event or infectious complications at the time of blood withdrawn. Antiphospholipid antibodies were assayed by ELISA commercial Kits. Normally distributed continuous variables were analysed using the Student's t test. To assess the normal distribution the Kolmogorov-Smirnov test on each sample data was applied. Non-normally distributed variables were analysed using the Mann-Whitney test. Correlation was assessed using the Pearson Correlation test. *Results.* In the cancer patients the IgA/IgG-anti-β2GPI antibodies mean levels were significantly higher than in the control group and highly significantly correlated with the IgM of both the anti-β2GPI and the anti-CL antibodies. The prevalence of antiphospholipid antibodies positivity was not significantly different between the groups. *Conclusions.* The higher IgA/IgG-anti-β2GPI mean plasma levels may identify a subset of cancer patients who are at high risk of developing thrombotic complication. Our findings seem then to suggest a further pathogenetic factor which may contribute to the hypercoagulability of our gastric cancer patients.

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COAGULATION FACTORS IX AND XI IN COLORECTAL CANCER PATIENTS

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There is evidence that high plasma levels of fibrinogen and coagulation factors (F) VIII, IX and XI are independent risk factors of venous thromboembolism. In our previous study, performed in cancer patients with non-metastatic gastrointestinal cancer, a significantly high levels of fibrinogen and FVIII was found. *Aim.* To determine, in colorectal cancer patients, the FIX and FXI plasma levels and their relationships with fibrinogen and FVIII to investigate their role in the pathogenesis of cancer thrombophilia. *Methods.* Fibrinogen, FVIII, FIX and FXI plasma activity was measured in 43 non-metastatic colorectal cancer patients and 56 matched control subjects. No one, in both group, had a history of previous thrombotic event. Coagulation factors activity was determined by coagulometric method. Normally distributed continuous variables were analysed using the Student's t test. To assess the normal distribution the Kolmogorov-Smirnov test on each sample data was applied. Non-normally distributed variables were analysed using the Mann-Whitney test. Correlation was assessed using the Pearson Correlation test. *Results.* FIX mean plasma levels were significantly higher than in the control group while no significant difference was found for the FXI. Moreover both FIX and XI were significantly and positively correlated with the FVIII plasma levels. *Conclusions.* Factor IX may be involved, as an acute-phase reactant, in the acute-phase response and then in the thrombophilic state of our group of patients suffering from non-metastatic colorectal cancer.

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ACUTE PHASE RESPONSE AND ANTIPHOSPHOLIPID ANTIBODIES IN COLORECTAL CANCER PATIENTS

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Aims. The acute-phase response is activated in cancer patients and the antiphospholipid antibodies(aPL)have been found in a large variety of malignancies. This study was addressed to investigate, in a group of colorectal cancer patients, the plasma levels and the prevalence of the most common aPL and their relationships with some plasma markers of inflammation, to better characterize some aspects of cancer thrombophilia. *Methods.* 83 cancer patients with non-metastatic colorectal adenocarcinoma and 56 matched control subjects were tested for the presence of the anti-cardiolipin (anti-CL) and anti-β2-glicoprotein I (anti-β2GPI) IgG/IgM/IgA antibodies, and of some acute-phase reactants, such as the fibrinogen, the factor VIII:C and the C4b-binding protein. Antiphospholipid antibodies were assayed by ELISA commercial Kits. *Results.* In the cancer patients the acute-phase reactants were significantly higher than in the control group as were the anti-CL IgG and the anti-β2GPI IgA antibodies mean levels. Moreover using the principal component analysis separately applied to the two set of variables, the two principal component resulted significantly and positively correlated (p=0.036). The prevalence of the aPL positivity was instead not statistically different between the groups. *Conclusions.* In patients with non-metastatic solid tumours there is an acute-phase response associated with higher plasma levels of the anti-CL IgG and the anti-β2GPI IgA antibodies that could represent a further pathogenetic mechanism for the thrombotic complications of the colorectal neoplastic disease.

P167

JAK2 MUTATION AND PRV-1 AND WT1 TRANSCRIPTION LEVELS IN PH NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS

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JAK2V617F mutation has been described in patients with polycythemia vera (PV) and in a subset of patients with essential thromocythemia (ET) and idiopathic myelofibrosis (IMF). Furthermore, PRV-1 over-expression has been found in many affected patients. RT PCR assessment of PRV1 and WT1 expression levels was performed in 330 patients [26 IMF, 156 PV, 122 ET, 16 Hypereosinophilic Syndromes (HES), 60 reactive conditions] as well as in 50 normal controls (NC). JAK2 mutation was also assessed in all patients. JAK2 mutation was found in 50% IMF, 93% PV, 55% ET, 6% HES but not in NC and in reactive conditions. PRV-1 over-expression was found in 84% IMF, 98% PV and 90% ET but not in HES. WT1 over-expression was found in 99% IMF, 45% PV, 42% ET and 100% HES. Low WT1 and PRV1 expression levels were found in NC and in reactive conditions. Mean WT1 transcription levels were: 306 copies in IMF; 155 in PV; 202 in ET and 161 in HES. Mean PRV1 transcription levels were: 144 copies in IMF; 423 in PV and 290 in ET. Both WT1 and PRV1 levels were higher in JAK2 mutated samples than in wild-type JAK2 samples. Increased PRV1 and WT1 levels, but not JAK2 mutation, were found in 34% IMF; WT1 over-expression, in the absence of both JAK2 mutation and WT1 over-expression, was found in 15% IMF. PRV-1 expression, in the absence of JAK2 mutation and WT1

expression, was found in 5% PV and 35% ET. WT1, but not PRV1 overexpression, was found in HES patients; JAK2 mutation was found only in 1 HES patient. This study shows that concomitant assessment of these molecular markers may be useful for a better diagnosis and patient stratification in this setting. Studies are in progress to correlate these data with clinical manifestations and prognosis.

P168

TUMOR CELL TISSUE FACTOR IS RELATED TO THE MALIGNANT PHENOTYPE: EVIDENCE FROM RETINOID-DIFFERENTED FRESH BLAST CELLS FROM ACUTE PROMYELOCYTIC (APL) AND NO PROMYELOCYTIC LEUKEMIA PATIENTS

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All-trans-retinoic acid (ATRA) downregulates the expression of the cellular procoagulant Tissue Factor (TF) *in vitro* and *ex vivo*. In a model of APL cell line NB4, this effect parallels the ATRA-induced cellular differentiation and is regulated principally through the retinoic acid receptor (RAR) α (Falanga et al. Blood, 1998). To understand whether TF of APL patient blasts is regulated by ATRA by a similar mechanism and whether its expression is related to the malignant phenotype, we have: 1. Evaluated the effect of ATRA (pan-RAR agonist) and synthetic retinoids selective agonists for RAR-α (Am580), RAR-β (CD2019) and RAR-γ (CD437) on TF expression and differentiation of APL blasts freshly isolated from bone marrow of 8 APL; 2. Compared these effects with those obtained by all retinoids on blasts from 3 patients with M5 acute myeloid leukemia, non- ATRA- differentiating leukemia. Blasts were incubated for 24h with 1 μmol/L of each retinoid and TF was characterized both as activity and antigen. Differentiation was evaluated as% increase in CD11b-positive cells by flow cytometry. The results (mean % inhibition) show that ATRA and RAR-α agonist AM580 significantly reduced TF activity and antigen (by 43% and 26%, respectively) of APL blasts. CD2019 and CD437 did not significantly affect APL blast procoagulants. Differentiation study demonstrated that in APL cells both ATRA (28.4% pos. cells) and Am580 (39.1% pos. cells) increased CD11b expression compared to control cells (12.5% pos. cells), while CD2019 and CD437 had no effect. Differently, the same treatment did not affect M5 blast TF, which remained constantly highly expressed, nor induced cellular differentiation. Therefore, in freshly isolated APL cells, ATRA confirmed to downregulate TF mainly through RAR-α. Importantly, this effect was selective for the APL phenotype and was dependent on the cellular differentiation and not on a direct effect of retinoids. Therefore TF represents a unique characteristics of malignant leukemic blasts.

P169

HEMOSTATIC ALTERATIONS IN A PEDIATRIC POPULATION WITH ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)

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Children with ALL are at increased thrombotic risk during remission induction therapy. Identification of a susceptible population is relevant for effective thromboprophylaxis. We started a multicenter, longitudinal, observational study in ALL children in order to identify: a) plasma haemostatic marker alterations at the onset of disease; b) possible effects on these markers of anti-tumor treatment; and c) possible risk marker(s) for symptomatic thrombosis.112 children treated according to the AIEOP ALL protocol 2000 by four AIEOP centres entered the study. At diagnosis (T0), and on days 24, 36, 52 of the protocol, the following plasma prothrombotic markers were evaluated: TAT; vWF-antigen and vWF-HMWM; TNF-α, IL-6 and PAI-1. Results are in Table as median (range) or mean±SD. Two thrombotic events (1 pulmonary embolism on day 35, and 1 cerebral venous thrombosis on day 22) occurred. Plasma marker analysis indicates coagulation and endothelial activation at T0, associated to inflammatory cytokines elevation. Significant ($p<0.05$) positive correlations were found between IL-6 and TNF-alfa, and IL-6 and vWF-Ag. All these markers significantly decreased during treatment. Differently, PAI-1, normal at T0, significantly increased until day 24 and 36, going back to baseline at the end of therapy. Therefore, inspite an overall improvement of hypercoagulability, a therapy-associated elevation of PAI-1, a recognized thrombotic risk factor, occurred. The low thrombotic rate did not allow to evaluate the predictive value.

Table.

Parameter	*T0*	*24th day*	*36th day*	*52nd day*	*p vs T0*
TAT (μg/L)	5.1 (1.2 - 300)	4.2 (1.4 - 60)	2.6 (1.4 – 42)	2.4 (0.8 – 19.5)	< 0.001
vWF:Ag (%)	117.5±37.3	109.2±34.8	110.2±34.2	108.2±24.4	NS
vWF:HMWM (%)	16.2±5.7	14.6±5.7	13.3±5.9	14.8±6.5	< 0.05
TNF-alfa (pg/mL)	15 (0.3 – 71.0)	6.8 (0 – 22.0)	8.0 (0.3 – 22.0)	6.4 (0 – 15.0)	< 0.001
IL-6 (pg/mL)	6.4 (0.3 – 272.0)	2.2 (0 – 94.0)	4.9 (0.4 – 40.0)	2.6 (0.1 – 66.0)	< 0.001
PAI-1 (ng/mL)	11.97±10.8	23.35±13.2	28.0±19.0	16.0±12.6	< 0.01

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FACTOR VIII LEVELS IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH THALIDOMIDE

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Venous thromboembolism (VTE) occurs in approximately 25% of patients with multiple myeloma (MM) receiving thalidomide and combined chemotherapy. The presence of prothrombotic risk factors in MM patients and the possible relationship with VTE occurrence is still controversial. Aim of this study was to investigate FVIII levels in patients with newly diagnosed MM, before and 4 months after thalidomide therapy, in relationship with MM status, response to therapy, and VTE occurrence. All patients received warfarin 1.25 mg/die as VTE prophylaxis during thalidomide treatment. 182 patients (M=100, median age 57 years) and 183 controls (M=97, median age 57 years) were examined. Baseline median FVIII levels were significantly higher in patients than in controls (2.22 IU/ml vs 1.61 IU/mL, respectively, $p<0.0001$). At baseline, 44% of patients had FVIII levels above the 90°percentile of the control group (2.39 IU/mL) and the prevalence did not significantly vary after the 4 month treatment (45.4%; $p=0.906$). The levels were higher in the 105 stage III (2.35 IU/mL) compared to the 77 stage I or II (2.03 IU/mL; $p=0.053$) MM patients. Median FVIII levels were not different in patients responders (77%) or not responders (23%) to therapy, both at baseline control (2.22 IU/mL vs 2.23 IU/mL, respectively, $p=0.853$) and at the 4 month control (2.36 IU/ml vs 2.10 IU/mL respectively, $p=0.9648$). During thalidomide treatment 18 patients (9.9%) experienced a VTE complication; 50% of them had high baseline FVIII levels (> 90° percentile), compared to 43.3% of those without VTE ($p=0.774$). In conclusion, FVIII levels were significantly higher in MM patients than in controls. Elevated FVIII levels seemed to be associated with more advanced disease stages. FVIII levels were not correlated with the responsiveness to the therapy. Moreover, elevated FVIII levels are not a risk factor for VTE occurrence in MM patients during treatment with thalidomide and combined chemotherapy.

P171

EVALUATION OF THE PRESENCE OF THROMBOPHILIC ALTERATIONS IN MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE

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Venous thromboembolism (VTE) has been observed in approximately 25% of patients with multiple myeloma (MM) receiving thalidomide and combined chemotherapy. A complete thrombophilic study was performed in patients with newly diagnosed MM, before and 4 months after thalidomide therapy to evaluate if the increased VTE risk was associated with the presence of thrombophilic alterations. All patients received warfarin 1.25 mg/die as VTE prophylaxis during thalidomide treatment. 185 patients (M=102, median age 57 years) and 183 controls (M=97, median age 57 years) were enrolled. At baseline, the prevalence of Factor V Leiden or G20210A prothrombin mutations was 3.2% and 2.7%, respectively; 21/185 (11.4%) patients showed low levels of antithrombin (AT) and/or protein C (PC) and/or protein S (PS) activity; 14/185 (7.6%) had an activated protein C resistance (APCR) not associated with Factor V Leiden mutation. Reduction in natural anticoagulants and acquired APCR were completely normalized after 4 months therapy in all patients but one. 17/185 patients (9.2%) experienced VTE during the 4 month treatment. The incidence of VTE was higher among patients with (27.3%) vs those without (7.6%; p=0.0987) thrombophilic polymorphisms. The presence of a polymorphism conferred a relative VTE risk of almost 4 times [Hazard ratio: 3.96 (95% CI 1.46-108.7), p=0.021]. The incidence of VTE in patients with reduced baseline levels of the natural anticoagulants or acquired APCR was not higher compared with those with normal tests. In conclusion, the prevalence of Factor V Leiden or G20210A prothrombin mutations in MM patients was not significantly higher than in controls. The presence of these mutations in MM patients during treatment with thalidomide and combined chemotherapy conferred a 4-times increased risk of VTE despite all the patients received a prophylactic treatment. Reduced levels of natural anticoagulants or acquired APCR seem to be transitory conditions and were not associated with increased risk of VTE.

Inflammation and Thrombosis: experimental

P172

PARNAPARIN REDUCES THE EXTENT OF DAMAGE IN RAT BRAIN ISCHEMIA: A NUCLEAR MAGNETIC RESONANCE IMAGING STUDY

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Background and rationale. For many years a protective effect of heparin against tissues reperfusion injury has been proposed. This effect is independent of its anticoagulant activity and is related to newly describe anti-inflammatory properties of heparin. Recent studies indicate that the inflammatory process plays a pivotal role in ischemic brain damage: the hallmark of the inflammatory reaction is the presence of activated phagocytic cells in the ischemic area. In the present study magnetic resonance imaging (MRI) has been used to investigate therapeutic intervention with Parnaparin, a low molecular weigh heparin, in a model of permanent focal cerebral ischemia. *Methods and Results*. Brain ischemia was induced in rats by permanent occlusion of the middle cerebral artery (MCAO) and the brain infarct size followed up in alive animals 2, 24 and 48 hours after MCAO using the trace apparent diffusion coefficient (DWI) maps and T2-weighted images. In vehicle treated rats, the infarct volumes increased by 34.4±11.3 (SD) % and 45.8±16.0 % after 24 and 48 hours, respectively, compared with the damage detected at 2 hours after MCAO. Parnaparin was administered intravenously at not hemorrhagic dosage (1 mg/Kg), 2 or 5 hours after MCAO and 24 hours thereafter. Treatment with Parnaparin 5 hours after MCAO significantly reduced the increase in brain infarct volume occurring at 24 and 48 hours with respect to vehicle treated animals (12.6±22.8 % and 24.1±43.87 %, respectively; p<0.05) and the accumulation of macrophages positive cells in the infarct area. This effect was not observed in animals treated 2 hours after MCAO. *Conclusions.* This study indicates that Parnaparin, beside its anticoagulant effects, exerts a neuroprotective role in the attenuation of brain damage after acute brain ischemia, probably due to its anti-inflammatory properties.

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ANTI-PROTEIN Z AND ANTI-FACTOR XII ANTIBODIES ASOCIATED TO HYPERCOAGULABILITY AND INFLAMMATION IN UREMIA

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Haemostatic alterations in end-stage renal failure are complex and involve several abnormalities in the coagulation and fibrinolytic systems. We have investigated the relationship between plasma levels of Factor VIII (FVIII), C-Reactive Protein (CRP), Anti-Protein Z antibodies (anti-PZ abs) and Anti-Factor XII antibodies (anti-FXII abs) in haemodialysis patients. FVIII, CRP, Anti-PZ abs and Anti-FXII abs were evalueted in 30 patients on maintenance haemodialysis (MHD) and in 60 blood donors (healthy controls). In particular, anti-PZ abs and anti-FXII abs were measured by a new, specific, commercially available enzyme-linked immunosorbent assay (Bouty, Italia). The study disclosed the presence of anti-PZ abs and anti-FXII abs in all patients on MHD. Anti-PZ IgG averaged 33.0+5.0 Arbitrary Units/milliliter (Au/mL) in MHD and 1.0+0.3 Au/ml in healthy controls (p=0.0001), Anti-PZ Ig M averaged 28+6.0 Au/ml in MHD and 1.5+0.4 Au/ml in Controls (p=0.0001). Anti-FXII abs averaged 9,3+0.2 Au/mL in MHD and 1,3+0.2 Au/ml in healthy controls (p=0.001). FVIII averaged 175+60.5 % in MHD and 105+45 % in healthy controls (p=0.01). C-Reactive Protein (CRP) averaged 1.15+0.19 mg/dL in patients on MHD and 0.7+0.10 mg/dL in healthy controls (p=0.001). However, Anti-PZ abs and anti-FXII abs levels did not correlate with FVIII and CRP levels. Data show elevated levels of such antibodies in all patients on MHD. No correlation was found among these parameters and coagulation cascade associated to chronic inflammatory state. Patients on MHD frequently experience thrombotic events that occur at different sites. The etiology is still unknown. Our findings demonstrate that other pathogenetic mechanisms, in addition to endothelial damage, may cause hypercoagulability associated with thrombosis in uremia. Studies on the antigens to which autoantibodies bind might provide a further insight into the patogenicity of thrombosis in patients on MHD.

P174

C-REACTIVE PROTEIN AND THE RISK OF FIRST EVER CARDIOVASCULAR EVENT IN HEALTHY ELDERLY PEOPLE

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Background and aim. Measurements of CRP are now considered to improve cardiovascular risk stratification beyond traditional risk factors in the general population, but its predictive ability to predict cardiovascular events in elderly people has not yet been established. The aim of this study was to assess the cardiovascular risk predictive role of CRP in healthy elderly subjects. *Materials and Methods.* We searched the MEDLINE database for all English language prospective studies published between January, 1966, and December, 2005 where the relationship between CRP concentrations and the risk of fatal and non fatal ischemic heart disease (IHD) and/or heart failure (HF) and/or cerebrovascular events (CVE) and/or total cardiovascular mortality (CM) was dealt. Additional references were identified by reviewing the bibliographies of retrieved articles. All potential studies derived from the MEDLINE search were independently reviewed. Individual studies had to meet the following criteria to be included: (1) elderly study population originating from a well-established cohort; (2) examination of the cross-sectional or longitudinal effects (or both) of CRP on cardiovascular end-points; and (3) appropriate consideration of and adjustment for potential confounders. *Results.* We identified about 100 potentially relevant studies. Of these, 12 studies of CRP enrolling a total of about 21,000 healthy elderly subjects (»12,000 females) were included because they met the predefined selection: 5 studies for stroke, 4 for cardiovascular mortality (CVM), 3 for acute myocardial infarction (AMI) or fatal coronary heart disease (CHD), and 3 for heart failure (HF) Mean follow-up of the studies was 6.1±2.7 years. Several studies had two or more endpoints of interest. Raised CRP concentrations were associated nearly to 70% with an increased risk of incident fatal or non- fatal CVE and IHD. High concentrations of CRP were also predictive of the HF development, while less evident was the association between CRP and CM. *Conclusion.* We conclude that high concentrations of CRP are associated with increased risk of fatal and non-fatal cardiovascular events also in elderly people, but this relation could be not strong such as in younger people

P175

ENHANCED SOLUBLE CD40L IN PATIENTS WITH THE METABOLIC SYNDROME: RELATIONSHIP WITH *IN VIVO* THROMBIN GENERATION.

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Aims. The metabolic syndrome is associated with proinflammatory and prothrombotic states. Aim of this study was to assess the behaviour of soluble CD40 ligand (sCD40L) and prothrombin fragment F1+2, a marker of thrombin generation, in metabolic syndrome.

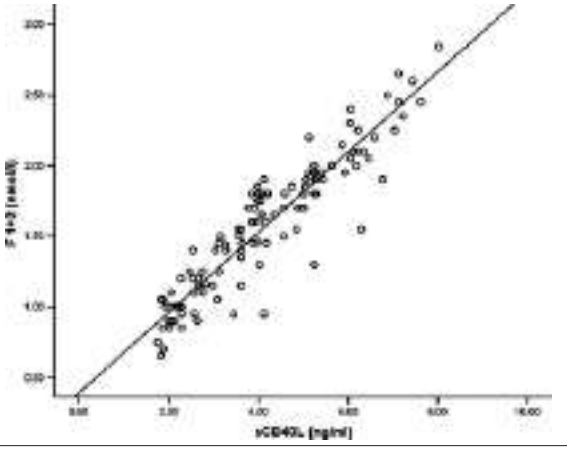

Figure 1.

Methods and results. One-hundred and six patients with the metabolic syndrome diagnosed according to ATPIII report, and 104 subjects without the metabolic syndrome were investigated. sCD40L and F1+2 plasma values were higher in patients with the metabolic syndrome (4.11±1.64 vs 2.61±0.89 ng/mL and 1.54±0.49 vs 0.87±0.21 nmol/l, respectively; $p<0.001$) and significantly correlated (r=0.925, $p<0.001$) Figure; stepwise multiple linear regression analysis showed that sCD40L was significantly associated with F1+2, female sex and waist circumference. *Conclusions.* Patients affected by the metabolic syndrome show enhanced values of plasma sCD40L and F1+2. The study provides further insight in the relationship among the metabolic syndrome, inflammation and thrombosis.

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NORMAL AND HIGH ANGIOTENSIN II LEVELS DIFFERENTLY MODULATE ACE GENE EXPRESSION IN HUMAN LYMPHOCYTES

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Angiotensin converting enzyme (ACE) is involved in inflammatory processes and an increased ACE activity and expression was reported in atherosclerotic plaques. ACE mRNA and enzymatic activity was demonstrated in unstimulated circulating lymphocytes in control subjects. The present study was aimed at evaluating whether angiotensin II (Ang II) plasma levels can modulate ACE gene expression of human lymphocytes. Human lymphocytes that had been obtained from peripheral blood of 15 healthy subjects (9 males 6 females, aged 27+4 years) by layering over Ficoll-Hipaque, had been incubated for 12, 18, 24, 30 and 36 hours with physiological or high Ang II concentrations (0,1 nM and 0,05 pM). Messenger RNA for ACE was studied by semiquantitative reverse transcriptase polimerase chain reaction (RT-PCR). PCR analysis was performed on serial 2-fold dilutions of cDNA for each sample using glyceraldeyde-3-phosphate deydrogenase (GAPDH) as internal standard..The last dilution giving a positive reaction for GAPDH was used to equalize the amount of cDNA in each PCR. At Ang II physiologic concentration (0,05 pM) lymphocyte ACE gene expression significantly increased ($p<0,001$) versus baseline at 12 hours , then slowly went back to baseline at 36 hours. In contrast, 0,1 nM Ang II concentration did not significantly modify ACE gene expression throughout the study. Our data show that in healthy subjects ACE gene expression of isolated lymphocytes is differently modulated by normal or high Ang II plasma levels. At high Ang II plasma levels an inhibitory feed-back mechanism does occur.

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ROLE OF INFLAMMATION IN MODULATING GRAFT TOLERANCE INDUCED BY MESENCHIMAL STEM CELLS

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Mesenchymal stem cells (MSCs) possess immunomodulatory properties and inhibit T-cell proliferation *in vitro*; this suggests that MSCs may be used for the prevention and treatment of graft-versus-host disease in organ transplantation. *In vitro* evidences suggested inflammatory environment could modulate the behaviour of MSC. We performed a case-control study to assess the effect of intravenous administration of MSCs in rats undergone skin allograft transplantation. The experimental design included 4 arms: group A, rats receiving only skin transplantation; group B, skin transplantation and cyclosporine A (CyA) immunosuppressive therapy; group C, skin transplantation, CyA and endovenous infusion of donor MSCs; group D, skin transplantation and MSCs infusion. For each arm, we evaluated the timing of allograft rejection and expression of 6 genes involved in immunomodulatory effect by real-time PCR. Rats of group C had significantly higher median value of skin allograft rejection: 30 (range 15-30) vs 18, 20 and 13 days for group A, B, and D, respectively. IFN-γ and IL-2 gene expression was significantly different among the 4 groups ($p<0.0001$ and $p=0.023$, respectively). IFN-γ mRNA levels were lower in group B ($p=0.004$) and group D ($p=0.002$) in comparison to group A. TNF-α mRNA gene expression was lower in B and more markedly in C group, in comparison to group A.

IL-10 mRNA levels were higher in group C in comparison to the other 3 groups (p=0.06). IDO mRNA levels in group B were lower than in both group A (p= 0.001) and group C (p=0.001). TGF-β mRNA levels were lower in CyA+MSCs group than in CyA (p=0.002), untreated (p=0.019), and MSCs (p=0.02) groups. Our data suggest an immunogenic role of MSC *per se* whereas MSC may significantly improve the graft tolerance in CyA treated immunodeficient animals. Inflammatory molecules and in particular TNF-α seem to play a pivotal role in modulating this phenomenon.

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MODULATION OF FIBRINOLYTIC PROPERTIES OF MICROVASCULAR ENDOTHELIAL CELLS BY LOW-MOLECULAR WEIGHT (LMWH) AND UNFRACTIONATED (UFH) HEPARINS

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Heparins are glycosaminoglycans largely used as anti-thrombotic drugs. In a previous study we demonstrated that both LMWH and UFH affect microvascular endothelial cells (EC) pro- and anti-coagulant properties. It is not known whether these drugs may influence the fibrinolytic properties of microvascular EC. Aim of this study was to evaluate the impact of the LMWH Dalteparin (DLT) and UFH on the fibrinolytic proteins of human microvascular EC (HMEC-1). HMEC-1 were incubated with LMWH or UFH (0.01 to 10 IU/mL), or vehicle (control cells),±10 µg/mL LPS, for up to 72h. At the end of incubation, conditioned media (CM) were collected and analyzed for t-PA and PAI-1 antigens (t-PA:Ag and PAI-1:Ag) by ELISA, or t-PA activity (t-PA:Act) by a chromogenic assay. The results show that in HMEC-1 CM (72h): 1. DLT induced a dose-dependent increase of t-PA:Ag (10 IU/ml DLT: 41.2±5.8% increase, p<0.05 vs Control) and t-PA:Act (10 IU/ml DLT: 35.4±3.8% increase, p<0.05) without affecting PAI-1:Ag levels; 2. DLT also significantly prevented the decrease of the EC fibrinolytic activity induced by LPS; 3. UFH did not affect t-PA:Ag levels but reduced PAI-1:Ag and this was associated to an increase in t-PA:Act (t-PA:Act: 10 IU/ml UFH: 27±3.2% increase, p<0.05 vs Control). A similar effect was obtained in the presence of LPS (t-PA:Act: 10 IU/mL UFH/LPS: 31.2±4.1% increase, p<0.05 vs Control/LPS). In conclusion, these data show that both LMWH and UFH increase the fibrinolytic potential of microvascular EC by means of different mechanisms. Overall this effect of heparins in the microcirculation may contribute to the antinflammatory role of these drugs.

P179

INTERPLAY BETWEEN PROTEIN Z AND INTERLEUKIN-6 DURING THE ACUTE PHASE OF THE CORONARY ARTERY DISEASE

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Introduction. Protein Z, a vitamin K-dependent glycoprotein, is a prothrombotic factor which serves as a cofactor for the inhibition of activated factor X. Low circulating levels of this protein have been found to be associated with prothrombotic clinical states. Actually, the relationship between protein Z and the acute-phase reaction has not been completely investigated since some authors reported that protein Z could act as a negative acute-phase reactant whereas others showed that protein Z was weakly influenced by inflammatory cytokines. We enrolled and prospectively followed 10 patients (8 males; median age: 64 years) with acute coronary syndromes who underwent primary PCI, and we evaluated the time-course of protein Z and interleukin-6 during and after the acute event, in order to give an insight into the relationship between protein Z and acute-phase state. All the patients were prospectively followed over several time-points (baseline i.e. before PCI, 36 hours, 72 hours, 1 month and 3 months after PCI) remaining free from any adverse events throughout the follow-up period. *Results.* Protein Z showed an increase of concentrations up to a peak at 72 hours after the acute event, so returning as similar as baseline at 3 months after the PCI. Interleukin-6 showed a parallel pattern but an earlier peak value (36 hours), with a strong positive correlation with protein Z at both baseline and 3 months of follow-up (R=0.85; p=0.002 and R=0.89; p=0.003, respectively). In addition, a linear regression analysis showed a modest, but significant, influence of interleukin-6 on protein Z levels (β = 0.019±0.005; p= 0.004). *Conclusions.* We found, in a group of patients prospectively investigated over several time-points during follow-up after PCI, an influence, albeit modest, of interleukin-6 on protein Z levels.

P180

CHANGES OF INFLAMMATORY MARKERS DURING FOLLOW-UP OF PATIENTS WITH HEART FAILURE UNDERGOING CARDIAC RESYNCHRONIZATION THERAPY

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Introduction. Inflammation plays an important role in the progression of atherosclerosis. During the last years, several studies demonstrated that high-sensitivity C-reactive protein (hsCRP), as well as other inflammatory markers, is able to predict the development of heart failure (HF) and of other cardiovascular adverse events. However, no data exist about predictive values of inflammatory markers [hsCRP and interleukin-6 (IL-6)] in HF patients on cardiac resynchronization therapy (CRT). Aim of this study was to evaluate the interplay between inflammation and adverse events in HF patients who underwent CRT. *Methods.* Before and 6 months after CRT we evaluated IL-6 and hsCRP serum levels on 140 patients with symptomatic HF (on optimized medical therapy; III-IV NYHA class) who underwent CRT. *Results.* IL-6 serum levels were significantly lower (p=0.02) after 6 months of follow-up with respect to baseline [5.4 (0.07-73.07) pg/mL vs. 6.8 (0.67-65.2) pg/mL], whereas no significant difference for hsCRP levels was observed. MACE were observed in 40 patients (28.6%): 22 due to cardiac causes, and 18 due to unplanned re-hospitalization. In MACE patients no significant differences between baseline and follow-up for IL-6 and hsCRP levels, were observed [IL-6: 7.5 (0.7-53.9) pg/mL vs. 5.6 (1.3-50.1) pg/mL for baseline and follow-up, respectively (p=0.4); hsCRP: 8.2 (0.6-10.2) mg/L vs. 6.4 (1.2-145) mg/L for baseline and follow-up, respectively (p=0.4)]. However, in patients who not occurred MACE at follow-up, a significant decrease for both parameters was observed [IL-6: 6.8 (1.2-56.1) pg/mL vs. 4.7 (0.1-73.1) pg/mL for baseline and follow-up, respectively (p=0.01); hsCRP: 5.3 (0.1-21.8) mg/L vs. 2.9 (0.2-34.8) mg/L for baseline and follow-up, respectively (p=0.001)].

P181

PLASMA OF CHRONIC URTICARIA PATIENTS SHOWS SIGNS OF THROMBIN GENERATION

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Background. Several aspects of the pathogenesis of chronic urticaria (CU) remain contradictory. Autologous serum skin test (ASST) and in-vitro histamine release assays (HRA) seem to look into distinct aspects of the disease and the specificity of ASST has been questioned. *Objective.* We investigated autologous plasma skin test (APST) as a more specific means to detect patients with autoreactive CU. As APST scored much more frequently positive than ASST the clotting process was investigated as well. *Methods.* 96 adults with CU underwent ASST and APST with Na citrate-anticoagulated plasma. Prothrombin fragment F1+2 was measured by a sandwich ELISA in Na citrate-anticoagulated plasmas from 28 patients with CU and 27 controls. *Results.* 51 patients (53%) scored positive on ASST whereas 86% scored positive on APST-Na citrate. Plasma levels of fragment F1+2 were higher in patients than controls (3.06 [SD3.36] vs 0.80 [0.34]; p<0.001) and were higher in ASST+/APST+ than in ASST-/APST+ patients (3.89 [SD 3.68] vs 1.33 [1.64] ; p = 0.058). F1+2 levels were directly related with urticaria severity (r= 0.37; p<0.05). *Conclusions*. Most CU patients are positive on APST-Na-citrate. CU is associated with the generation of thrombin, a serine protease able to activate mast cells and to cause relevant increase in permeability of endothelium. These findings open new insights into the pathogenesis of CU and suggest new therapeutic opportunities for this disease.

P182

PLATELET P-SELECTIN EXPRESSION, PLATELET-PMN AND PLATELET-MONOCYTE AGGREGATE FORMATION UPON STIMULATION, ARE POSITIVELY CORRELATED IN A GENERAL POPULATION

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Platelet-leukocyte interactions may play an important role in both inflammatory and thrombotic processes. The aim of this study was to correlate new markers of cell activation and adhesion in a general population. Venous blood was collected on citrate from 155 subjects (84 F, 71 M, 16-89 years old), recruited from the general population in the frame of an epidemiological study. Platelet P-selectin, platelet-PMN and platelet-monocyte aggregates were measured in whole blood, both at baseline and after stimulation with both ADP (5 μM) and collagen (2 μg/mL) for 10 min, at 37°, under stirring at 1000 rpm. Fixed samples, labelled with fluorescence-conjugated MoAb, were analysed by flow cytometry. PMN and monocytes were characterized by CD45 and side scatter light, platelets by CD42b and CD61, when measuring P-selectin expression and mixed aggregates, respectively. Table 1 reports both baseline values and data obtained after blood stimulation. Both baseline P-selectin and platelet-PMN aggregates significantly correlated with age (p=0.014, r=0.2 and p=0.038, r=0.17). P-selectin was higher in the oldest tertile than in the youngest, while a similar non significant trend was observed with both types of mixed aggregates (Table 2). No difference was found in any baseline parameter between males and females (Table 1). When data were analysed as the ratio of stimulated/baseline values, P-selectin positively correlated with both platelet-PMN (p<0.0001, r=0.6) and platelet-monocyte aggregates (p<0.0001, r=0.5). P-selectin expression and platelet-PMN aggregates, but not platelet-monocyte aggregates, were significantly higher in females vs males (p=0.002 and p=0.02 respectively). P-selectin ratio inversely correlated with age (p=0.003, r=-0.24) and was significantly lower in the highest tertile as compared to the lowest one (p=0.02); this finding might be related to the higher baseline values in the oldest subjects (Table 2). These data offer new insight into the role of platelets and leukocytes in inflammation and thrombosis.

Supported by Telethon, contract GGP04198.

Table 1. Percentage of double fluorescence-positive cells (means±SD).

	BASELINE		*STIMULATED*	
	F	*M*	*F*	*M*
Platelet P-selectin	3.5±4.7	3.0±2.5	24.9±11.3	19.6±12.1
Platelet-PMN aggregates	6.8±9.5	5.5±7.4	28.8±24.7	18.3±20.3
Platelet-monocyte aggregates	8.6±12.5	7.7±10.9	33.8±31.7	22.2±23.1

Table 2. Percentage of double fluorescence-positive cells at baseline (means±SD) in 3 age-groups.

Age tertiles	*Platelet P-selectin*	*Platelet-PMN aggregates*	*Platelet-monocyte aggregates*
16-35	2.3±2.2	4.4±5.0	6.3±8.5
36-54	3.2±3.7	5.6±7.5	8.7±12.4
55-89	4.3±5.0 *	8.7±11.9	9.7±13.8

**significantly different from lowest tertile (p=0.016).*

P183

D-DIMER LEVEL IN CEREBROSPINAL FLUID OF PATIENTS WITH MENINGO-ENCEPHALIC INFECTION

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D-dimer is a degradation product of cross-linked fibrin and represents a reliable marker of fibrin formation and degradation. Considering that fibrin is a hall-mark of inflammatory reactions we evaluated the presence of D-dimer in cerebrospinal fluid (CSF) of patients with suspected meningo-encephalic infection (MEI). CSF was obtained from 20 patients with clinical signs of meningo-encephalic infection (fever, headache, altered level of consciousness,and symptoms and signs of cerebral dysfunction) and 20 subjects undergoing spinal anaesthesia for minor surgery (control group). D-dimer was measured on fresh CSF by enzyme linked fluorescent assay (VIDAS). Other assays (protidorrachia , glicorrachia, leukocyte count) were carried out by routine laboratory methods. D-dimer levels in CSF were below the detection limit (< 45 ng/mL) in all controls. On the contrary, D-dimer was detectable in 18 out of 20 patients with suspected MEI (median: 95 ng/mL; range: 55 - 9800). Regression analysis showed that D-dimer concentration in CSF was inversely correlated with glicorrachia (r=-0.58; p=0.007) and positively correlated with protidorrachia (r=0.63; p=0.003) and leukocyte count (r=0.68; p=0.001). Subsequent analyses (rachiculture, MRI, nucleic acid detection, serological tests) confirmed the diagnosis of MEI in the 18 patients with positive D-dimer in CSF (14 viral menigitis, 2 bacterial menigitis, 1 viral encephalitis and 1 bacterial encephalitis) while excluded the diagnosis in the 2 patients with negative D-dimer. Interestingly, another laboratory index suggestive of MEI, such as the presence of leukocytes in CSF, was negative in 2 patients affected by viral meinigitis. These results suggest that D-dimer in CSF is an early marker of meningo-encephalic inflammation. The origin and the patophysiological significance of this fibrin product as well as the usefulness of D-dimer testing in CSF remains to be established.

P184

PLATELET-LEUKOCYTE INTERACTIONS IN WHOLE BLOOD: DOWN-REGULATION BY A LOW MOLECULAR WEIGHT HEPARIN (PARNAPARIN)

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Parnaparin (PNP), a LMWH, prevents *washed* polymorphonuclear leukocyte (PMN) activation and interaction with platelets.We tested the effect of PNP on platelet-leukocyte aggregates formed in whole blood in the presence of platelet stimuli. Citrated blood from healthy subjects was stimulated with 2 μg/mL collagen and 50 μg/mL ADP under dynamic conditions (3 min, 37°C). Platelet-PMN and platelet-monocyte aggregates, fibrinogen (fg) binding on all three cells and PMN CD40 were determined by three colour flow-cytometry and results expressed as % of positive events (mean±sem,n=4-5). PNP and unfractionated heparin (UFH) were added to blood immediately before stimulation. Platelet-PMN aggregates rose from 9.9±3.2% (in the absence of agonists) to 19.5±1.2%, while platelet-monocyte aggregates increased from 2.8±0.7% to 11.4±2.8% (p<0.05). PNP (0.3-0.8 IUaXa/mL) significantly reduced the extent of both mixed cell aggregates in a concentration-dependent manner, reaching the lowest values of 7.3±2.2% and 5.5±3.8%, for platelet-PMN and platelet-monocyte, respectively at 0.8 IU/mL. UFH failed to prevent either type of aggregates. Fg binding increased after stimuli from 4.9±0.6% to 25.8±8.1% on PMN; from 6.9±0.9% to 57.8±4.5% on monocytes and from 4.3±0.6% to 27.9±2.3% on platelets. PNP prevented fg binding in a concentration-dependent manner, reaching at 0.8 IU/mL the minimal values (PMN 8.2±3.7%,monocytes 12.5±3.5%, platelets 4.8±0.7%). UFH significantly affected fg binding only at 0.8 IU/mL. CD40 expression on PMN, increased upon stimulation (48.9±3.7% vs. 19.8±2.3%), was also significantly prevented by all PNP concentrations (20.12±1.3% at 0.8 IU/mL). In conclusion, in a whole blood system in dynamic conditions, PNP prevents platelet-leukocyte stable aggregate formation, integrin activation on platelets and leukocytes (both PMN and monocytes) and an inflammation marker. Besides its anticoagulant effects, PNP might contribute to down-regulate inflammatory mechanisms underlying thrombosis.

Oral Communications

C001

REGISTRO INFORMATIZADO DE LA ENFERMEDAD TROMBO EMBÓLICA (RIETE)

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Anticoagulant therapy is the treatment of choice for most patients with VTE. The proceedings of the sixth American College of Chest Physicians (ACCP) Consensus Conference provide an extensive critical review of the literature related to the management of patients with VTE and lay the scientific groundwork for the standard of care, based largely on data from randomized controlled clinical trials. But a number of patients are often excluded from clinical trials (for example due to pregnancy, renal insufficiency or high risk of bleeding). Thus, there is no evidence about what would be the best therapeutic approach for these patients. However, physicians need to be aware of factors that influence a patient's response to anticoagulant therapy. Prognostic tools that give quantitative probabilities of adverse events, and are of practical value to physicians, are clearly required. A history of bleeding has consistently been shown to be predictive of major bleeding associated with antithrombotic therapy, while the presence of a serious co-morbid condition, such as malignancy, liver or renal insufficiency also predicts hemorrhagic events. The relationship between older age or pregnancy and anticoagulant-associated bleeding remains controversial. Paradoxically, many of these high-risk patients are excluded from the randomized clinical trials upon which the recommendations for the treatment of VTE are based. By contrast, the RIETE registry has already collected data on VTE treatment in many of these patient subgroups. Although patient numbers for some subsets within the RIETE database are currently small, numerical differences in outcomes suggest the possibility of clinically relevant treatment differences. The data already obtained indicate that standard recommended therapy for VTE (UFH or LMWH during the acute phase followed by a vitamin K antagonist for at least 3 months) may not be optimal in some subgroups of patients. Further, the risk of bleeding complications may not be directly dose-related in certain patient groups. More patients are needed to investigate fully these observations. *Inclusion Criteria.* Consecutive patients with symptomatic, acute deep-vein thrombosis (DVT) or pulmonary embolism (PE), confirmed by objective tests (contrast venography, compression ultrasonography, helical computed tomography [CT] scan, or impedance plethysmography for suspected DVT; pulmonary angiography, lung scintigraphy, or helical CT-scan for suspected PE), are enrolled in RIETE. Patients are excluded if they are participating in a therapeutic clinical trial or if they are not available for the 3-month follow-up. *Start Date and Duration.* March 2001, no limits. *Design.* The parameters recorded by the registry comprise details of each patient's baseline characteristics; clinical status, including any coexisting or underlying conditions such as chronic heart or lung disease, recent bleeding complications, and abnormal creatinine levels; risk factors for VTE (cancer, immobilization, surgery, previous VTE, pregnancy, estrogen therapy, postpartum, long travel or leg varicosities); use of antiplatelets, anti-inflammatory drugs or NSAIDs; thromboprophylaxis received prior to enrollment; the type, dose, and duration of treatment received on VTE diagnosis, and clinical outcome (recurrent VTE, fatal PE, major bleeding, minor bleeding, fatal bleeding, overall death, osteoporotic bone fractures, thrombocytopenia, and other adverse events) during the first 3 months of therapy. After hospital discharge, all patients are followed-up for at least 3 months. During each visit, any signs or symptoms suggesting either DVT or PE recurrence or bleeding complications were noted. Each episode of clinically suspected recurrent DVT or PE was documented by repeat compression ultrasonography, venography, lung scanning, helical CT scan or pulmonary angiography. An Adjudication Committee is the responsible to confirm that any event developing during the follow-up has been confirmed by objective tests. All patients provide oral informed consent to participate in the registry, according to the requirements of the ethics committee within each hospital. Data are recorded on a computer-based case report form by a RIETE registry coordinator at each participating hospital and submitted to a centralized coordinating center through a secure website. The coordinators also ensure that eligible patients are consecutively enrolled. Patient identities remain confidential because they are identified by a unique number assigned by the study coordinator center that is responsible for all data management. Study end points are adjudicated by the attending physicians. At regular intervals, data quality is monitored and documented electronically to detect inconsistencies or errors, which are resolved by the local coordinators. Data quality is also monitored by periodic visits to participating hospitals by contract research organizations, which compare the medical records with data on the secure website, as is the case of most clinical trials. In the event of substantial or unjustifiable inconsistencies, patients enrolled from that center are not included in the database. A full data audit is performed at periodic intervals. In the RIETE registry, selection bias is avoided by including consecutive patients with objectively confirmed, symptomatic, acute VTE who were referred to study centers. Enrolled patients are treated according to standard practice, and prospective follow-up is completed for all patients. Objective criteria are strictly applied for the diagnosis of initial and recurrent VTE, including contrast venography and pulmonary angiography if indicated, and major bleeding was classified according to widely accepted and validated criteria. *Intervention.* Data are recorded on a computer-based case report form by a RIETE registry coordinator at each participating hospital and submitted to a centralized coordinating center through a secure website. The coordinators also ensure that eligible patients are consecutively enrolled. Patient identities remain confidential because they are identified by a unique number assigned by the study coordinator center that is responsible for all data management. Study end points are adjudicated by the attending physicians. *Statistics.* The type of analysis will depend on the study. There are no limits in the sample size. *Sponsors.* The RIETE registry is an independent registry, partially supported by Sanofi-Aventis in Spain and Red Respira from the Instituto Carlos III, Spain (RedRespira-ISCiii-RTIC-03/11). Neither Sanofi-Aventis or The Spanish Ministery of Health have right to acceed to the database, and there is no payment per patient recruitment.

C002

ASPIRIN AFTER SIX MONTHS OR ONE YEAR OF ORAL ANTICOAGULANTS FOR THE PREVENTION OF RECURRENT VENOUS THROMBOEMBOLISM AND CARDIOVASCULAR EVENTS IN PATIENTS WITH IDIOPATHIC VENOUS THROMBOEMBOLISM. THE WARFASA STUDY

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Background. Aspirin prevents recurrent venous thromboembolism and cardiovascular events when given after six or 12 months of oral anticoagulation in patients with idiopathic venous thromboembolism. *Inclusion criteria.* Patients will be eligible for inclusion in the study if they meet the following criteria: a) first episode of symptomatic, objectively confirmed idiopathic proximal deep vein thrombosis and/or pulmonary embolism; b) initial treatment with unfractionated heparin or low-molecular-weight heparin (or effective alternative) followed by a vitamin K antagonist (target INR 2.0-3.0). All patients will receive 6 or 12 months of oral anticoagulant treatment. Patients initially treated with thrombolytic therapy who received warfarin therapy are eligible for inclusion. *Exclusion Criteria.* Subjects with any of the following will be excluded: a) permanent risk factors for venous thromboembolism: patients known to have antiphospholipid antibodies or lupus anticoagulant (based on local laboratory criteria) or to have homozygous factor V Leiden or homozygous prothrombin G21210A or heterozygous factor V Leiden plus heterozygous prothrombin G21210A or antithrombin III deficiency; patients with active malignancy ; b) temporary risk factors for venous thromboembolism; c) any recurrence of venous thromboembolism or bleeding episode during the established 6-month period of oral antico-

agulant treatment; d) allergy or intolerance of aspirin; e) clear indication for aspirin or other anti-platelet therapy (e.g. clopidogrel, ticlopidine); f) clear indication for long-term anticoagulant therapy (e.g. recurrent idiopathic venous thromboembolism, prosthetic heart valve); g) treatment with non-selective COX-1/2 non-steroidal anti-inflammatory drugs; h) life expectancy less than 6 months; i) active bleeding or at high risk of bleeding (gastrointestinal bleeding within the past 12 months; endoscopic diagnosis of peptic ulcer disease or ulcerative esophagitis within the past 6 months unless there is documented endoscopic evidence of healing; intracranial bleeding within the past year; known bleeding diathesis); j) anticipated non-adherence to study medications; k) inability to attend follow up because of geographic inaccessibility; l) failure to provide informed consent. *Start Date and Duration.* December 2004 - two years of accrural - postulated end date December 2007. *Design.* Multicenter, randomized, double-blind, placebo-controlled trial. *Intervention.* Aspirin, 100 mg daily or placebo, given for at least two years. *Statistics.* The primary analysis will be performed on the intention-to-treat basis. The analysis will be conducted by means of a LOCF approach. If any significant unbalance between active and placebo in terms of drop-out or lost to follow-up, a Worst Rank analysis will also be performed. By using a sample size of 1198 patients with a patient accrual over two years, equal allocation to aspirin or placebo, a follow-up of at least two years, and a mean placebo event rate of 6.0% per year throughout the course of the study, we will have 80% power to detect a relative risk reduction 35% in favor to aspirin compared with placebo. Expecting a drop-out rate of 15%, a total of 1378 patients will be enrolled. *Sponsor.* Clinical Research Unit of the University of Perugia

C003

EVALUATION OF THE BAYPAD DIAGNOSTIC SUPPORT SYSTEM IN PULMONARY EMBOLISM

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Background. Numerous decision trees and scoring systems have been proposed for the diagnosis of pulmonary embolism (PE). With few exceptions, these are the product of a consensus opinion among experts rather than a quantitative evaluation. In addition, these proposals are often in disagreement one with another, also with regard to the type of examination to be used. At this time, therefore, there does not exist a diagnostic strategy that is held to be completely satisfactory and, in the past, some Authors have even questioned whether it would be possible to create such a universal strategy. One of the major difficulties is due to the variability of the clinical presentation, exactly because it legitimates the suspect of PE to an excessively various extent. In this regard, the contribution of a quantitative evaluation of the clinical probability to the implementation of an efficient diagnostic strategy is well established. Recently, a number of Authors have proposed the use of helical computed tomography, but the most accurate version of this technique is available only among a minority of Centres. As a result, the diagnosis of PE is still based on a combination of techniques (d-Dimer test, ultrasound of the lower limbs), together with an evaluation, albeit approximate, of the clinical probability of PE. The BayPAD diagnosis support system offers an estimate of the PE risk in light of the clinical characteristics of an individual case. In addition, it is able to suggest further diagnostic tests and to order these by the usefulness of the data they produce. In doing this, the system uses a Bayesian network as a physiological / pathological model of PE. Bayesian networks are graphical probability models that are particularly suited to situations in which the availability of some data makes the knowledge of other information irrelevant. For this reason, these networks have been shown to be very useful tools in the field of medical diagnosis and they are used in a clinical context. Once the list of tests available in a centre has been specified and the practicality of these for use on a given patient assessed, the algorithm provides for the introduction of all the elements that resulted in a suspected diagnosis, first among these being the results of the patient's medical history and physical examination. BayPAD then proposes further diagnostic testing unless the probability of PE is greater than 95% or less than 5% and no further tests are valid from a cost point of view. *Start Date and Duration.* January 2007, one year. *Design.* This project has to be regarded as an observational study. All cases in which, in the doctor's opinion, the possibility of pulmonary embolism cannot be excluded, are eligible. *Design.* The study aims to involve: Accident and Emergency Departments (above all if equipped for the short-term hospitalisation of the patient); General Medicine Departments; Pneumology Departments; Cardiology Departments. *Intervention.* Patient data: only the clinical observations and the results of the diagnostic procedures provided for by the model are to be collected. Of these data, only the observations obtained with the tests requested by the doctor (and selected or not on the basis of the BayPAD suggestions) will be input as soon as they are available. Opinion of participating doctor: these are the tests the doctor would ask for before receiving the BayPAD suggestions. Doctor's decision: the tests requested by the doctor after having received the BayPAD suggestions. Reasons for not accepting the BayPAD suggestions, to be reported only in the case of none of the tests suggested by BayPAD as a first choice being accepted by the doctor. Further information: profile of participating doctor; profile of participating centre. Confirmation of diagnosis: the evaluation of BayPAD's performance is based on checking the accuracy of the diagnosis in each patient. The use of angiopueumography to this end will not be necessary, however. As an alternative, an evaluation group of expert radiologist will evaluate the correctness of the diagnosis of PE made on the basis of a direct visualisation of the embolous (for example, as evidenced by spiral CT, echocardiography or angiopueumography). In the case of the diagnosis of pulmonary embolism not being based on this type of data, scintigraphy follow up will be used to record of the resolution of perfusion. The confirmation of the diagnosis in which pulmonary embolism is excluded will be given by a negative scintigraphy examination or, if this is not available, a follow up of the clinical condition of the patient. *Statistics.* 550 cases are required to obtain a result with an accuracy confidence limit of 95%, range 5%.

C004

ITALIAN REGISTRY OF MYH9-RELATED DISEASE

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Background. MYH9-related disease (MYH9-RD) is an autosomal dominant disorder caused by mutations of MYH9, the gene for the heavy chain of non-muscle myosin IIA (NMMHC-IIA). At birth all patients present macrothrombocytopenia, giant platelets, and cytoplasmic aggregates of NMMHC-IIA in granulocytes recognizable by specific antibodies. These aggregates are often evident upon routine staining of blood films as basophilic "Döhle-like" inclusions. Most of MYH9-RD patients subsequently develop, in childhood or adult life, sensorineural hearing loss and/or presenile cataracts and/or interstitial glomerulonephritis that can lead to end-stage renal failure. MYH9-RD includes four autosomal dominant syndromes that have been previously regarded as distinct entities: May-Hegglin anomaly, Sebastian, Fechtner, and Epstein syndrome (OMIM 155100, 605249, 153640, and 153650, respectively). Mutations of MYH9 have also been identified in patients with isolated congenital macrothrombocytopenia, with neither leukocyte inclusions nor additional clinical findings. Many questions regarding the diagnostic, clinical and prognostic aspects of MYH9-RD are still to be solved. (a) MYH9-RD is often misdiagnosed and its prevalence is unknown. Most of patients received a previous incorrect diagnosis and undue treatments. Diagnostic difficulties derive partly from the unavailability of the specific diagnostic procedures in non-specialized Institutions and inadequate information on the disease. (b) Due to the small size of the case series analyzed so far, the clinical phenotype of MYH9-RD is poorly defined. For instance, the incidence, age of onset, severity, and natural history of glomerulonephritis, sensorineural deafness and cataracts are largely unknown. Different other abnormalities have been described in MYH9-RD patients, but it is not clear if they are less frequent manifestations of MYH9 mutations or they derive from fortuitous associations of different diseases. (c) The investigations performed so far did not evidence any genotype/phenotype correlation for patients with MYH9 mutations. However, these studies do not allow to conclude that genotype is not a determinant of the MYH9-RD phenotype, since they are based on small case series and short follow-ups. (d) Clinical picture of MYH9-RD is often different in patients belonging to the same pedigree. One of the possible explanations is the different role of polymorphic variants of genes interacting with MYH9. Several strong interactors of MYH9 have been identified so far. Elucidation of the role of their polymorphisms requires the identification of wide pedigrees suitable for analysis of transmission of haplotypes among generations. The rationale for the creation of a national registry of MYH9-RD is that the collection of a large database of homogeneously studied patients could answer many of these open questions. Institution of a national registry will

improve the information on and the diagnosis of the disease. Finally, the pathogenesis of the disorder is still unknown, and no therapy is at present available. The registry could represent a logistic tool to provide case series of homogeneously studied patients for biological studies on disease pathogenesis, and to identify subjects that could benefit from specific therapeutic approaches. *Inclusion Criteria:* (a) patients with inherited and/or congenital macrothrombocytopenia; or (b) patients with macrothrombocytopenia, whenever a genetic origin cannot be excluded on the basis of medical history; or (c) patients with chronic interstitial glomerulonephritis and/or sensorineural deafness and/or cataracts, whenever association with thrombocytopenia is found. Exclusion criteria: (a) established diagnosis of any other form of inherited macrothrombocytopenia; or (b) proven X-linked or autosomal recessive inheritance; or (c) refusal of consent. *Start Date and Duration.* October 2006/no limit. *Design.* The study will retrospectively and prospectively enrol all eligible cases according to inclusion/exclusion criteria. For all the enrolled patients will be performed the search for NMMHC-IIA aggregates in granulocytes by immunofluorescence analysis of blood smears (centralized). Whenever this test will result positive, the patient will undergo the examinations for definition of genotype and phenotype and respective data will be included in the database. Mutational screening of MYH9 gene will be centralized. Definition of phenotype includes personal and familiar medical history, physical examination, automated and phase-contrast microscopy platelet count, bleeding time, standard urinanalysis, quantitative 24-hours proteinuria, serum creatinine and liver enzymes, audiometric examination, ophtalmological evaluation (examinations performed by referring Institution). The protocol includes periodic repeats of examinations listed at point (b) according to a scheduled follow-up. *Intervention.* Clinical data will be collected by the referring physicians by a predisposed form, which will be send to coordinating centre (Pavia). It is scheduled a first data collection at diagnosis and an updating once at year. Enrolment implies the shipping of blood smears and blood or gDNA samples. *Statistics.* The enrolment of 125 patients will allow to define genotype-phenotype correlations for the 5 most frequent MYH9 mutations - responsible for about 85% of cases (type I error, 0.05; power, 0.80). *Sponsor.* None.

C005

INCIDENCE AND MANAGEMENT OF HEPARIN-INDUCED THROMBOCYTOPENIA WITH THROMBOSIS (HIT/T) OR WITHOUT THROMBOSIS (HIT) IN HOSPITALIZED PATIENTS

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Background. Heparin-induced thrombocytopenia is an antibody-mediated syndrome associated with heparin exposure, a falling platelet count and a high risk of thrombosis. Cardiovascular patients are at increased risk of HIT due to wide use of heparin in this population. Should HIT be suspected, heparin must be avoided in most situations, and anticoagulation with an alternative anticoagulant should be instituted. *Start Date and Duration.* First patient in: October 2006 - Last patient's last visit: December 2008. *Study Design*. Prospective patients' data review. Study will include all hospitalised patients with clinical symptoms of HIT including new thrombosis and thrombocytopenia. Patients with a positive anti-PF4 assay and a decrease in platelets to <100.000/mmc or 50% from baseline will be considered HIT positive.Platelet count will be monitored daily during treatment, as well as anti-PF4/heparin antibodies. *Objectives and end-points.* To activate an HIT/T registry which will provide information on incidence of HIT/T associated with the use of unfractionated heparin and low molecular weight heparin, on the outcomes of these patients, on the adverse events associated with the syndrome and finally on the efficacy and safety of alternative anticoagulation regimen. *Intervention.* Therapeutic or prophylactic regimen will be instituted according to patient's clinical evidence. Patients with HITT will be submitted to objective tests for the assessment of the extension of venous and/or arterial thromboembolism before randomisation and one week after the initiation of treatment. *Sponsor.* None.

Posters

P001

A REGISTRY OF VENOUS THROMBOEMBOLISM IN RENAL TRANSPLANTED PATIENTS

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Background. Renal transplantation (RT) provides the best long-term treatment for chronic renal failure, but the long-term outcome of RT recipients appears to be influenced by the occurrence of many complications. Among them an increased risk of venous thromboembolism (VTE) has been reported. It is well known that in RT patients there is an imbalance of haemostatic system, leading to a persistent hypercoagulable state that may play a role in VTE events. The nature of clotting activation is probably multifactorial including both classic risk factors, such as surgery, and specific factors related to RT, such as immunosuppressive therapy, hyperhomocysteinemia, or CMV infection. The incidence of VTE reported in previous studies is widely different, ranging from 0.6 to 25%. A retrospective cohort study performed using the United States National Registry have found a 1.5% incidence of VTE in RT patients followed for 3 years. However, this study likely produced an underestimation of disease prevalence, because it was performed on Medicare claims and with a short follow-up. Other observational studies performed in clinical setting, reported an incidence of VTE of 9% in RT patients during the 10 years following. In a previous study we have demonstrated a high incidence of recurrent VTE (50%) after antithrombotic prophylaxis withdrawal in RT patients with previous VTE. Guidelines on the treatment of acute VTE events and its duration are quite well established (FCSA; ACCP); however, no specific recommendations are available on secondary prophylaxis of VTE in this subgroup of patients. If confirmed, the high risk of VTE recurrence in RT patients requires strategies for its prevention and possibly the need for prolonged, probably life-long, treatment. *Inclusion Criteria.* All RT patients with a symptomatic or asymptomatic episode of VTE (deep vein thrombosis and pulmonary embolism) objectively confirmed should be reported. Deep vein thrombosis should be diagnosed by Doppler ultrasound (DUS) or venography. Pulmonary embolism should be diagnosed by either perfusion-ventilation or lung scanning, helical CT scan or pulmonary angiography. *Start Date and Duration.* October 2006, duration 3 years. *Design.* The registry is a multicenter, observational study designed to record data on VTE in RT patients to obtain information on: i) Epidemiology; ii) Type and timing of thromboprophylaxis; iii) Treatment complications; iv) VTE recurrences; v) Total and cardiovascular mortality. The study includes: the retrospective registration of VTE cases verified in RT patients followed by the participating Centre in the previous 3-5 years, and the prospective registration of all new VTE episode. 1. All the first VTE episodes occurred in the cohort of RT patients followed by the single Centre in the last 5 years and all clinical data available will be registered. Recurrent VTE episodes in these patients during the last 5 years also will be registered. 2. All new VTE episodes will be registered. The patients with previous VTE should be followed up at periodic intervals (3-6 months) to register the eventual VTE recurrence and clinical outcome. Differently from randomized trial, there is no imposed experimental intervention: management is determined solely by responsible physician who will register data. Reported data will reflect real-world approaches and clinical outcomes of RT patients in participating Centres. The improvement of the knowledge about VTE in RT patients should be useful to identify the best treatment for these patients.

Intervention. The parameters recorded comprise details of each patient's baseline characteristics: clinical status, type of event, timing and type of thromboprophylactic treatment, adverse events during thromboprophylactic treatment, recurrent VTE episode. Each participating Centre gives data on RT patients followed up. *Statistics.* Descriptive and qualitative statistical analysis will be employed. *Sponsor.* None.

P002

ITALIAN REGISTRY OF PEDIATRIC STROKE

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Background. Arterial Ischemic Stroke (AIS) in children is very rare, with a reported incidence ranging from 0.063 to 0.12 per 10,000 children per year and an AIS/SVT ratio of 3:1. Nevertheless, stroke is an important cause of mortality and chronic morbidity in children. Of the hundreds of children who suffer a stroke each year, more than half will have permanent motor or cognitive disability. Perinatal stroke occurs between the 28th week of gestation and 1 month of age, whereas childhood stroke is observed later. Neonates accounted for 25% of all cases of AIS in the Canadian Registry, which is equivalent to an incidence of 93/105 live births. No data regarding this problem are available in Italy. Since stroke is quite infrequent in children and newborns, the result is often delayed recognition of the problem and the inability to intervene early with medication that could reduce subsequent neurological deficits. Although rare, the incidence of stroke in children is similar to the incidence of brain tumours, for which a coordinated research approach has significantly decreased the mortality and morbidity. *Inclusion criteria.* All children between 1 month and 16 yrs of age with documented AIS. For prospetive study informed consent from parents will be obtained. Newborns after the 28th week of gestation will be enrolled. *Exclusion Criteria*. Newborns and children with no documented AIS and lack of informed consent for prospectively enrolled patients. *Start date and Duration.* January 2007, three years. *Design.* The registry will retrospectively and prospectively enroll all children with AIS. A pre-study survey will be carried out among all participating centres to collect information about their diagnostic facilities. Informed consent to participate in the study will be obtained from the parents for prospectively enrolled patients. The study form will be filled in directly at diagnostic work up, during hospitalisation, and at discharge. With regards to retrospective analysis, all the clinical records of children diagnosed with AIS between 1996 and 2006 will be reviewed and the data will be collected using a specific form The follow up form for all patients (regardless of retrospective or prospective enrolment) will be filled in at later check-up visits. We plan to call back all retrospectively enrolled patients to have them undergo investigation for any prothrombotic conditions that were not carried out during previous admissions. Standard diagnostic tests (PS, PS and AT, as well as FV G506Q and Pr G20210A mutations), radiologic and cardiologic diagnostic procedures (CT scan, MRI and /or angio MRI, echo Doppler and echocardiogram) will be performed on site. Additional testing for prothrombotic or metabolic alterations with possible prothrombotic roles (P-Selectin, lipoprotein (a), PAI-1, homocysteine), as well as assays that are not routinely performed at all centres will be centralised in the Giannina Gaslini Hospital laboratory. DNA will be stored in order to carry out extensive work up, both for all the reported mutations which may play a role in determining thrombotic risk, as well as for any newly described mutations. Presently, two guidelines for the treatment of AIS in children are available in the literature, i.e., the UK guidelines (ASA 5 mg/kg/day from onset) and the Chest guidelines (LMWH 1 mg/kg every 12 hours for 7 days followed by ASA 3-5 mg/kg/day). We strongly suggest that all participants comply with one of the two available guidelines (which will both be provided to the participating centres). Since there are no existing guidelines for the treatment of AIS in newborns, no standardised treatment is scheduled and each participating centre will continue following its own policy. *Intervention.* Three data collecting forms will be used: one to retrospectively record events, one to prospectively collect data regarding acute events, and one for the follow up of both. A specific database will be set up for data entry and processing. A web based registry will likely be established in a later phase of the study. *Statistics.* Descriptive and analytical statistics for paired data will be employed. Sponsor. None.

P003

ITALIAN REGISTRY OF VENOUS THROMBOSIS IN CHILDREN

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Background. Thromboembolic disease in children has become more frequent over the last decade. Advances in tertiary care paediatrics have paradoxically resulted in rapidly increasing numbers of children who are diagnosed with venous thrombosis. Newer imaging modalities, such as Doppler ultrasound (US), CT scans, and MRI, are more widely available than ever before and have improved diagnosis. Use of central venous catheters has become standard in the care of critically ill children and has contributed greatly to the increased rate of thrombotic events. However, the incidence of CVL-related venous thromboembolisms (VTEs) reported in the literature varies, reflecting different underlying conditions, the use of different diagnostic tests, and different indexes of suspicion. Finally, children who are born prematurely or who develop life-threatening or chronic medical conditions are surviving longer because of advanced medical therapies. These intensive therapies can be complicated by events, such as thrombosis; we are beginning to realize the extent of this problem. The estimated incidence of symptomatic VTE in children is 5.3 per 10,000 hospital admissions. The development of national and international registries helped to increase awareness of thrombosis in children and focus attention on the serious need for objective data regarding epidemiology, aetiology, diagnosis, treatment and outcome. No data regarding these issues are available in Italy. As far as newborns are concerned, an international registry of symptomatic VTEs in neonates reported an incidence of 2.4 per 1,000 admissions to neonatal intensive care units. A German prospective, nationwide, 2-year registry reported an incidence of symptomatic neonatal TE (which included CNS events) to be 0.51 per 10,000 births, with approximately half of the cases being VTE and half being arterial thrombosis. Also these points should be outlined in Italy. There is no published information on the incidence of recurrent VTE in neonates. There is also no published information on the incidence of Post Thrombotic Syndrome (PTS) in neonates. Potentially, neonates are at an increased risk for PTS because the fibrinolytic system is physiologically suppressed. The long-term follow-up of neonates with VTE is critically important to determine the frequency and severity of PTS. *Inclusion Criteria.* All children between 1 month and 16 yrs of age and newborns after the 28th week of gestation with documented venous thrombosis with informed consent from parents will be enrolled. *Exclusion Criteria.* Newborns and children with no documented venous thrombosis. Missing informed consent. *Start Date and Duration.* January 2007, three years. *Design.* The registry will prospectively enroll all children with venous thrombosis. A pre-study survey will be carried out among all participating centres to collect information about their diagnostic facilities. Informed consent to participate in the study will be obtained from the parents. The study form will be filled in directly at diagnostic work up, during hospitalisation, and at discharge. The follow up form for all patients (regardless of retrospective or prospective enrolment) will be filled in at later check-up visits. Standard diagnostic tests (PS, PS and AT, as well as FV G506Q and Pr G20210A mutations), radiologic and cardiologic diagnostic procedures (CT scan, MRI and /or angio MRI, echo Doppler and echocardiogram) will be performed on site. Additional testing for prothrombotic or metabolic alterations with possible prothrombotic roles (P-Selectin, lipoprotein (a), PAI-1, homocysteine), as well as assays that are not routinely performed at all centres (anti Xa) will be centralised in the Giannina Gaslini Hospital laboratory. DNA will be stored in order to carry out extensive work up, both for all the reported mutations which may play a role in determining thrombotic risk, as well as for any newly described mutations. *Intervention.* Two data collecting forms will be used: one to prospectively collect data regarding acute events and one for the follow up. A specific database will be set up for data entry and processing. A web based registry will likely be established in a later phase of the study. *Statistics.* Descriptive and analytical statistics for paired data will be employed. *Sponsor.* None to date

P004

GTR: GLANZMANN'S THROMBASTHENIA REGISTRY - TREATMENT OF GLANZMANN'S THROMBASTHENIA. A PROSPECTIVE OBSERVATIONAL REGISTRY

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International Expert Panel: Giovanni Di Minno, Italy; Yves Laurian, France; Man-Chiu Poon, Canada; Rainer Zotz, Germany

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Background. Glanzmann's thrombasthenia (GT) is a rare (1/1,000,000) congenital bleeding disorder due to deficiency/dysfunction of the platelet glycoprotein IIb/IIIa receptor. Bleeding tendency in GT patients is highly variable and also treatment demands vary considerably. Mild-moderate bleeding can be often be controlled by local measures or hemostatic agents or by antifibrinlytic agents. When such approaches fail or for severe bleeding, and for prophylaxis for surgery, platelet concentrate transfusions is the standard therapy. About 15-30% of patients develop anti-HLA or anti-IIb/IIIa antibodies and become refractory to platelet transfusions. Over the last decade, activated recombinant Factor VII (rFVIIa) has documented efficacy in GT patients for treatment of bleeding and hemostatic cover of invasive procedures and surgery. Recently rFVIIa has been licensed within European Union for GT patients with allo-imunization and/or past/present refractoriness to platelet transfusion. *Inclusion Criteria:* Patients (males and females) with GT of any age. Informed consent. Treatment for bleeding or surgical coverage considered necessary by physician. No limit to the number of treatments for each patient. *Exclusion criteria.* Acquired thrombasthenia. *Start Date and Duration.* Q1 2005, 6 years. *Design.* Prospective observational multi-center multi-national registry collecting data on the use of rFVIIa and other hemostatic agents in GT patients with platelet refractoriness. *Intervention.* Web-based standardized data collection (www. glanzmann-reg.org). Patient Baseline information (demographics, clinical history, with emphasis on anti-HLA anti-IIb/IIIa and platelet reractoriness status). Bleeding episode (cause, type, onset of symptoms). Surgery or invasive procedure (type, indication, date and time). Treatment (rFVIIa +/- antifibrinolytics and/or platelets, DDAVP +/- antifibrinolytics). Efficacy evaluation. Complications/adverse events. *Statistics.* No limit for sample size. All treatment allocated subjects for whom the efficacy endpoint is known. All patients will be included in the safety analysis. *Sponsor.* Novo Nordisk.

P005

OPTIMAL DURATION OF ANTITHROMBOTIC THERAPY IN CANCER PATIENTS WITH DEEP VEIN THROMBOSIS: THE ROLE OF RESIDUAL VEIN THROMBOSIS. THE "CANCER DACUS" STUDY

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Background. The optimal choice and duration of antithrombotic therapy in cancer patients with deep vein thrombosis (DVT) of the lower limbs is still unresolved. Recent guidelines suggest that patients with active cancer should be treated for at least 6 months. New investigations have highlighted the role of Low Molecular Weight Heparin (LMWH) instead of warfarin for the long treatment of DVT, since heaprin has shown a safer and more efficacious profile than oral anticoagulants (Lee a et al. N Engl J Med. 2003 Jul 10;349(2):146-53). Individual parameters (such as D-dimer or evaluation of residual vein thrombosis -RVT-) are becoming useful tools for deciding the optimal duration of oral anticoagulants (Palareti et al. Thromb Haemost. 2002 Jan;87(1):7-12., Siragusa S et al. JTH 2005;3:OR176). These parameters show that persistently negative D-dimer or absence of RVT after 3 month of warfarin are related to a very low risk of recurrency, respectively. In cancer patients, the detection of RVT, as individual parameter for deciding the duration of antithrombotic therapy, seems the most suitable approach because of its reproducibility and absence of variation by chemotherapy and tumor staging (such as D-dimer). However, no randomized trials have evaluated its role for establishing the optimal duration of antithrombotic therapy in cancer patients with an episode of DVT. *Inclusion criteria.* Cancer patients with a first episode of DVT with a life expectancy of at least 1 year. Absence of symptoms of PE. Exclusion criteria: recurrent DVT, life expectancy < 1 year, indication for long-term anticoagulation, refusal of consent. *Start date and Duration.* 2006-2008. *Design.* It is proposed a randomized, controlled trial with 3 arms of intervention. All patients with a first episode of DVT of the lower limbs will be treated with LMWH for 6 months. At this time, the presence/absence of RVT will be detected. Patients with RVT will be randomly assigned to continue (for additional 6 months) or to stop LMWH therapy. Those without RVT will stop RVT. All patients will be followed-up for 2 years for evaluating the occurence of recurrent DVt or Pulmonary Embolism (PE) and/or major or minor bleeding. *Intervention.* All patients with a first episode of DVT of the lower limbs will be treated with LMWH for 6 months (full dosage for the first month then dose reduced by 75% in the following 5 months) from the index DVT. At this time, the presence/absence of RVT will be detected as previously reported (Siragusa et al. JTH 2005;3:OR176). Patients with RVT will be randomly assigned to continue (for additional 6 months, group A1) or to stop LMWH therapy (group A2). Those without RVT will stop RVT (group B). All patients will be followed-up for 2 years from the index DVT for evaluating the occurence of recurrent DVT or Pulmonary Embolism (PE) and/or major or minor bleeding. RVT will be investigated at the common femoral vein and at the popliteal vein; if any of these two sites will found to have the presence of RVT, the patient will be considered as belonging to group A and consequently randomized (A1 continue LMWH or A2 stop LMWH). Any sign or symptom suggestive of recurrent DVT and/or PE have to be confirmed by objective testing. Interpretation of events will be independently reviewed by clinicians unaware of patients allocation. *Statistics.* It has been estimated that in patients with RVT the risk of recurrent DVT is about 20% in comparison to those without RVT who have an estimated risk of recurrency of 5%. It has been estimated that 300 patients are required to reach a power of 80% for documenting a difference of 15% between at least two groups (Bonferroni method with error I -0.05- for multiple comparisons). *Sponsor.* None.

Disclosure index

SISET is committed to providing unbiased, balanced and objective scientific information. All presenters were asked to disclose any relationships with corporate entities that might affect or appear to affect the objectivity of their presentations. Below is a list of abstracts on which the authors have provided such disclosures.

Oral Communications

C055

NCX-4016, BUT NOT ASPIRIN, PREVENTS THE ACUTE HYPERGLYCEMIA-INDUCED ENHANCEMENT OF SHEAR STRESS-INDUCED PLATELET ACTIVATION IN TYPE 2 DIABETES MELLITUS (TD2M)

Gresele P, Marzotti S, Guglielmini G, Falcinelli E, Giannini S, Minuz P, Ballabio M, Bolli GB

C068

SOLUBLE E-SELECTIN AND TISSUE FACTOR PATHWAY INHIBITOR, BUT NOT C-REACTIVE PROTEIN, CORRELATE WITH CAROTID INTIMA-MEDIA THICKNESS (C-IMT) IN STABLE CORONARY PATIENTS: THE BASELINE DATA OF THE MIAMI STUDY

Porta B, Baldassarre D, Camera M, Amato M, Arquati M, Tremoli E, Cortellaro M, on behalf of the MIAMI Study Group

Posters

P032

SINGLE BOLUS OF RECOMBINANT FACTOR VIIA FOR PROPHYLAXIS OF BLEEDING DURING SPONTANEOUS DELIVERY IN A PATIENT WITH FACTOR VII DEFICIENCY

Imbimbo V, Spina M, Guarino C, Esposito E, Abagnale I, Pinotti M, Cimino E, Coppola A

Proposed Research Protocols

P004

GTR: GLANZMANN'S THROMBASTHENIA REGISTRY - TREATMENT OF GLANZMANN'S THROMBASTHENIA. A PROSPECTIVE OBSERVATIONAL REGISTRY

Di Minno G, Coppola A, Margaglione M

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