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XX Congress of the Società Italiana per lo Studio dell'Emostasi e della Trombosi (SISET) (Italian Society for Studies on Hemostasis and Thrombosis) Firenze, Italy, September 25 - 28, 2008

ABSTRACT BOOK

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Società Italiana per lo Studio dell'Emostasi e della Trombosi

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

(Italian Society for Studies on Hemostasis and Thrombosis)

Florence, Italy, September 25-28, 2008

ABSTRACT BOOK

SISET

XX Congress of the
Società Italiana per lo Studio dell'Emostasi e della Trombosi
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New submission and tracking system

On September 1st, 2008, Haematologica adopted Bench>Press as its new web-based manuscript submission and tracking system. Below you find some new instructions regarding submission of papers.

Original Articles across all areas of experimental and clinical hematology are the prototype of papers the journal is looking for. *Brief Reports* are just shorter papers, which must provide conclusive findings: preliminary observations or incomplete findings cannot be considered for publication. *Case Reports* are no longer considered for publication: please do not submit them as *Brief Reports*, unless they include peculiar studies such as those concerning molecular basis of disease. *Review Articles* are typically solicited by the Editors, but the journal may also consider reviews submitted on authors' own initiative: pre-submission inquiries are welcome. *Decision Making and Problem Solving* papers typically include meta-analyses, guidelines and position papers by scientific societies. *Letters to the Editor* should typically refer to a recent article; letters not about a journal article may also be considered, but the authors should be aware that the journal can publish only a small minority of them due to space constraints. *Editorials & Perspectives* are typically solicited by the Editors to accompany an accepted manuscript: the Editors discourage submission of non-invited manuscripts and welcome pre-submission inquiries.

A non-refundable fee of Euro 50 is due on submission of *Original Articles, Brief Reports, Decision Making & Problem Solving* articles, unsolicited *Review Articles*, unsolicited *Editorials* and unsolicited *Perspective Articles*. No submission fee is required for *Letters to the Editor* or *Responses to a Letter to the Editor*. If a submission fee is required, the authors will be asked to pay it online using a credit card in order to complete the submission process. Authors are solicited to have all required information (see below) ready at the time of submission.

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Registration of clinical trials. Haematologica fully adopts the ICMJE policy on clinical trial registration (Obligation to Register Clinical Trials: http://www.icmje.org/#clin_trials). The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Haematologica requires, as a condition of consideration for publication of a clinical trial, registration in a public trials registry. The journal considers a trial for publication only if it has been registered before the enrollment of the first patient. This policy applies to trials that started recruiting on or after July 1, 2005. As regards trials that started recruiting before July 1, 2005, Haematologica considers for publication those that have been registered before September 13, 2005. Haematologica does not advocate one particular registry, but requires authors to register their trial in a registry that meets several criteria. The registry must be accessible to the public at no charge. It must be open to all prospective registrants and managed by a not-for-profit organization. There must be a mechanism to ensure the validity of the registration data, and the registry should be electronically searchable. An acceptable registry must include a minimum of data elements (http://www.icmje.org/#clin_trials). For instance, ClinicalTrials.gov <www.clinicaltrials.gov>, sponsored by the United States National Library of Medicine, meets these requirements. Before proceeding with the submission of any clinical trial that has not been registered according to the above ICMJE criteria, the authors should carefully consider that Haematologica would be unable to publish it. The journal welcomes *ad hoc* pre-submission inquiries.

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Oral Communications

Atherothrombosis and Inflammation: Physiopathological Aspects

C001

GENE EXPRESSION PROFILING OF PERIPHERAL BLOOD PERTURBATION IN ABDOMINAL AORTIC ANEURYSM. IDENTIFICATION OF NOVEL PATHOPHYSIOLOGICAL MECHANISMS

Giusti B, Rossi L, Lapini I, Magi A, Pratesi G, Lavitrano M, Biasi GM, Fatini C, Pulli R, Pratesi C, Gensini GF, Abbate R

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Background. The pathogenesis of abdominal aortic aneurysm (AAA) remains poorly understood. Due to the multifactorial nature of AAA, microarray technologies are ideal tools to screen genes involved in AAA pathophysiology. Aim of this study was to investigate gene expression profile of peripheral blood of AAA patients by using microarray technology to provide insight into AAA systemic pathophysiology. *Results.* Microarray data on 14.000 transcripts analyzed in 10 AAA patients and 10 matched controls were confirmed in two different AAA (n=36) and control (n=36) populations by real-time PCR and were integrated with functional data. We identified 91 genes differentially expressed in AAA patients with respect to controls. Gene Ontology analysis identified that patients differentially express a large variety of transcripts involved in oxygen transport, red cell mechanical stability regulation, and lipid metabolic process. In particular, we demonstrated, for the first time in humans, that the downregulation of low density lipoprotein receptor-related protein 5 (LRP5) gene is associated with increased levels of lipoprotein (a), a well known atherothrombotic risk factor. Moreover, a large panel of erythrocyte genes resulted differentially expressed in patients showing a relationship with the alteration of haematological parameters such as hematocrit and erythropoietin. *Conclusions.* These data indicate that hyperexpression of adult, fetal and embryonic haemoglobin chain genes and of genes involved in erythrocyte mechanical stability, as well as downregulation of LRP5 participate in pathophysiological mechanisms of AAA. Our data underline the power of microarray in identifying further pathophysiological hypotheses for AAA.

C002

CAROTID ARTERY DISEASE: IDENTIFICATION OF NOVEL PATHOPHYSIOLOGICAL MECHANISMS BY GENE EXPRESSION PROFILING OF PERIPHERAL BLOOD CELL PERTURBATION

Rossi L, Lapini I, Magi A, Pratesi G, Capalbo A, Pulli R, Lavitrano M, Biasi GM, Pratesi C, Gensini GF, Abbate R, Giusti B

Department of Medical and Surgical Critical Care, University of Florence,and Department of Heart and Vessels, Azienda Ospedaliero-Universitaria Careggi, Florence; Vascular Surgery Unit, Department of Surgery, University of Rome Tor Vergata, Rome; Department of Surgical Sciences, University of Milano-Bicocca, Italy; Department of Vascular Surgery, University of Florence, Italy

Carotid artery disease (CAS) is the most frequently identified cause of ischemic stroke and is mostly due to atherosclerotic disease. Inflammation plays an important role in the pathogenesis of atherosclerosis. Aims of this study were to investigate the systemic gene expression profile of patients affected by CAS versus control subjects, and validate and extend microarray data in two further independent populations of patients and controls. Total RNAs were extracted from whole peripheral blood of 46 patients affected by CAS and 46 controls comparable for age and sex. We determined the expression of 14,000 genes by two colors microarray technology in n=10 pooled RNA from patients and n=10 pooled RNA from controls and validated data by real time PCR in n=36 CAS patients and n=36 controls. After data processing and application of the filtering criteria, the differentially expressed genes between CAS patients and controls were 82: 61 genes resulted up-regulated and 21 down-regulated. Gene ontology analysis indicated an alteration of the following biological processes: immune response, oxygen transport, cytoskeleton organization and lipidic metabolism. Some biological process found in CAS and the relative associated genes resulted similarly altered in patients affected by atherosclerotic lesions in an other district (abdominal aortic aneurysm patients). In particular we focused our attention in validating genes associated with the biological process peculiarly observed in CAS patients. These genes encod for the major histocompatibility complexes, expressed by the human leukocyte antigens (HLA) or are involved in the immune response (HLA-DPA1, HLA-DPB1, HLA-DQB1, HLA-DRB3, IFIT1, IGKV1D, TRBJ2-1, DBNL, HLA-B). This study provides new insights into the regulatory mechanisms controlling the development and the progression of plaque emphasizing the central role of inflammatory and immune cells.

C003

OXIDATIVE STRESS IS ASSOCIATED WITH ARTERIAL DYSFUNCTION AND ENHANCED INTIMA-MEDIA THICKNESS IN CHILDREN WITH HYPERCHOLESTEROLEMIA. POTENTIAL ROLE OF NADPH OXIDASE

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Background. Endothelial dysfunction and intima-media thickness (IMT) are precocious manifestations of hypercholesterolemia but the mechanism is unclear. *Objective.* Aim of the study was to analyse the interplay among endothelial dysfunction, IMT and oxidative stress in hypercholesterolemic children (HC). *Methods.* We performed a cross-sectional study comparing flow mediated dilation (FMD), IMT, lipid profile, urinary isoprostanes as markers of oxidative stress, and platelet expression of gp91phox, the catalytic unit of NADPH oxidase, in a population of 50 HC (mean age 10.0±3.7 years) and 50 normocholesterolemic children (NC) (mean age 9.2±3.5 years). Four children with hereditary deficiency of gp91phox were also studied. *Results.* HC had reduced FMD (6.2±2.4 *vs.* 9.2±2.5 per cent; $p<0.001$) and enhanced IMT (0.45±0.07 *vs.* 0.40±0.06 mm; $p=0.002$), urinary isoprostanes (86.9±51.6 *vs.* 45.9±25.6 pg/mg of creatinine; $p<0.001$) and gp91phox platelet expression (4.4±3.8 *vs.* 2.0±1.7 mean fluorescence; $p<0.001$) compared to controls. At bivariate analysis, FMD was correlated with LDL cholesterol (r=-0.448; $p<0.001$), IMT (r=-0.356; $p=0.01$), urinary isoprostanes (r=-0.388; $p=0.005$) and platelet gp91phox (r=-0.314; $p=0.02$). Stepwise multiple linear regression analysis showed that in HC, FMD and IMT were significantly associated with LDL cholesterol and urinary isoprostanes; also, gp91phox platelet expression was an independent predictor of urinary isoprostanes. Children with gp91phox hereditary deficiency showed down-regulation of platelet gp91phox and reduced urinary excretion of isoprostanes. *Conclusions.* The study suggests that gp91phox -mediated oxidative stress may have a pathogenic role in the anatomic and functional changes of the arterial wall occurring in children with premature atherosclerosis.

C004

PHOSPHODIESTERASE -TYPE IV-CAMP-PKA AXIS REGULATES SFK-PYK2 PATHWAY, PMN/ PLATELET ADHESION AND PMN ACCUMULATION AT THE SITE OF VASCULAR INJURY

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Pharmacological modulation of leukocyte (PMN) recruitment on

platelets (PLT) adherent at the site of vascular injury represents a novel approach to reduce the progression of atherothrombosis and to prevent neointimal proliferation and restenosis post-angioplasty. We previously established that Src family kinase (SFK) activity is required for Mac-1-dependent firm adhesion of PMN to PLT, *in vitro*, and for PMN accumulation at the site of vascular injury, *in vivo*. The focal adhesion kinase, Pyk2, was suggested as downstream effector of SFK. In the present study we used a flow adhesion assay and a murine model of arterial injury, to confirm the role of Pyk2 in PMN recruitment on PLT. When sheared on adherent PLT, 75% of interacting PMN arrested and became firm adherent. Pharmacological blockade of Pyk2 activity by Tyrphostin A9 significantly reduced to 38%±7 (mean±sem, n=5) the fraction of firm adherent and slightly affected the number of interacting PMN. PMN accumulation at the site of injury, was evaluated one hour after guidewire-induced de-endothelization of femoral artery, in mice. Fewer PMN accumulated in Pyk2-null, respect to wild-type mice (0,5±0,05 and 13±6 PMN/microscopic field, respectively). Intimal hyperplasia was measured four weeks after damage. The intimal/media ratio was reduced in Pyk2-null, respect to wild-type mice (0,07±0,01 and 0,7±0,13, respectively). Then, we explored the effect of phosphodiesterase(PDE)-type IV blockade on PMN adhesion to PLT, *in vitro* and *in vivo*. Selective inhibitors (rolipram, RO 201724 and zardaverine) of PDE-type IV, but not inhibitors of PDE-III or -V, dose-dependently reduced PMN-PLT adhesion both, in mixed cell suspension and in flow assays. Moreover, blockade of PDE-IV inhibited Pyk2 phosphorylation. The effect of PDE-IV blockade on PMN adhesion and Pyk2 phosphorylation was prevented in PMN treated with inhibitors of PKA activity. The effect of PDE-type IV blockade on PMN accumulation was analyzed *in vivo*, one hour after damage. At this time, fewer PMN accumulated at the site of endothelial denudation, in rolipram (10mg /Kg i.p.)-treated, respect to untreated mice(2.2±0.5 and 34±7 PMN/field, respectively). These results indicate that PDE-cAMP-PKA axis negatively regulates SFK-Pyk2 activity and PMN adhesion to activated PLT. PDE-type IV may be a novel pharmacological target to reduce PMN accumulation at the site of arterial injury.

C005

WHOLE BLOOD PROCOAGULANT ACTIVITY AS A POSSIBLE CELLULAR MARKER OF CARDIOVASCULAR RISK

Napoleone E, Zurlo F, Gozdzikiewicz J, De Curtis A, Costanzo S, Vohnout B, Iacoviello L, Donati MB, Lorenzet R

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Introduction. The aim of this work was to test whole blood (WB) procoagulant activity (PCA) as a possible marker of cardiovascular risk in a general population. *Methods.* The study population included 252 consecutive apparently healthy subjects (aged 45±19 SD; 42% men) randomly recruited from the general population of a Southern Italian region in the framework of an epidemiological study. At enrolment, WB was drawn and incubated for 2h at 37°C with or without bacterial endotoxin (LPS) or tumor necrosis factor-α (TNF-alpha). At the end of incubation, PCA of intact cells was assessed by a one-stage clotting time. In validation experiments, a monoclonal anti-tissue factor (TF) antibody (IL, Milan, Italy) was added to WB 30 min before the clotting assay. Plasma TF levels were assessed by ELISA (IL, Milan, Italy). Analysis, adjusted for sex and age, compared the 4th with the 1st quartiles of PCA distribution. *Results.* Basal, LPS- and TNF-α-stimulated WB PCA was significantly higher in the subjects who had a familial history either of myocardial infarction or of stroke ($p<0.0001$). Subjects whose basal and LPS-stimulated WB PCA was in the 4th quartile, had a trend for higher levels of CRP. However, only the results with TNF-α-stimulated WB PCA were significant ($p=0.036$). Surprisingly, lower levels of cholesterol, LDL and HDL were associated with higher WB PCA both in basal and stimulated conditions. No significant association was found between WB PCA and: BMI, waist to hip ratio, blood pressure, blood glucose and smoking habits. Subjects with stronger basal WB PCA tended to have increased levels of plasma TF antigen.About 70% of WB PCA following stimulation of WB with LPS was, attributable to TF. *Conclusions.* In a large population sample WB PCA was found positively associated with a cardiovascular risk factor, such as CRP, while its inverse association with cholesterol levels warrants further study. WB PCA, an assay reflecting cellular TF expression, may be developed as a simple and reliable test for large scale epidemiological studies.

Supports: Telethon GGP04198 and MIUR 1588-19/11/2004.

C006

ENDOTHELIAL DYSFUNCTION IN HIV-INFECTED PATIENTS: EFFECTS OF HAART

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Since the introduction of highly active antiretroviral therapy (HAART), in the mid '90s, human immunodeficiency virus (HIV) infection has changed from a rapidly fatal disease into a chronic condition. With the prolongation of the average lifetime new causes of morbidity and mortality have emerged in HIV-infected patients, in particular ischemic cardiovascular disease. It is still debated whether cardiovascular complications are a consequence of the HIV infection itself or of HAART which is known to induce alterations of the lipid profile. Endothelial dysfunction is a precocious marker of atherosclerosis with predictive value for cardiovascular events. Aim of our study was to evaluate markers of endothelial and platelet activation in HIV-infected patients compared with healthy subjects, and to assess whether they are affected by HAART therapy, and to assess if there is a difference between treatment with protease inhibitors (PIs) or non-nucleoside reverse transcriptase inhibitors (NNRTs). In a retrospective, case-control study, the time course of some endothelial and platelet activation markers (sVCAM-1, MCP-1, sCD40L and sP-sel) was examined in 56 HIV-infected patients before and during (3, 6, 12 and 24 months) HAART therapy with PIs (n=28) or NNRTs (n=28). Data were compared with those obtained from 28 healthy age- and sex-matched controls. At diagnosis (baseline) plasmatic levels of soluble P-selectin (sP-sel), vascular cell adhesion molecule-1 (sVCAM-1) and monocyte chemoattractant protein-1 (MCP-1) were significantly higher in HIV positive subjects than in controls (422.6±28.3 *vs.* 235.2±24.2 ng/mL, 1105.9±90.9 *vs.* 676.3±58.9 ng/mL and 279.6±33.9 *vs.* 158.8±23.0 pg/mL, respectively, $p<0.05$) while those of sCD40L were within the normal range. During the 24 months of follow up, sVCAM-1 and MCP-1 progressively decreased, without significant differences between patients treated with PIs or with NNRTIs. On the contrary, sP-sel levels remained elevated during treatment. A subgroup of 10 untreated HIV-infected patients was studied at diagnosis and after 12 months. In these patients too levels of sP-sel, sVCAM-1 and MCP-1 were higher than in healthy controls at baseline, but remained elevated after 12 months of untreated infection. Our results show that HIV infection itself, and not its pharmacological treatment, induces endothelial dysfunction and that a short-term treatment with HAART reduces some parameters of endothelial dysfunction.

C007

RAPID AND REVERSIBLE INCREASE OF INDUCIBLE CYCLOOXYGENASE (COX-2) IN MONOCYTES OF PATIENTS WITH TYPE II DIABETES MELLITUS (T2DM) EXPOSED *IN VIVO* TO HYPERGLYCEMIC SPIKES

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T2DM is associated with a two-to-four fold increase of vascular complications and an important prognostic factor is post-prandial hyperglycaemic spikes. Monocyte inducible COX-2 has been advocated as a player in the pathogenesis of ischemic events. Aim of our study was to assess whether acute, short-term hyperglycaemia affects COX-2 expression in monocytes of patients with T2DM, and to evaluate the kinetics of the phenomenon. 120 patients with T2DM (HbA1c 8±1.14%; FBG 180±9 mg/dL; age 67±9 years) and 50 age and sex-matched healthy controls (HbA1c 5.9±0.3%; FBG 94±10 mg/dL; age 62±13 years) were studied. COX-2 in purified peripheral blood monocytes was measured by Western Blotting. COX-2 was expressed in monocytes of 87.5% of T2DM patients (105/120), and in 4% of normal volunteers (2/50; $p<0.01$). Average monocyte COX-2 was 0.38±0.06ng/microgram total proteins in T2DM. A statistically significant correlation was present between monocyte COX-2 and FBG ($r^2=0.27$; $p=0.05$) or HbA1c ($r^2=0.25$; $p=0.05$). Ten patients with T2DM underwent a 4 hrs hyperglycaemic clamp (blood glucose 13.9 mM): COX-2 expression in monocytes increased two-fold (from 0.45±0.1 to 0.88±0.18 ng/microgram total proteins; $p<0.001$). Five poorly controlled T2DM subjects, kept under strict metabolic control for 3 months (HbA1c decreased from 8.1±0.4% to 7±0.6%; $p<0.01$) showed a significant decrease of COX-2 in monocytes (from 0.18±0.06 to 0.12±0.05 ng/microgram total proteins; $p<0.05$). Four T2DM subjects in good metabolic control (HbA1c: 6.35±0.4%) underwent 4 hrs of hyperglycaemia and subsequently to 4 hrs euglycaemia. COX-2 expression in monocytes increased after hyperglycaemia (from 0.37±0.18 to 0.79±0.16 ng/microgram total proteins), and strikingly decreased after further 4 hrs euglycaemia (0.79±0.16 to 0.16±0.06 ng/microgram total proteins). Monocytes isolated from healthy volunteers cultured in 22 mM (high) glucose-containing medium for 4 hours showed a significant increase of COX-2 (from 0 to 0.6±0.15 ng/microgram total protein, $p<0.001$). A further four hours incubation in a 5.5 mM (normal) glucose-containing medium reduced COX-2 (to 0.3±0.11 ng/microgram total protein; $p<0.01$) and incubation for additional 20 hours abolished it ($p<0.01$). Acute, short-term hyperglycaemia induces a rapid, reversible, upregulation of monocyte COX-2; increased monocyte COX-2 may contribute to the pathogenesis of cardiovascular complications in T2DM and to aspirin non-responsiveness.

Thrombophilic Syndromes

C008

INHERITED OR ACQUIRED THROMBOPHILIC ALTERATIONS IN PATIENTS WITH SUPERFICIAL VEIN THROMBOSIS

Legnani C, Cini M, Monteduro P, Lo Manto G, Frascaro M, Boggian O, Palareti G

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Inherited or acquired thrombophilic alterations (TA) are associated with increased risk of deep vein thrombosis (DVT), but whether they are also risk factors for SVT is uncertain, mainly due to the small number of patients included in the published papers. We evaluated the prevalence of inherited and acquired TA in a large population of patients with previous SVT events. All consecutive patients with a coding of leg SVT between the years 1990-2007 were sorted from our database. Patients with a previous history of DVT, pulmonary embolism (PE) or SVT associated to varicose veins were excluded. A total of 692 patients (SVT group; 497 females; mean age: 44 y, range: 15-91y; 236 patients with recurrent SVT episodes) were included. The TA prevalence in the SVT group was compared with that in 576 apparently healthy subjects (Control group; 346 females; mean age 48 y, range: 18-86 y) and in 1142 patients with DVT events, with/without PE (DVT group; 603 females; mean age: 51 y, range: 11-87 y). The following TA were considered: Antithrombin (AT), Protein C (PC) or Protein S (PS) deficiency; Factor V Leiden (FVL) and G20210A prothrombin (PT) mutation, Lupus Anticoagulant and/or increased antiphospholipid antibody levels (anticardiolipin and anti beta 2 Glycoprotein I) (LA); increased homocysteine (Homo) and Factor VIII (FVIII) levels. The prevalence of the TA in the three examined groups and the univariate odds ratios are reported in the Table 1. No significantly differences were found in the prevalence of the thrombophilic alterations in patients with recurrent vs those with a single SVT event. In conclusion, though TAs in SVT patients are less prevalent than in DVT patients, they are however significantly more frequent (with the exclusion of PT mutation) than in Control group and are associated with increased risk for SVT. The laboratory search for these alterations is recommended in patients with SVT.

Table 1. Prevalence (n, %) and OR (95%CI) of inherited and acquired thrombophilic alterations.

	SVT group N=692	*DVT group N=1142*	*Control group N=576*
AT, PC or PS deficiency	12 (1.7%)	32 (2.8%)	1 (0.2%)
OR	10.1 (1.31-78.3)	16.6 (2.26-121.6)	1 (Ref)
FVL mutation	70 (10.1%)	153 (13.4%)	18 (3.1%)
OR	3.49 (2.05-5.93)	4.80 (2.91-7.90)	1 (Ref)
PT mutation	34 (4.9%)	99 (8.7%)	29 (5.0%)
OR	0.97 (0.59-1.62)	1.79 (1.17-2.74)	1 (Ref)
Combined inherited alterations	8 (1.2%)	26 (2.3%)	0
LA	20 (2.9%)	32 (2.8%)	5 (0.9%)
OR	3.40 (1.27-9.11)	3.29 (1.28-8.49)	1 (Ref)
High Homo #	50/282 (17.7%)	185 (16.2%)	59 (10.2%)
OR	1.89 (1.26-2.84)	4.09 (2.68-6.25)	1 (Ref)

C009

ENDOGENOUS THROMBIN POTENTIAL (ETP) ANALYSIS OF LUPUS ANTICOAGULANT PLASMA WITH THE BMS (DADE BEHRING SIEMENS) SYSTEM

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Dade Behring Siemens has developed a chromogenic assay for endogenous thrombin potential (ETP) analysis using Innovin® as a source of phospholipids (PL, f.c.10 micromol) and tissue factor (f.c. 300 pmol). We evaluated ETP parameters (T-lag, T-max, C-max, AUC), and

the difference (sec) T-max-T-lag (δ-T) in plasma samples from subjects with lupus anticoagulants (LA, n=97), thrombosis patients with lupus anticoagulants on oral anticoagulant treatment (LA-OAT, n=38), controls and unselected outpatients with normal PT and APTT (control group, n=153), and patients with atrial fibrillation on oral anticoagulant treatment (AF, n=97). Diagnosis of LA was by abnormal prolongation of a LA-sensitive APTT (PTT-LA, Stago) in a 1:1 plasma mixture plus prolongation of kaolin clotting time (KCT) and its normalization or near-normalization with the addition of PL. In the entire population, age explained only a minor part of the variation in ETP parameters. Average T-lag and T-max were longer, and C-max and AUC were reduced in LA subjects, although to a much lesser degree than observed in LA-OAT and AF patients. Relative to the control group, LA subjects had a 36-fold higher chance of a prolonged δ-T and a 3.8-fold higher chance of a reduced AUC (MH-OR adjusted for gender and age). Relative to LA patients, LA-OAT and AF patients had twice the probability of a prolonged δ-T, together with a 40-fold to 300-fold higher probability of a reduced AUC. As a result, the probability of a prolonged delta-T together with a normal AUC was 28-fold higher in LA subjects, but was not significantly different in LA-OAT patients, AF patients and controls. A prolonged δ-T together with a normal AUC was observed in 39 LA subjects, 2 LA-OAT patients, 5 controls and 0 AF patients; PTT-LA, KCT, KCT+PL and anti-β2GPI IgG were not significantly different in LA subjects with and without this characteristic. Interestingly, AUC values were higher in LA-OAT patients than in AF patients in spite of similar INR values (not determined with Innovin). These ETP results, obtained at a very high tissue factor concentration, provide an algorithm for the identification of LA activity in plasma samples.

C010

ABSENCE OF THE JAK2 EXON 12 MUTATIONS IN PATIENTS WITH SPLANCHNIC VENOUS THROMBOSIS AND WITHOUT OVERT CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background. Thrombosis of major abdominal veins has been reported in 5-10% of the patients with polycythemia vera (PV) or essential thrombocythemia (ET). Conversely, molecular hallmarks of chronic myeloproliferative disorders (CMD) can be recognized in a substantial portion of patients with splanchnic venous thrombosis not meeting all the criteria for diagnosis of PV or ET. We have previously reported the presence of the JAK2 V617F mutation in the absence of overt signs of CMD in 21% of overall patients with splanchnic venous thrombosis and in 32% of patients with extrahepatic portal vein thrombosis (De Stefano *et al.,* J Thromb Haemost 5: 708, 2007). Recently additional JAK2 exon 12 mutations have been reported in PV patients JAK2 V617F-negative, two of them with splanchnic venous thrombosis (Scott *et al.,* N. Engl J Med 356: 459, 2007; Colaizzo *et al.,* Blood 110: 2768, 2007) Aims: The present study is aimed to investigate the prevalence of the JAK2 exon 12 mutations among patients with splanchnic venous thrombosis and without overt CMD. *Patients and Methods.* We investigated JAK2 exon 12 mutations in 52 patients (M/F 27/25) with splanchnic venous thrombosis and without overt CMD who had been previously tested for the JAK2 V617F mutation and resulted negative. The median age at the thrombotic event was 45 years (range 18-79). Thrombosis involved the extrahepatic portal vein in 31 patients, the superior mesenteric vein in 13, the hepatic veins in 7, and the splenic vein in 1. DNA samples was obtained from peripheral blood granulocytes. We screened the JAK2 exon 12 mutations according to the original reports by Scott *et al.* (2007) for F537-K539delinsL, H538QK539L, K539L, and N542-E543del, and by Colaizzo *et al.* (2007) for R541-E543delinsK. We tested 46 patients for the F537-K539delinsL mutation, 42 for H538QK539L, 45 for K539L, 40 for N542-E543del, and 50 for R541-E543delinsK. Thirty-six patients were screened for all the 5 mutations. *Results*. We did not find any mutation of the JAK2 exon 12 in this sample of patients with abdominal thrombosis and JAK2 V617F negative. *Conclusions.* Apparently the JAK2 exon 12 mutations are not frequently detectable in patients with splanchnic venous thrombosis, unlike the JAK2 V617F mutation.

C011

INFLUENCE OF THE JAK2 V617F HOMOZYGOUS OR HETEROZYGOUS MUTATION AND OF INHERITED THROMBOPHILIA ON THE THROMBOTIC RISK AMONG PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background. It is uncertain whether the JAK2 V617F mutation is associated with an increased risk of thrombosis in patients with Philadelphia-negative chronic myeloproliferative diseases (CMD). It is unknown whether inherited thrombophilia is an additive risk factor in the patients with the JAK2 mutation. Aims: The present study is aimed to investigate the thrombotic risk associated with the JAK2 mutation and thrombophilia in ET patients. *Patients and Methods.* We studied 132 patients with ET (M/F 46/86, median age 53 years, range 20-92). Forty-five patients had had a major thrombotic event (34%). Arterial vessels were involved in 27 cases (cerebrovascular disease in 16, acute coronary syndrome in 7, peripheral arterial thrombosis in 4); thrombosis involved venous vessels in 18 cases (splanchnic veins in 9, deep veins of the legs in 7, cerebral veins in 1, retinal vein in 1). All patients were investigated for the presence of the JAK2 V617F mutation by PCR and sequencing analysis, defining homozygosity (Homo) or heterozygosity (Hetero) as a mutant allele burden higher or lower than 50%. Laboratory investigation for inherited thrombophilia (deficiency of antithrombin, proteins C and S, factor V Leiden [FVL], prothrombin G20210A [PT-A]) was carried out in all patients. *Results.* The JAK2 mutation was detected in 83 patients (62.8%), with Homo in 8 cases (6%). Seven patients carried thrombophilia (4 FVL and 3 PT-A). The relative risk (RR) for thrombosis was 2.1 (95%CI 1.1-3.8) in JAK2 mutated patients in comparison with wild-type (WT) patients; in Homo and Hetero the RR was 3.7 (95%CI 1.8-7.2) and 1.9 (95%CI 1.0-3.5) in comparison with WT patients. The RR of Homo in comparison with Hetero was 1.9 (95%CI 1.2-3.2). The patients with mutation had a RR in comparison with WT patients without thrombophilia of 4.4 (95%CI 2.2-8.8) in the presence of thrombophilia and of 2.1 (95%CI 1.1-4.0) in the absence of thrombophilia. Among the patients with mutation, those with thrombophilia had a RR of 2.1 (95%CI 1.3-3.4) in comparison with those without thrombophilia. *Conclusions*. In ET patients the thrombotic risk is higher in the presence of the JAK2 mutation. The magnitude of the increase in risk is dependent on the mutant allele burden, being higher in homozygotes. The concomitant presence of inherited thrombophilia produces a further increase in the thrombotic risk, yet further studies on larger patient cohorts are needed to confirm this finding.

C012

PATIENTS WITH PRIMARY ANTIPHOSPHOLIPID ANTIBODY SYNDROME AND WITHOUT ASSOCIATED VASCULAR RISK FACTORS PRESENT A NORMAL ENDOTHELIAL FUNCTION

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Primary antiphospholipid antibody syndrome (PAPS) is characterized by venous or arterial thrombosis and positive antiphospholipid antibodies. Some chronic inflammatory autoimmune conditions have been associated with accelerated atherosclerosis, however it is controversial whether PAPS patients have accelerated atherosclerosis. Endothelial dysfunction is an early event in the natural history of atherosclerosis. Aim of our study was to compare endothelial function of patients with PAPS and no associated risk factors with that of age- and sex-matched controls. Patients with PAPS, carefully selected to exclude all known risk factors for cardiovascular diseases, estrogen therapy, pregnancy, intake of drugs affecting endothelial function, vitamins or antioxidants, were included in a case-control study. Controls were age- (±5 years) and sex-matched subjects with the same exclusion criteria but without PAPS. Flow-mediated dilation (FMD) of the brachial artery and some plasmatic markers of endothelial and platelet activation were measured. Twenty PAPS cases (mean age 42±4.0 years, 11 females) and 39 controls (mean age 41±2.9, 22 females) were studied. FMD was 5.7±0.8% in cases (95% CI: 4.1-7.3) and 6.8±0.5% (5.7-7.9) in controls (p=NS). Plasma von Willebrand factor was 128±11.3% and 134.2±16.1% in cases and controls, respectively (p=NS). Soluble P-selectin and soluble CD40L were 94.1±4.9

ng/mL and 0.7±0.1 ng/mL in cases and 87.7±4.0 ng/mL and 1.0±0.2 in controls, respectively (p=NS). In a substudy, circulating progenitor and mature endothelial cells were comparable between the two groups. In conclusion, endothelial function in patients with PAPS and no associated risk factors is similar to that of age- and sex- matched controls. These data suggest that the alterations leading to thrombosis in PAPS are not the consequence of accelerated atherogenesis but rather concern primarily the clotting system.

C013

HYPERHOMOCYSTEINEMIA IS AN INDEPENDENT RISK FACTOR FOR VENOUS THROMBOEMBOLISM RECURRENCE AFTER A FIRST EPISODE OF PULMONARY EMBOLISM

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Introduction. The role of thrombophilic abnormalities in increasing the risk of venous thromboembolism (VTE) recurrence after a first episode of pulmonary embolism (PE) is controversial. We evaluated the relation between the rate of VTE recurrence after PE and thrombophilic abnormalities. *Methods.* One-hundred-forty-two consecutive patients [74 males and 68 females, median age 59 yr (range 23-89 yr)] with a first objectively confirmed episode of PE were followed up for a median time of 24 mo. (range 2-114 mo.) after discontinuation of oral anticoagulation. Thrombophilic risk factors evaluated were antithrombin, protein C, free protein S, APC-resistance, factor V Leiden, prothrombin G20210A polymorphism, fasting homocysteine, lupus anticoagulant, anticardiolipin antibodies, factor VIII activity, lipoprotein(a). *Results.* During follow-up 19 recurrences (13.4%) were observed of which 9 PE and 10 deep vein thrombosis (DVT). The median time of recurrence was 20 mo. (range 2-114 mo.). Patients with VTE recurrence were significantly older than those without (p=0.015). Recurrences were more frequent in patients whose initial PE was unprovoked (18%) than in those whose initial PE was provoked (2%) [unadjusted OR 4.9 (95% CI, 1.1-22.2), p=0.03)]. A trend of association was observed after adjustment for age and sex (p=0.06). Elevated homocysteine levels (>95th percentile) were found in 53% of the patients with recurrence and in 26% of those without (p=0.03). After multivariate analysis, hyperhomocysteinemia was the only thrombophilic abnormality associated with a significantly higher risk of recurrence [OR 3.1 (95% CI, 1.1-8.7), p=0.03]. The association between hyperhomocysteinemia and VTE recurrence was even stronger when considering only the patients with unprovoked PE [OR 4.8 (95% CI, 1.4-15.8), p=0.01]. *Conclusions.* Hyperhomocysteinemia is an independent risk factor for VTE recurrence after a first episode of unprovoked or provoked PE. Homocysteine measurement may prove useful in the risk stratification of VTE recurrence after acute PE.

C014

HIGH THROMBIN GENERATION MEASURED IN THE PRESENCE OF THROMBOMODULIN IS ASSOCIATED WITH AN INCREASED RISK OF RECURRENT VENOUS THROMBOEMBOLISM

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Background. The assessment of the risk of recurrent venous thromboembolism (VTE) is important to determine the optimal duration of secondary prophylaxis. The risk can be estimated by measuring individual parameters reflecting hypercoagulability. Because of the large numbers of such putative parameters, the assessment in individual patients is complex. Application of global assays reflecting the pro-/anti-coagulant balance *in vivo* would be desirable. *Objectives.* To investigate the relationship between recurrent VTE and thrombin generation (TG). *Patients and Methods.* Two-hundred-fifty-four patients were followed up after a first episode of unprovoked, objectively documented VTE for a period of 2.7 years after discontinuation of treatment with vitamin K antagonists. TG was measured one month after discontinuation of treatment as endogenous thrombin potential (ETP), peak thrombin and lag-time in the presence or absence of thrombomodulin. The study outcome was objectively documented symptomatic recurrent VTE. *Results.* Patients with ETP or peak (measured in the presence of thrombomodulin) of >960 nM*min or >193 nM had hazard ratios (HR) (95% CI) for recurrent VTE of 3.41 (1.34-8.68) or 4.57 (1.70-12.2) as compared to those with an ETP <563 nM*min or peak <115 nM. Patients with lag-time <14.5 min had HR of 3.19 (1.29-7.89) as compared to those with lag-time >20.8 min. HR for ETP, peak or lag-time measured in the absence of thrombomodulin were smaller than those measured in the presence of thrombomodulin. *Conclusions.* The measurement of TG helps to identify patients at higher risk of VTE recurrence. The increased risk may be better appreciated if the test is performed in the presence of thrombomodulin.

Platelets: Biochemistry and Physiology

C015

CHARACTERIZATION OF PLATELET NITRIC OXIDE PRODUCTION TRIGGERED BY PLATELET ADHESION UNDER FLOW USING MICROSCOPIC IMAGE SEQUENCE ANALYSIS

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Nitric Oxide (NO) is a powerful vasodilator and a platelet inhibitor produced by endothelial cells upon stimulation by soluble mediators or by shear stress. Platelets contain constitutive NO synthase (NOS 3) and several soluble stimuli (collagen, ADP, thrombin, beta-adrenoreceptor agonists) activate the synthesis of NO by platelets. We have previously shown that high shear stress may also trigger NO production by human platelets. To better investigate the production of NO by platelets under flow and to evaluate its role in the modulation of platelet adhesion we have now analyzed concurrently the production of NO and the formation of platelet aggregates under flow using human platelets loaded with an intracellular, fluorescent probe for NO (DAF-FM diacetate). DAF loaded platelets, resuspended in plasma together with autologous washed red cells at the final count of 50000/μL, were perfused over immobilized type I fibrillar collagen for 3 minutes at the wall shear rate of 3000 s-1. Fluorescent platelets were visualized with an inverted microscope equipped with epifluorescent illumination (Diaphot-TMD; Nikon Instech, Kanagawa, Japan)and images were continuously videorecorded using an intensified CCD videocamera (C-2400-87; Hamamatsu Photonics, Shizuoka, Japan) at 25 images/sec and analyzed off-line. The deposition of quinacrine-loaded platelets onto immobilized collagen was also investigated using platelets from healthy humans or from wild type or NOS3 KO mice. After perfusion, the number of adhering DAF fluorescent platelets, i.e. producing NO, was 64.4±16.4 platelets/field (mean±sem,n=5; an optical field corresponding to 0.007 mm^2). In samples pretreated with L-Arg (1 mM), the substrate of NO synthase, fluorescent platelets increased by 28.5±1.5%(p<0.001 *vs.* control), while in samples treated with L-NMMA, a competitive inhibitor of NO synthase, the number of fluorescent platelets decreased by -25±3.5%(p<0.003 vs CTRL). The total surface covered by quinacrine-loaded human platelets was of 17-20%, and was unchanged by pretreatement with L-Arg but it was significantly increased (+35%, p<0.0006) by pretreatment with L-NMMA. With quinacrine-loaded platelets from wild-type the surface covered was 27.2±2.6% while with platelets from eNOS KO mice surface coverage was 39.8±3.2% (p<0.001). In conclusion, we show here for the first time that NO production can be visualized in single platelets undergoing adhesion to collagen at high shear rate. Moreover, we show that platelet- derived NO influences aggregate formation and stabilization. These data provide new insights into the role of platelet-derived NO in the modulation of thrombus growth in health and disease.

C016

GP91PHOX –DEPENDENT ISOPROSTANE GENERATION ENHANCES PLATELET RECRUITMENT AND GP IIB/IIIA ACTIVATION

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Background. Isoprostanes stem from arachidonic acid interaction with reactive oxygen species (ROS) and seem to be implicated in platelet activation. However the underlying mechanisms have not been fully elucidated. *Aim.* to analyze platelet activation and its relationship with platelet production of isoprostanes in healthy subjects (n=8) and in patients (n=8) with hereditary deficiency of gp91phox, the central core of NADPH oxidase (X-CGD). *Methods.* Platelet recruitment, and gp IIb/IIIa activation urinary and platelet isoprostanes production as well as platelet and serum thromboxane formation were analysed. *Results.* Platelet recruitment, gp IIb/IIIa activation and platelet isoprostane production were higher in healthy subjects compared with X-CGD patients. No difference was detected in both serum and platelet thromboxane formation. Urinary excretion of isoprostanes were almost completely absent in xCGD patients compared to healthy subjects. To evaluate the role of isoprostanes in platelet activation further *in vitro* experiments were performed. *In vitro* isoprostanes dose dependently (0.001-0.1 mM) increased platelet recruitment and gp IIb/IIIa activation in healthy subjects (HS). Incubation of platelets with aspirin only partially inhibited platelet recruitment and gp IIb/IIIa activation whereas completely inhibited platelet thromboxane formation. Addition of the isoprostane receptor inhibitor (SQ29,545) to aspirin-treated platelets stimulated with isoprostanes almost completely suppressed recruitment and gp IIb/IIIa activation. *Conclusions.* These data suggest that 1)isoprostanes stimulate gp IIb/IIIa activation and in turn platelet recruitment independently from thromboxane formation; 2)platelet isoprostanes formation seemed to be generated prevalently by NADPHoxidase activation as they were almost completely absent in NADPH deficient patients.

C017

PLATELET-DERIVED NITRIC OXIDE REGULATES ARTERIAL BLOOD PRESSURE IN MICE

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Nitric Oxide (NO) produced by endothelial cells (EC) is an important regulator of arterial vascular tone. Competitive inhibitors of nitric oxide synthase (NOS), such as L-NAME, enhance blood pressure (BP) in humans and increase peripheral arterial resistance both in humans and mice. eNOS is present also in blood platelets. Aim of our study was to assess whether platelet-derived NO participates in the regulation of arterial BP. Mice lacking the eNOS gene (NOSIII) were compared to wild type (WT) mice of the same background, C57BL6/J. BP was measured by the non invasive, computerized, tail-cuff method and, in some selected experiments, by intraarterial measurements though a cathether inserted in the carotid artery. BP in eNOS-/- was significantly higher than in WT mice (135±3 vs 93±6 mmHg, p<0.001) while BP in eNOS+/- mice was only slightly enhanced (103±3 mmHg). Platelets isolated from either eNOS-/- or WT mice were cross-transfused into WT or eNOS-/- recipient mice made deeply thrombocytopenic by the injection of a rabbit anti-mouse antiserum. Moreover, chimeric mice were generated by bone marrow transplantation: eNOS-/- and WT recipient mice were lethally irradiated and transplanted with bone marrow from WT and eNOS-/-, respectively. BP of WT mice transfused with eNOS-/- platelets increased significantly (from 93±3.5 to 112±2, p<0.01) while the BP of eNOS-/- mice transfused with WT platelets decreased significantly (from 130±1.6 to 101±3.6, p<0.01). BP of eNOS-/- mice reconstituted with bone marrow from WT animals decreased starting 2 weeks following bone marrow transplantation (-21%, p<0.01) while BP of WT mice transplanted with eNOS-/- bone-marrow increased (+17%, p<0.01). L-NAME (50 mg/kg i.v.) infusion in WT animals enhanced BP by 54% while it increased it by only 22% in WT mice rendered deeply thrombocytopenic. Interestingly, profound platelet depletion (platelet count <95% of basal) enhanced BP by 41% in eNOS+/- mice (from 102+/-3.2 to 145+/-11 mmHg, p<0.05) while it did not affect BP of WT or of eNOS-/- mice. Our data show that besides EC-derived NO, which is a known powerful regulator of BP *in vivo*, platelet-derived NO plays an important role in the regulation of arterial blood pressure. These results may be relevant to human hypertension, a condition in which indeed a defective platelet-derived NO production has been reported.

C018

PLATELETS EXPRESS MRNAS FOR MATRIX METALLOPROTEINASES AND THEIR INHIBITORS: PROTEIN EXPRESSION AFTER PLATELET ACTIVATION?

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Platelets express several matrix metalloproteinases (MMPs) and some of them are secreted upon activation and play an important role in hemostasis and thrombosis, either by their priming (MMP-2) or inhibitory (MMP-9) activity on platelets. Over the last few years it has been shown that platelets can translate a subset of megakaryocyte-derived mRNAs into new proteins upon activation. The aim of our study was to evaluate the presence of mRNA for MMPs and tissue inhibitors of MMPs (TIMPs) in platelets and to assess whether they are associated with protein synthesis during platelet activation. We extracted purified mRNA from CD45-leukocyte depleted platelet preparations. The retrotranscribed cDNA was used as a template in PCR reactions using gene specific primers for MMP-1, MMP-2, MMP-3, MMP-9, MMP-14, TIMP-1, TIMP-2, TIMP-3, TIMP-4. For the protein expression studies, purified platelets (1×10^9 cells/mL) were left quiescent or were activated with

human thrombin (0.1U/ml) for 2 hours. Intracellular and secreted MMP-1, TIMP-1 and TIMP-2 was measured by ELISA whereas zymography was used to assess MMP-9, in resting and stimulated platelets. We found that platelets contain mRNA for MMP-1, MMP-9, TIMP-1 and TIMP-2. On the contrary MMP-2, MMP-3, MMP-14, and TIMP-4 mRNAs were not detectable and TIMP-3 mRNA was found in only 50% of the samples. In regard to proteins, MMP-1 was constitutively present in resting platelets and secreted upon 30 minutes of activation but total protein remained unchanged over the 2 hours observation period. TIMP-1 was also present in resting platelets and secreted upon 30 minutes activation but in contrast to MMP-1, TIMP-1 increased in the supernatant during the 2-hours activation period. TIMP-2 was not detected in resting platelets but its levels in the supernatant increased within 30 minutes of platelet activation. MMP-9 was not detected in platelets by ELISA or zymography. These results demonstrate that platelets express a subset of mRNAs for matrix metalloproteinases and their inhibitors. Activated platelets also accumulate TIMP-1 and TIMP-2 protein upon activation, suggesting that these two mRNAs may be translated in a signal-dependent fashion. In conclusion, mRNA translational regulation allows platelets to synthesize TIMPs proteins without mRNA transcription, processing, or nuclear export and may play an important role for the activity of platelets in atherosclerosis and inflammation.

C019

INHIBITION OF P2Y12 ON HUMAN MEGAKARYOCYTES REDUCES PROPLATELET FORMATION

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Platelets and megakaryocytes (MKs) express two P2Y receptors for ADP, the Gq-coupled P2Y1 and the Gi-coupled P2Y12. In the present study, we analysed the effects of inhibition of P2Y1 and P2Y12 receptors, alone and in combination, on MK differentiation and proplatelet formation (PPF). MKs were differentiated from cord blood-derived $CD34^+$ cells for 12 days. Mature MKs were harvested and incubated with apyrase (an ADP scavenging enzyme), MRS2179 (a specific P2Y1 antagonist), MeSAMP (a specific P2Y12 antagonist) or Wortmannin (an inhibitor of phosphatidylinositol-3-kinase (PI-3K)), at 37°C for 30 minutes, before being seeded. Control cell samples were incubated in parallel with the corresponding volumes of PBS. MK differentiation and PPF were evaluated by phase contrast and fluorescence microscopy upon cell staining with anti-tubulin and anti-CD41 antibodies. The overall MK maturation was not affected by any tested inhibitor, while apyrase, the P2Y12 antagonist and Wortmannin reduced PPF by about 50%. The P2Y1 antagonist did not exert any inhibitory effect either by itself or when added to cells in combination with the P2Y12 antagonist. These data suggest that the interaction of autocrine ADP with P2Y12 on the MK membrane regulates PPF through a PI3-K driven mechanism. The addition of epinephrine to MK after their incubation with the P2Y12 antagonist did not normalize PPF, indicating that, at variance with some platelet functions, stimulation of the inhibitory G protein Gz by epinephrine does not compensate for the loss of P2Y12 mediated function. No tested inhibitor affected the morphological structure or tubulin distribution of MK-derived proplatelets. In conclusion, the results of our study show that the interaction of ADP with its P2Y12 receptor on MK plays a relevant role in *in vitro* proplatelet formation. Since treatment with anti-P2Y12 drugs, such as clopidogrel, or congenital P2Y12 deficiency are not associated with thrombocytopenia, the clinical relevance of our results needs to be investigated in further studies.

C020

RESVERATROL STIMULATES PLATELET NITRIC OXIDE PRODUCTION AT CONCENTRATIONS ATTAINABLE IN HUMAN PLASMA WITH MODERATE WINE CONSUMPTION

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The mechanisms through which moderate wine consumption reduces ischemic cardiovascular events are not yet fully unravelled. Grape extracts or mixtures of the polyphenols contained in wine were previously shown to increase nitric oxide (NO) production by various cells, however little information is available on the effects of resveratrol, the main polyphenol of wine, on platelet NO production. We investigated the effects of resveratrol, at the concentrations attainable after moderate wine intake, on platelet NO production and the mechanism of this activity. Twenty healthy volunteers were studied before and after 15 days of controlled white- or red-wine intake (300 mL/day). After wine intake plasma resveratrol concentration and the release of NO by stimulated platelets (measured as nitrite plus nitrate) increased significantly (plasma resveratrol: 0.72±0.3 to 1.33±0.3 μM for white-wine and 0.71±0.02 to 1.72±0.1 μM for red-wine; nitrite plus nitrate: 10.6±2.4 to 19.8±4.8 μM, for white wine and 13.9±3.4 to 24.1±3.6 μM, for red wine, $p<0.05$). Resveratrol, at the concentrations generated in plasma by wine intake (0.5 μM), was incubated *in vitro* with washed platelets and several parameters related to NO and signal transduction were measured. Resveratrol enhanced significantly collagen-stimulated NO production (control=25.8±12.0 pmol/108plts; resveratrol=78.6±22.5 μM, $p<0.05$) and the activity of platelet nitric oxide synthase, measured as 3HL-citrulline production, (control=16.9±8.7 pmol/108 plts; resveratrol= 44.4±8.2 pmol/108plts, $p<0.05$). Resveratrol increased the phosphorylation of AKT, an activator of eNOS, (from 0.25±0.06 to 0.85±0.20 MFI, $p<0.005$) as well as the phosphorylation of VASP, a marker of the NO biologic activity (from 0.84±0.05 to 1.28±0.09 MFI, $p<0.005$). On the contrary, resveratrol decreased the phosphorylation of p38MAPK, a proinflammatory pathway in human platelets (from 1.84±0.16 to 0.82±0.08 AU, $p<0.005$), as well as the activity of NADPH Oxidase (from 9±1 to 4.13±1.2 RLU/mg protein, $p<0.005$) and the generation of O2- radicals, as detected by cytochrome C reduction. In conclusion, resveratrol, at concentrations attainable *in vivo* after moderate wine intake, activates platelet eNOS and in this way it blunts the platelet proinflammatory pathway linked to p38MAPK, thus inhibiting ROS production and ultimately platelet function. This activity may contribute to the beneficial effects of moderate wine intake on ischemic cardiovascular disease.

C021

SYK-MEDIATED TYROSINE PHOSPHORYLATION OF THE TRANSPORTER SERT CONTROLS THE SEROTONIN (5-HT) TRANSPORT IN HUMAN PLATELETS

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Serotonin (5-HT) is a neurotransmitter which regulates a variety of vascular, smooth muscle and platelet functions. Platelets take up 5-HT from blood plasma by means of a plasma membrane transporter (SERT), which transfers 5-HT from extra-cellular to cytosolic compartment. A second carrier, i.e. vesicular monoamine transporter (VMAT), which is inhibited by reserpine, transports 5-HT from the cytosol into the dense granules. SERT contains, in the cytosolic domains, multiple consensus sites for various protein kinases. We have previously found that the 5-HT accumulation in human platelets was regulated by the protein kinase C and Tyr-kinase Src activities.[1,2] This study showed that the 5-HT accumulation in human, reserpine-treated, platelets decreased upon cellular exposure to piceatannol and Syk-inhibitor II, two structurally unrelated inhibitors of the Tyr-kinase Syk, which reduced the Vmax of 5-HT transport. By contrast, the protein Tyr-phosphatase inhibitor pervanadate increased the 5-HT uptake by platelets. Platelet treatment with the Syk-inhibitors also caused a decrease of the thapsigargin-induced efflux of serotonin from platelets. The Tyr-phosphorylation extent of SERT immuno-precipitated from membrane extracts decreased upon platelet pre-treatment with the Syk-inhibitors, and enhanced upon cellular pre-treatment with pervanadate. *In vitro* kinase activity towards the Syk-specific substrate alpha-sinuclein was detectable in the anti-SERT immuni-precipitates. Platelet treatment with the Syk-inhibitors also caused a reduction of the imipramine binding to platelets. It was concluded that the Syk-mediated Tyr-phosphorylation of SERT regulates the 5-HT transport in platelets by affecting its binding sites, and might represent a potential tool in the therapeutic strategies of the neurologic disorders linked to anomalous organic distribution of serotonin. *Methods.* Platelets were isolated from blood samples as previously described.[1] 5-HT transport was determined by radioisotopic techniques, while immunoblotting procedures were adopted for detecting the SERT Tyr-phosphorylation in platelet fractions and immuno-precipitates.[2]

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Von Willebrand Disease and ADAMTS-13

C022

RELEVANCE OF CHLORIDE BINDING TO VON WILLEBRAND FACTOR IN TYPE 2B VON WILLEBRAND DISEASE PATIENTS

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Background. In von Willebrand disease type 2B (VWD2B) the abnormal VWF spontaneously binds to circulating platelets, usually resulting into loss of high molecular weight multimers (HMWM) and thrombocytopenia. In these patients VWF:RCo/Ag ratio is indicative of reduced HMWM and roughly correlated with the severity of VWD2B defect. Physiological concentrations of Chloride ions (Cl-) inhibit the hydrolysis of VWF by ADAMTS-13. Cl- acts as an allosteric effector and its specific binding to VWF A1 domain results into a reduced availability of A2 to be proteolised by ADAMTS-13. Type 2B VWF R1306W showed a reduced affinity to Cl- (>KdCl-) and an increased (>kcat/Km) susceptibility to proteolysis by ADAMTS13.[1] *Aims*. To determine if other VWD2B mutations affect Cl- binding by changing the proteolysis of mutant VWF by ADAMTS-13. *Methods.* Five distinct mutant recombinant (r)A1-A2-A3 proteins (Table 1), have been prepared along with the WT. Determination of KdCl- of each rA1-A2-A3 was achieved by intrinsic protein fluorescence, performed as a function of both Cl- concentrations and temperature. Study on the kinetic rates of hydrolysis of each rA1-A2-A3 by ADAMTS-13 was evaluated using HPLC methods. *Results and Conclusions*. The results (Table 1) confirmed that affinity of Cl- (KdCl) was decreased in all mutant rA1-A2-A3s compared to the WT at different extent, with an inverse correlation to the catalytic specificity (kcat/Km) of ADAMTS-13 interaction. Higher VWF:RCo/Ag ratios were found associated with lower values of kcat/Km in our studied patients. This finding strongly support that the reduced affinity of type 2B VWF to Cl- might contribute to the depletion of HMWM.

Reference

1. De Cristofaro,R. Molecular mapping of the chloride-binding site in VWF. J Biol Chem 2007;281: 30400-11.

Table 1.

Table (patients)	*WT* ---	*R1341Q* (4)	*R1341W* (3)	*H1268D* (1)	*V1316M* (4)	*R1306W* (15)
K_dCl^- (mM)	156	191	359	481	526	540
kcat/Km ($M^{-1}s^{-1}$)	7.25×10^4	8.8×10^4	1.2×10^5	1.39×10^5	1.6×10^5	1.95×10^5
VWF:RCo/Ag ratio (range)	0.7 – 1.2	0.8 (0.77-0.84)	0.7 (0.6-0,8)	0.5	0.3 (0.2-0.7)	0.5 (0.25-1.0)

C023

MODULATION OF VON WILLEBRAND A1 DOMAIN FUNCTION BY THE HOMOLOGOUS DOMAINS A2 AND A3

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Introduction. Soluble von Willebrand factor (VWF) has a low affinity for platelets, but conditions of high shear rate (>10,000 1/s) enable platelet aggregation mediated by binding of the A1 domain (VWF-A1) to glycoprotein (GP) Ibα. Thus, hemodynamic forces regulate VWF function, possibly through conformational changes that expose functional A1 domain sites. The antibiotic ristocetin can bypass these shear-induced effects, and provides a flow-independent assessment of VWF-A1 function. In contrast to multimeric VWF, dimeric VWF-A1 can mediate platelet aggregation in a laminar flow field with low shear rate (600 1/s), suggesting that other domains in the native molecule may regulate the interaction with platelets. In this study, we have investigated the role of the homologous domains A2 and A3, which are thought not to interact with GP Ibα, in regulating VWF-A1 function. *Methods*. We expressed in insect cells and purified 3 recombinant VWF fragments comprising residues 445-733 (VWF-A1), 445-909 (VWF-A1A2) or 445-1111 (VWF-A1A2A3); all were obtained as secreted dimers owing to the presence of a portion of domain D3 (residues 445-497) that forms interchain disulfide bond(s). The dimeric fragments were tested for the ability to mediate ristocetin and shear-induced platelet aggregation. *Results*. All 3 fragments in solution supported ristocetin-mediated platelet aggregation at equimolar A1 domain concentration. By comparison, VWF-A1 was twice and thrice more effective than VWF-A1A2 and VWF-A1A2A3, respectively, in supporting shear-induced aggregation at the shear rate of 600 1/s. *Conclusions.* Domains A2 and A3 negatively modulate the function of VWFA1 with respect to GP Ibα-mediated platelet aggregation, and the effect is abolished by the negatively charged ristocetin. These results provide the framework to study the regulation of VWFA1 function without the complexity of VWF multimerization.

C024

THE VON WILLEBRAND DISEASE TYPE 2A (IIH): A UNIQUE VARIANT OF VON WILLEBRAND FACTOR DUE TO COMBINED 2A(IIC)/2N DEFECTS

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Von Willebrand disease (VWD) type 2A/IIH is characterized by the loss of the high molecular weight multimers in plasma/platelets and of the *triplet structure* of von Willebrand factor (VWF) (Am J Hematol 1989; 32:287). We have recently found three missense mutations in the propositus: 604C>T (R202W, D1 domain), 2546G>A (C849Y, D' domain) and 4748G>A (R1583Q, A2 domain): R202W and R1583Q in the same allele being found in the propositus' daughter. Mutation 2546G>A is at last nucleotide of exon 19 and RT-PCR analysis showed an alternative splice site product which predicted the nonsense mutation C849X; mRNA sequences predicting both mutations C849Y and C849X were identified in patient's platelets. The three missense mutations were expressed alone, together and with the wild-type (WT). The amounts of recombinant (r)VWFs from mutants C849Y, C849Y/WT and hybrid R202W-R1583Q/C849Y rVWFs showed reduced levels in conditioned media and increased levels in cell lysates. A normal multimeric pattern was found in R1583Q rVWF, while mainly dimers and intermediate molecular weight multimers in R202W and C849Y respectively. Both hybrids R202W-R1583Q/WT and C849Y/WT rVWFs had an almost normal set of multimers, whereas hybrid R202W-R1583Q/C849Y rVWF showed markedly reduced multimerization with an intermediate pattern between R202W and C849Y multimers. To evaluate cleavage of VWF R1583Q by ADAMTS-13, two expression vectors, producing the WT or R1583Q VWFs A1-A2-A3 domains, were made (J Biol Chem 2007; 281: 30400). Digestion of both proteins with rADAMTS-13 showed a similar susceptibility to cleavage, not supporting an effective role of R1583Q mutation. Recently we found patient's plasma VWF to show a partially reduced VWF:FVIII binding, therefore C849Y rVWF was tested for its capacity to bind FVIII and resulted markedly reduced. Based on these findings *in vitro*, the type 2A/IIH VWD previously reported is actually due to combined type 2A/IIC (R202W) and type 2N (C849Y) defects since these mutations affect VWF multimerization, intracellular survival and VWF:FVIII binding.

C025

DISTINCT VON WILLEBRAND FACTOR A1 DOMAIN INTERACTIONS WITH GLYCOPROTEIN IBα DURING PLATELET ADHESION AND AGGREGATION

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Background and Aim. Glycoprotein (GP) Ibα interacts with surface-bound von Willebrand factor A1 domain (VWF-A1) and mediates platelet tethering and rolling. With VWF in solution, the interaction occurs only above a threshold shear rate and results in aggregation. Hemodynamic forces may induce conformational changes required to *activate* VWF-A1; yet, platelet adhesion to immobilized VWF occurs even at low shear rates, suggesting that soluble and immobilized VWF may interact with platelets through distinct mechanisms. We have tested this hypothesis by studying the ability of selected VWF-A1 mutants to sup-

port platelet adhesion or aggregation, respectively, under controlled flow conditions. *Methods.* We expressed in insect cells and purified a series of VWF-A1 fragments comprising residues 445-733, 1 with native sequence and 8 with single or multiple substitutions of positively charged amino acid residues in helices α4 and/or α5. None of the substituted residues contribute to contacts with GP Ibα in the known crystal structures of the corresponding complex. The fragments were dimeric (d) owing to the presence of interchain disulfide bond(s). *Results.* Native dVWF-A1 in solution supported platelet aggregation in a laminar flow field with low shear rate (600 1/s). Of the 8 mutants, 5 had variably decreased function (up to 80% less aggregation) and 2 had increased function (up to 200% increase in aggregation). The same results were observed with platelet-rich plasma in suspension or by measuring thrombus formation with whole blood perfused over immobilized VWF-A1. In contrast, as judged by the number of tethered platelets and their rolling velocities, all mutants supported adhesion as well as or better that the native VWFA-1 at all shear rates tested (500-25,000 1/s). *Conclusions.* These results provide the first structural evidence for the existence of different VWF-A1 conformers that can modulate adhesive properties with distinct effects on platelet adhesion to a surface or platelet-platelet aggregation.

C026

EVALUATION OF ASSAYS TO MEASURE ADAMTS13 ACTIVITY IN PATIENTS' PLASMA

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Several assays have been developed to measure ADAMTS-13 activity. Although the performance characteristics of these methods were recently evaluated (Tripodi A, *et al.* JTH 2004), little information is currently available on concordance of these assays in the measurement of plasma ADAMTS-13. The main goal of this study was to investigate whether widely available assays concordantly measure the same amount of ADAMTS-13 activity in plasma. ADAMTS-13 activity using residual collagen binding activity (CBA) and FRET assays were measured in plasma samples of 72 healthy subjects and 124 patients affected with thrombotic microangiopathies. The Spearman's rho and the Kappa statistics were used to assess the correlation and agreement between assays after categorisation of plasma samples into 5 subgroups with different levels of ADAMTS-13 activity. The measurement of ADAMTS-13 activity by CBA and FRET assays showed a correlation of 0.85 (p<0.0001). The concordance between the two assays was confirmed in 81% of samples (Kappa=0.68, p<0.0001). Major discrepancies were observed in 10 samples: ADAMTS-13 activity was consistently lower as measured by FRET assay compared to CBA (Table 1, grey area). Only 3 of these discordant data could be ascribed to plasmatic hyperbilirubinemia (Meyer SC, *et al.* JTH 2007). Additionally, in other three patients with severe haemolysis, ADAMTS-13 activity was not measurable using the FRET assay due to a non-parallelism of serially diluted plasma samples, in contrast to the consistent CBA measurement. In conclusion ADAMTS-13 activity determined by CBA and FRET assays confirmed an overall good concordance. The major advantage of FRET assay compared with CBA is that the results are available within 1h. However, the disadvantage is related to the use of fluorescent probes, which may be influenced by plasma factors such as bilirubin interfering to the fluorescence emission and consequently to the results of FRET assay.

Table 1.

	FRET assay					
TADAMTS-13 activity (%)	under detection limit (<3)	3-10	11-20	21-44	normal range ≥45	Total
CBA assay						
Under detection limit (<6)	33	0	0	0	0	33
6-10	6	2	1	0	0	9
11-20	3	6	2	1	0	12
21-49	1	2	2	11	5	21
Normal range (≥50)	1	0	3	6	111	121
Total	44	10	8	18	116	196

C027

EFFECT OF SITE-SPECIFIC OXIDATION OF VWF ON ITS CATALYTIC INTERACTION WITH ADAMTS-13: PROTHROMBOTIC IMPLICATIONS OF OXIDATIVE STRESS IN DIABETES MELLITUS

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Objective. Oxidation of selected amino acid side chain, such as those of tyrosine and methionine could strongly affect the function of proteins and enzymes. The cleavage of VWF by ADAMTS-13 takes place at the Tyr1605-Met1606 peptide bond in the A2 domain. Tyrosine (Tyr) and methionine (Met) aminoacids are the most reactive groups for the oxidant species, and could generate oxidized derivatives such as nitro-Tyr and sulfoxy-Met. We tested the hypothesis that the oxidant stress occurring in diabetes may involve the above aminoacids in VWF, negatively affecting the interaction with ADAMTS13. *Methods and Results.* The hydrolysis by recADAMTS13 of the VWF peptide from 1596 to 1668 (VWF73), and of the same peptide containing nitro-Tyr and sulfoxy-met at positions 1605 and 1606 were studied using a fluorescence quenching method (FRETS). Multimeric pattern of VWF purified in 20 patients with type 2 diabetes mellitus (T2DM) and hydrolyzed by ADAMTS13 *in vitro* was studied by SDS-agarose electrophoresis and compared with that of controls. The nitro-Tyr content of VWF of T2DM subjects and controls was also quantified by ELISA. The kcat/Km of cleavage of VWF73 containing nitro-Tyr was reduced about 20-fold compared to wild type VWF73. Likewise, hydrolysis of full length VWF in T2DM subjects, where nitro-Tyr was increased by 40%, was significantly lower than in controls. *Conclusions.* These results showed that the presence of nitro-Tyr at position 1605 of VWF negatively affects the interaction with ADAMTS13. The oxidant stress involves VWF of T2DM patients, inhibiting its *ex vivo* hydrolysis by ADAMTS13. This phenomenon may contribute to the accumulation of ultra-large VWF multimers, favoring the occurrence of the thrombotic microangiopathy in this clinical setting.

C028

VON WILLEBRAND FACTOR- GLYCOPROTEIN IBALPHA INTERACTIONS PLAY A ROLE IN GENERATING PLATELET MICROPARTICLES: DATA FROM *EX VIVO* AND *IN VITRO* STUDIES

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Microparticles (MP) are circulating submicroscopic cell fragments expressing procoagulant phospholipids and haemostatic proteins such as tissue factor. Several evidences support a pro-haemostatic role of MP and suggest that the axis VWF-platelet GpIb might be critical in their generation. The aim of this study was to measure the levels of platelet-derived MP (PMP) and tissue factor-expressing MP (TFMP) in relationship with VWF levels and multimeric composition as well as shear stress exploiting *ex vivo* and *in vitro* natural models. PMP and TFMP were analyzed by flow cytometry and defined as Annexin V and GpIIb/IIIa or Tissue Factor positive events falling in a gate defined by 1 Ìm beads. MP were enumerated adding a known number of 7 Ìm beads to each test tube. In the first set of experiments, levels of PMP and TFMP were measured before and after DDAVP administration in a 9 patients with mild hemophilia A. FVIII:C, VWF:Ag, PMP and TFMP were measured in plasma at baseline and 30 min, 1, 2, 4, 8 and 24 h after DDAVP 0.3 μg/kg, subcutaneously. A significant increase in the levels of PMP and TFMP (p<0.05 at all time points vs baseline) was observed after DDAVP; peak levels were achieved after 2-8 h. Correlation was found between the increase of VWF:Ag and FVIII:C levels and the peak levels of MP (r=0.62 and r=0.7, respectively). We then studied 27 patients with different types of von Willebrand disease (VWD) and 15 normal controls: citrated platelet rich plasma was exposed to increasing shear stress (from 0 to 90 dyne) for 45 seconds in a cone and plate viscometer and generated PMP were then enumerated by flow cytometry. In patients with type 1 VWD shear generated-PMP increased at the same levels observed in normal controls, while in type 3 patients no PMP increase was observed. Interestingly while type 2B and 2M patients generated PMP at normal levels after shear stress exposure, type 2A patients had the same behavior of type 3 patients. This study support the hypothesis that ultralarge VWF multimers released by the endothelium interact with platelets and trigger MP generation, both *in vivo* and *in vitro*.

Venous Thromboembolism: Diagnosis

C029

OUTCOME COMPARISON OF TWO DIAGNOSTIC STRATEGIES FOR SUSPECTED DEEP-VEIN THROMBOSIS IN SYMPTOMATIC PATIENTS: TWO-POINT VERSUS WHOLE-LEG ULTRASONOGRAPHY

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Background. Patients with clinically suspected deep-vein thrombosis of the lower extremities are currently investigated with either compression ultrasonography of the proximal veins only, repeating the test within one week in those with initial normal result and abnormal D-Dimer (limited ultrasonography), or ultrasonography of the entire deep vein system (extended ultrasonography). We sought to compare the safety and the cost implications of the two strategies. *Methods.* 2098 consecutive outpatients with suspected deep-vein thrombosis were randomized to limited or extended ultrasonography. We assessed the prevalence of thrombosis, and the 3-month incidence of symptomatic venous thromboembolism occurring in patients with initial normal diagnostic workup. In addition, we determined the cost implications of managing 100 patients with either strategy. *Results.* The initial workup led to the diagnosis of thrombosis in 231 of the 1045 patients (22.1 percent) allocated to limited ultrasonography, and in 278 of the 1053 (26.4 percent) randomized to extended ultrasonography (p=0.022). Subsequent symptomatic venous thromboembolism developed in 7 of the remaining 814 patients (0.9 percent; 95 percent confidence interval, 0.3-1.8) in the limited ultrasonography, and in 9 of the 775 (1.2 percent; 0.5-2.2) in the extended ultrasonography group (p=0.69). The limited strategy saved US $ 2,150 for every 100 symptomatic patients investigated. *Conclusions.* The two diagnostic strategies appear equally safe. As compared to extended ultrasonography, limited ultrasonography leads to the detection of a significantly lower rate of thrombosis and is less expensive, but implies the need for one fourth of patients to visit the diagnostic service twice.

C030

DIAGNOSIS AND MANAGEMENT OF VENOUS THROMBOEMBOLISM: RESULTS OF A QUESTIONNARIE ON CURRENT CLINICAL PRACTICE

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Background. The application of research evidence into daily clinical practice is not always a straightforward process. Venous thromboembolism (VTE) is certainly one of the best examples in which diagnosis and management are extensively studied, and current guidelines are evidence-based. We developed a standardized questionnaire to explore clinical practice patterns in the management of VTE by Italian expert physicians, in particular about the use of pre-clinical probability and D-dimer testing and on the home treatment of pulmonary embolism (PE). *Methods.* A standardized questionnaire was sent to all 643 members of the Società Italiana per lo Studio dell'Emostasi e della Trombosi (SISET) by e-mail. A reminder by air-mail was sent two months later. Three groups of questions were administered: general information, diagnostic process for both deep venous thrombosis (DVT) and PE, and home-therapy of PE. A web page was developed to answer the questionnaire anonymously. *Results.* Three hundred and ninety-four members (61.3%) were physicians potentially involved in daily clinical management of VTE. One hundred and thirty-two (33.5%) physicians replied partially or fully to the questionnaire. Eighty-three (63%) were males. Fifty-four (40.9%) were aged between 46 and 55 years. One hundred and fifteen (89.9%) declared to be VTE experts. For DVT diagnosis, 74 (58.3%) physicians answered they always measure D-dimer; 4 (3.0%) do never measure it; only 11 (9.1%) take notice of D-dimer before visiting the patients; 38 (30.3%) use only clinical judgment and do not apply clinical prediction rules to assess pre-clinical probability of disease. For PE diagnosis, 83 (68.9%) physicians always measure D-dimer, whereas 3 (2.4%) do never measure it; 14 (12.1%) take notice of D-dimer before visiting patients; 51 (42.4%) use only clinical judgment and do not apply clinical prediction rules to assess pre-clinical probability. Twenty-two (18.2%) physicians declared to always treat patients with PE at home, and 45 (37.2%) to never treat them at home. *Conclusions*. The diagnostic approach to VTE among expert physicians appears to be heterogeneous; in particular there is no widespread use of clinical prediction rules, in particular when PE is suspected. The majority of expert physicians appear to consider the possibility of treating at home patients with PE.

C031

PRESENCE OF RESIDUAL THROMBOEMBOLI AT LEAST SIX MONTHS AFTER A FIRST EPISODE OF SYMPTOMATIC PULMONARY EMBOLISM: DO PERFUSION SCINTIGRAPHY AND CT-ANGIOGRAPHY AGREE?

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Introduction. The natural history of residual thromboembolic obstructions after pulmonary embolism (PE) and the clinical utility of imaging techniques at follow up are not completely known; in particular, to our knowledge, there are no clinical studies that compared the accuracy of computed tomography (CT) and perfusion scintigraphy (Q scan) in detecting residual thromboemboli after at least 6 months from a first episode of symptomatic PE. *Methods.* Consecutive patients on anticoagulant treatment for a first episode of symptomatic PE diagnosed by CT scan and/or Q scan underwent CT and Q scan after 6 to 12 months from the index event. Exclusion criteria were previous PE, recurrent symptomatic PE or deep vein thrombosis (DVT) during the follow-up period. All diagnostic tests were independently assessed by one expert radiologist unaware of the clinical status of the patients. The results of the tests were classified as no signs of recanalization or reperfusion, partial resolution or complete resolution. The agreement beyond chance between tests was assessed by kappa statistics. *Results.* Twenty-five patients (14 males, 56%, mean age 59.8) were included after a mean of 7.2 months (217 days, standard deviation 58 days) after acute PE. PE was diagnosed by CT alone in 13 cases, by high probability Q scan alone in 2 cases and by both CT and Q scan in 10 patients. Eleven episodes (44%) were considered idiopathic, 11 (44%) were secondary to transient risk factors, 3 (12%) patients had cancer; concomitant DVT was detected in 17 (68%) patients. At follow-up, Q scan showed no signs of reperfusion in 1 patient (4%), partial resolution in 13 patients (52%) and complete resolution in 11 (44%). CT showed no signs of recanalization in 0 patient, partial resolution in 17 patients (68%) and complete resolution in 8 (32%). Complete resolution was present in both tests in 1 patient (4%), partial resolution was present in both tests in 6 (24%) patients, with a k agreement between tests <0.2 (slight). *Conclusions.* Partial resolution of thromboemboli after a mean period of 7.2 months from a first episode of PE is present at Q scan or at CT in more than 50% of patients, confirming the results of previous studies. However, the results of our study show for the first time that the agreement between these two tests is low. Thus, caution should be used when interpreting the results of either of these tests to monitor patients with previous PE.

C032

D-DIMER LEVELS AT DIAGNOSIS IN RELATION TO THE SITE AND EXTENSION OF LEG DEEP VEIN THROMBOSIS (DVT)

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D-dimer (Dd) assay is extensively used to exclude DVT in symptomatic patients (pts) due to its high negative predictive value; increased Dd levels are currently considered not useful in this diagnostic strategy. Recent data showed a possible use of high Dd levels to increase the clinical probability of pulmonary embolism in symptomatic pts. We investigated the relationship between Dd levels and presence/absence, site and extension of DVT in the legs. 786 consecutive pts referring to the emergency vascular room of our outpatient service for symptoms of leg DVT received an ultrasonography investigation (CUS), pre-test clinical probability (PCP) assessment (according to Wells) and blood sampling for Dd assay (STA Liatest D-dimer, Diagnostica Stago, on a STA Com-

pact instrument). All the pts who resulted as negative for DVT received a phone call after 3 months. Very high Dd levels (>2000 ng/mL) are: a) present in most pts with ilio-femoral-popliteal DVT, but are rare (<10%) in subjects without DVT, b) highly associated with proximal DVT, even in pts who have an unlikely PCP. Though Dd assay should be used for its high negative predictive value, very high levels in symptomatic outpatients should be evaluated with attention due to their strong association with proximal DVT.

Panel A: rates (%) of pts with increased Dd levels in relation with DVT diagnosis.

Dd level (ng/mL)	*Iliac-femoral DVT N=40*	*Femoral-popliteal DVT N=64*	*Popliteal DVT N=59*	*Isolated Distal DVT N=140*	*No DVT N=483*
> 1000	100	98.4	83.0	37.8	23.6
> 2000	90.0	78.1	54.2	17.5	9.1

Panel B: rates (%) of pts with increased Dd levels in relation with the PCP.

	Proximal DVT		*Distal DVT*		*No DVT*	
	Likely	*Unlikely*	*Likely*	*Unlikely*	*Likely*	*Unlikely*
PCP	N=137	N=26	N=81	N=59	N=204	N=279
> 2000	73.5	57.8	17.3	13.6	14.2	5.4

C033

IMPROVEMENT OF THE SPECIFICIY AND DIAGNOSTIC UTILITY OF A D-DIMER TEST FOR DEEP VEIN THROMBOSIS EXCLUSION USING DIFFERENT CUT-OFF LEVELS ACCORDING TO AGE AND GENDER

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D-dimer (Dd) levels increase with age for several factors (reduced renal clearance, > fibrinogen levels and presence of occult disease). In healthy subjects Dd levels are approximately 4 times higher in the highest age quartile. The specificity and diagnostic utility of Dd measurement for venous thromboembolism (VTE) exclusion is therefore lower in older patients. The aim of this study was to evaluate if the use of different cut-off levels according to age and gender might improve the specificity of a Dd assay for DVT exclusion. From Jan to Dec 2007, 643 patients (245 males; mean age 64.5 y, range 12-84 y) referred to our outpatient clinic for suspected leg DVT. The exclusion criteria were: previous DVT event, symptoms lasting > 15 days, anticoagulant treatments already underway at presentation, pregnancy. Blood was sampled from the patients at presentation and tested by technicians unaware of the patient's clinical diagnosis. Dd were measured by the STA Liatest D-dimer (Diagnostica Stago) on a STA Compact instrument.

Table 1. D-dimer levels (mean±SD) and specificity values (95%CI) in different groups according to age and gender. Sensitivity value was 100% for all cut-offs and all groups.

	Females		*Males*	
	≤≤70 y n=196	*>70 y n=200*	*≤70 y n=149≤*	*>70 y n=98*
D-dimer level	0.93±1.66	1.64±2.00	1.52±2.40	1.81±2.77
Cut-off = 0.50 mg/mL	74.1 (66.9-80.5)	32.9 (25.8-40.7)	66.9 (57.8-75.2)	33.3 (22.9-45.1)
Specifically calculated cut-off [cut off]	81.6 (75.0-87.1) [0.65 mg/mL]	53.7 (45.7-61.5) [0.85 mg/mL]	70.2 (61.3-78.2) [0.55 mg/mL]	69.3 (57.6-79.4) [0.90 mg/mL]

Patients were classified as DVT positive/negative according to the results of the following diagnostic work-up: pre-test clinical probability (PCP), Dd measurement and compression ultrasound (CUS) to assess proximal DVT, to be repeated after 5-7 days in patients with a negative initial CUS and likely PCP or altered Dd (>0.50 mg/mL). All negative patients received a phone call after 3 months. A proximal DVT was diagnosed in 109 patients (16.9%). Using the recommended cut-off (>0.50 mg/mL) the sensitivity of the Dd test was 100% and the specificity was 54.1% (95%CI 49.8-58.3). However, Dd levels resulted significantly higher in patients aged >70 y (1.71±2.29 mg/mL) *vs.* those £ 70 y (1.18±2.02 mg/mL; p=0.002) especially in females (Table 1; p=0.0001). Dd levels were also significantly higher in males (1.64±2.55 mg/mL) than in females (1.29±1.87 mg/mL; p=0.049). The specificity of Dd test using age and gender specific cut-off values increased from 54.1% to 68.7% (95%CI 64.6-72.7; p=0.000) (Table 1). In conclusion, the use of different cut-off levels according to age and gender significantly increases the specificity while does not affects the sensitivity.

C034

OUTCOME OF PATIENTS WITH SUSPECTED SYMPTOMATIC DEEP-VEIN THROMBOSIS (DVT) AND A NORMAL ULTRASOUND-BASED INITIAL DIAGNOSTIC WORKUP. A PROSPECTIVE STUDY

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Background. Of out-patients with suspected DVT only 20% have the disease confirmed, and the rest is labelled as *DVT-free*, after the initial diagnostic work-up. Even some of these patients maintain symptomatic, little is known about their fate within the following 3 months (only 1-2% of them will eventually suffer a subsequent DVT). In a prospective cohort study we assessed the outcome of symptomatic patients in whom DVT was not initially confirmed. *Methods*. Consecutive symptomatic outpatients were enrolled after objective DVT exclusion. At baseline we gathered information on patients' symptoms and risk factors for DVT; and sought to establish a likely cause for leg complaints. Patients were then scheduled for a 3-months follow-up visit. *Results*. 602 subjects were included (mean age 63, 31% male). Commonest leg complaints were pain (80%), oedema (70%), tension (50%), skin redness (34%); the more frequent risk factors were age >65 (52%), varicose veins (24%), obesity (20%), history of leg trauma/fracture (16%), cancer (9%). A likely explanation for leg symptoms was found in 78%; such as erysipelas (14%), superficial phlebitis (13%), trauma (12%), arthrosis (9%), muscle tear (8%). After 3-months follow-up, 47% of the patients remained asymptomatic, 46% was improved or stable and 7% worsened. The initial suspect of leg complaints was confirmed in 85% and excluded in 15%. In the latter case a new diagnosis was established in 7% (DVT 1%). The mortality rate was 1.6%, without fatal thromboembolic events. *Conclusions*. In patients *DVT-free* after the initial diagnostic workup, a differential diagnosis may be established on clinical grounds in more than 2/3 of the cases, usually due to local causes. The 3-months prognosis is usually benign, with only <10% with worsening symptoms and a <2% mortality rate.

C035

USEFULNESS OF TRANSTHORACIC ECHOCARDIOGRAPHY IN PATIENTS WITH A FIRST EPISODE OF PULMONARY EMBOLISM FOR THE DETECTION OF CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION

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Chronic Thromboembolic Pulmonary Hypertension (CTPH) is a rare complication of pulmonary embolism (PE). The incidence of symptomatic CTPH is estimated 1-3% after 1 year. Aim of our study was to evaluate the incidence of CTPH by using transthoracic echocardiography performed before the end of standard warfarin therapy and thereafter in patients who developed persistent dyspnea. We followed 155 consecutive patients (males 75; females 80), median age 59 years (19-87) after a first episode of PE. PE was isolated in 61 patients and associated with deep vein thrombosis (DVT) in 94; 101 patients had a spontaneous episode, in 54 the event was related to the presence of removable risk factors. In 2 asymptomatic young women (1.2%) oral anticoagulant treatment (OAT) was maintained for the detection of CTPH at echocardiography performed 6 months after the event (rV/rA gradient 50 mmHg and 55 mmHg respectively). OAT is still ongoing in 25 patients. Follow-up started after stopping warfarin (total period 241 pt/yrs, median 15 months (1-114). During follow-up 9 patients (rate 7%) had recurrence (3 PE and 6 DVT). At transthoracic echocardiography, 4 patients had a slight increase in pulmonary systolic pressure (defined as rV/rA gradient 28-32 mmHg) without dyspnea on exertion, therefore warfarin was withdrawn. These patients had neither recurrence nor worsening of echocardiographic findings was detected during follow-up. One patient developed exertion dyspnea 3 years after warfarin withdrawal. A transthoracic echocardiography showed an increase of pulmonary artery pressure and a pulmonary angiography confirmed the presence of CPTH. In our series CPTH was found in 3 patients (1.9%): 2 cases with early onset and 1 case after 3 years. Transthoracic echocardiography is a simple non-invasive method to identify symptomatic and asymptomatic patients who develop CTPH after a first episode of PE.

Anticoagulant Therapy I

C036

A MULTICENTRE RANDOMISED CLINICAL END-POINT STUDY OF PARMA 5 COMPUTER- ASSISTED ORAL ANTICOAGULANT DOSAGE

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Background and Design. The demand for oral anticoagulant treatment is growing worldwide thus increasing the need for efficient computer dosage programs. Recently, the larger multicentre clinical end-point study ever performed, to assess the safety and effectiveness of computer-assisted dosage, has been completed. It included 13,219 patients, randomized to be followed with 2 different computer programs or without the computer aid, by experienced medical staff. We present here the subset of patients followed by the PARMA 5 computer program. The PARMA computer-dosage program has been used for this purpose for several years and a new version, PARMA5, has been assessed in this multicentre clinical end-point study which has compared its safety and effectiveness with manual dosage by experienced medical staff. Patients were enrolled at 19 centres with known interest in oral anticoagulation in 6 EU countries. The study target recruitment was 8,000 patient-years, randomised to medical staff or computer-assisted dosage with PARMA 5. Safety and effectiveness were to be compared. *Results.* 10,421 patients were recruited (15,369 patient-years) in a 5-year study. International normalised ratio (INR) tests numbered 167,791 with manual and 160,078 with PARMA 5 dosage. Success in *time in target INR range* was significantly greater with PARMA 5 compared with experienced medical staff. Overall, with PARMA 5 assisted dosage there was a non-significant reduction in clinical events; however, in the 2542 patients with deep vein thrombosis/pulmonary embolism, bleeding and/or further thrombotic events were significantly reduced (p=0.005). *Interpretation.* Safety and effectiveness of PARMA 5 assisted dosage has been demonstrated, at centres with established interest in oral anticoagulation. Significant reduction of further thrombotic or bleeding events has also been observed in patients with established deep vein thrombosis/pulmonary embolism.

C037

RISK OF STROKE IN ATRIAL FIBRILLATION PATIENTS ON WARFARIN: PREDICTIVE ABILITY OF RISK STRATIFICATION SCHEMES FOR PRIMARY AND SECONDARY PREVENTION

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Atrial fibrillation (AF) patients are widely heterogeneous in terms of ischemic stroke risk. Several risk stratification schemes have been developed. We performed a prospective study on 622 AF patients on long-term OAT, evaluating the agreement among the different schemes and their correlation with adverse events recorded during follow-up. Patients at low risk were similarly distributed among different models. Instead, patients classed at medium risk were 71% by CHADS2 score, 41% by NICE and 3.4% by ACCP. As a consequence patients classed at high risk were 21.9% by CHADS2, 54% by NICE and 92% by ACCP. CHADS2 and NICE scores were associated to the best predictive accuracy. Patients on treatment for secondary prevention were included in high risk groups by all models, whereas for patients on primary prevention disagreement among the different models was observed, in particular for patients at medium and high risk. During follow-up 32 major bleeding (1.35% pt/yrs) and 39 thrombotic events (1.64% pt/yrs) were observed. Among patients on OAT for secondary prevention, all events occurred in high risk group. Instead, in patients on primary prevention, adverse events were distributed homogeneously between moderate and high risk groups. In conclusion, AF patients on secondary prevention are always included in high risk groups, therefore stratification by available models does not give further information. Differently, wide discrepancies in

stroke risk distribution were evident for patients on primary prevention. The incidence of adverse events during follow-up seems to confirm these results. Stratification of AF patients on primary prevention needs to be improved.

C038

NOT IRRELEVANT RISK OF BLEEDING IN ATRIAL FIBRILLATION PATIENTS ON WARFARIN WAITING FOR ELECTIVE CARDIOVERSION

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Systemic embolism is the most serious complication of cardioversion and may follow external or internal direct current pharmacologic and spontaneous cardioversion of AF. In addition, the immediate post-cardioversion period is associated with increased risk for thrombus formation. For this reason adjusted-dose anticoagulation (target INR 2.5) is recommended for patients with AF lasting ≥48 h or of unknown duration for 3 weeks before elective cardioversion and for at least 4 weeks after. No information is available about bleeding risk related to this practice. For this reason, we performed a prospective study on 95 AF patients [67 males, 28 females; median age 68 (38-89) years, total follow-up period 37.6 patient years (pt-yrs)] starting warfarin for elective cardioversion. The quality of anticoagulation levels and the occurrence of bleeding and thrombotic events were recorded. Patients spent 15.5%, 61.5% and 19.5% of time below, within and above the intended therapeutic range respectively. When we observed the INR levels, we found that 38 patients (40%) had at least in one occasion INR>4.5, 14 (18%) of them had at least once INR≥6.5, that was corrected by vitamin K administration. During follow-up no thrombotic complications were recorded. Instead, 2 patients had fatal cerebral haemorrhage (rate 5.4% pt-yrs): 1 male, 73 years, INR at the event 3.0, had spontaneous intracerebral haemorrhage; 1 female, 80 years, INR at the event 1.9, had post-traumatic sub-dural haematoma. In conclusion, our results show that AF patients on warfarin for elective cardioversion are exposed to a not irrelevant risk of bleeding. Efforts are required to limit time of exposure to warfarin for cardioversion.

C039

INCIDENCE OF MAJOR BLEEDINGS ASSOCIATED WITH THE USE OF FIXED DOSE SUBCUTANEOUS LOW MOLECULAR WEIGHT HEPARIN COMPARED TO INTRAVENOUS ADJUSTED DOSE UNFRACTIONATED HEPARIN FOR TREATMENT OF ACUTE THROMBOTIC EVENTS: A METANALYSIS

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Background. Compared to Unfractionated Heparin (UFH), Low molecular Weight Heparins (LMWH) proved to be at least equally effective antithrombotic drugs in various clinical settings. However, it is still unclear whether the incidence of bleeding complications associated with UFH and LMWHs differ. OBJECTIVES. Objective of this meta-analysis is to compare the bleeding risk associated with the use of fixed dose, subcutaneous LMWHs to that of adjusted dose intravenous UFH in the treatment of acute venous and arterial thrombotic events. Major hemorrhage was the primary end point of our meta-analysis. *Methods.* We searched electronic databases MEDLINE, EMBASE and the Cochrane Central Register of Controlled Trials, for randomized controlled trials comparing subcutaneous, fixed weight-adjusted doses LMWHs to intravenous, APTT-adjusted doses of UFH, for the treatment of acute venous or arterial thrombotic events. *Results.* Thirty-four studies were included. A total of 13608 patients (48.5%) received UFH and 14466 patients (51.5%) received LMWHs. Overall, a trend toward a lower incidence of major bleedings was observed among LMWH-treated patients than in UFH-treated ones (odds ratio, 0.84; 95% confidence interval [CI], 0.67-1.04). However, the higher safety of LMWH compared to UFH was observed in patients with venous thromboembolism only (odds ratio, 0.72; 95% CI, 0.51-1.02), as the risk of bleeding complications in patients with acute coronary syndromes treated with LMWH or UFH was not significantly different (odds ratio, 0.87; 95% CI, 0.63 to1.20). CONCLUSION. Our study shows a non statistically significant trend toward a lower risk of major bleeding complications associated with the use of LMWHs, compared to that of UFH, for treatment of patients with acute venous thrombotic events.

C040

THE ROLE OF GENERAL PRACTITIONERS IN THE MANAGEMENT OF ORAL ANTICOAGULANT THERAPY IN PARMA AREA

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An ever increasing number of patients are treated with Oral Anticoagulants (OA), with ever increasing workload on Anticoagulation Centers (AC), which demands for alternative models of service delivery. After the good results obtained in a pilot study carried out in Parma in the years 2000-2002, in which General Practitioners (GPs) have been directly involved in AO surveillance, in order to reduce access of stable patients to AC and to improve patients' quality of life, since 2004 we proposed access to the project to all GPs of the Parma area, obtaining adhesion from 80 out of 300 GPs. The proposed model provides a decentralisation of delivery service through GPs, who are directly involved in full management of their own patients. Three important tools are used to allow GPs manage OA therapy of their own patients:a) near-patients test devices; b) computerized decision support system (P.A.R.M.A. Program); c) complete education program (e-learning program, via a dedicated web site) to provide an opportunity for GPs to increase their knowledge in managing patients receiving OA. GP role in the model: a) patients attend the GP office; b) capillary blood is taken and analysed with a near-patient testing device; c) dosing is provided directly by the GP using a computerised decision support system (P.A.R.M.A. system vers 5.7), through internet connection to a central database; d) all patient data are stored in the central database and GP can only see data of his own patients. To assess the efficacy of the model, an analysis of quality of OA treatment has been performed. In 2007, GPs managed 1140 patients and delivered a total of 16,419 prescriptions. In the same period, all AC of the Parma area managed 7129 patients for a total of 135,965 prescriptions, therefore GPs delivered 10,8% of total OA prescriptions. We investigated the proportion of INR in therapeutic range obtained by GPs in comparison to the performance of the expert physicians of the four AC of the Parma area. In 2007, the percentage of INR in the therapeutic range was 59,1% for GPs and 56,3% for AC (data calculated only in stabilized patients, more than 90 days in Oral Anticoagulant Therapy). GPs are able to provide to their own patients a similar OA management than AC. We can conclude that GPs manage OC therapy with a satisfactory treatment quality, reducing access of stable patients to AC and improving patients' life quality.

C041

A NEW TOOL TO IDENTIFY POSSIBLE INTERACTIONS WITH ORAL ANTICOAGULANTS

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Background. Oral anticoagulants (OA's) are one of the most common causes of drug-induced adverse events. Interactions with anticoagulant effect might increase risk for bleeding and thrombosis recurrence but reports on OA's interactions are generally represented by small case series and single case reports. *Aim of the study.* To provide information on possible OA's interactions with drugs, illness and lifestyle and to implement targeted interventions. *Methods.* This study sample was drawn from 1.500 patients monitored in our Anticoagulation Clinic (AC). A patient had to have been a member of the AC for at least one year and must have had a *significant* INR variation. We defined as *significant* any INR variation outside the therapeutic range and greater or lower than 2.0 between two consecutive visits. The intensity of the anticoagulation had to have been within the therapeutic range for more than 65% of the time in the prior 12 months. For each significant INR variation we interviewed the patient to elicit the possible causes of the interaction. *Results.*

A total of 112 significant INR variations were retrieved over the two years study period (October 2005-September 2007). The incidence rates were similar over the two years (4,06 and 3,46% respectively, p=0.46). Variations were more frequent in female patients (p=0.01) and in acenocoumarol-1 mg users compared to warfarin-treated patients (p<0.001) or to acenocoumarol-4 mg users (p<0.01). Table 1 shows the full range of causes of the significant INR variations and the type of INR variation. Among the 14 interactions due to antibiotics, six were from quinolones, three from cephalosporins, two from amoxicilin/clavulanate, two from cotrimoxazole, one from fluconazole. Among analgesics we found four significant INR variations due to paracetamol, four to ibuprofen and one to ketorolac. There could be a bias on the number of variations resulting from ibuprofen as it is the most prescribed analgesic drug in the AC. We could not identify the cause of significant variations in 24% of patients but we intend to adjust for liver disease and hyperthyroidism in this category. *Conclusions.* This method of identifying possible OA's interactions has the potential to address problems that have not been revealed by traditional methods. It could be generalised for other AC and a multicentric study could be easily coordinated. With these data we will design a targeted educational project intended to reduce drug interactions in our patients.

Table 1.

	INR variations	
Causes of significant INR variations (n)	*increased*	*decreased*
No cause identified (28)	25	3
Antibiotics (14)	11	3
Congestive heart failure (11)	10	1
Analgesic (9)	9	0
Diarrohea/Constipation (7)	6	1
Antiarrhythmics (4)	4	0
Vacation (4)	4	0
Influenza vaccine (3)	3	0
Increased physical activity (3)	3	0
Low-Carbohydrate diet (3)	1	2
Corticosteroids (3)	3	0
Antihistamines (3)	2	1
Non-compliance (3)	3	0
Febrile illness (2)	2	0
Lansoprazole (2)	2	0
Allopurinol (2)	2	0
Phenobarbital withdrawal (1)	1	0
Papaya powder (1)	1	0
Levodopa (1)	1	0
Methimazole withdrawal (1)	1	0
Sildenafil (1)	1	0
Cholesterol-lowering drugs (1)	1	0
Ursodesossicholic acid (1)	1	0
Fibrate (1)	1	0
Glibencamide (1)	1	0
Heparin eye drops (1)	1	0
Malgadrate (1)	1	0

C042

SAMPLE CARRYOVER IN PT-INR DETERMINATION. IS IT AN ISSUE IN OAT CONTROL?

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Introduction. The quality of Oral Anticoagulant Therapy (OAT) control rely on the adequacy of the treatment and on the reliability of the laboratory analytical result. OAT is monitored through PT-INR (prothombin time - International Normalized Ratio) determination, which is generally performed using automated analysers that ensure highly precise and accurate analytical performance. Nevertheless, accuracy may be impaired by various analytical variables. We report on the effect of sample carryover (CO) in PT-INR determination. *Method.* Blood, collected into vacuum tubes containing 0.109 M sodium citrate (Becton Dickinson), was centrifuged at 2000 x g for 10 min. PT-INR test was carried out on ACL Elite (Instrumentation Laboratory - IL) using Recombiplastin (IL). Plasma samples from OAT patients at different intensities of anticoagulation were tested in the following analysis sequence: OAT sample (5 replicates) - Normal plasma (1 replicate) - OAT sample (5 replicates). The PT-INR test was then repeated increasing up to three the number of the washing cycles for the sample needle during plasma loading. The sample carryover was calculated as follows: [(OAT2-OAT1)/OAT1]*100, where OAT2 is the first PT-INR result of OAT sample analysed immediately after the normal plasma, and OAT1 is the pre-carryover mean PT-INR value of the same OAT sample analysed before the normal sample. *Results.* Analysis of OAT samples after a normal sample resulted in shortened coagulation times and therefore in lower PT-INR values. There was a linear relationship between CO and INR values: in fact, the carryover was 5.71% for PT-INR 3.19 and 19.1% for PT-INR 7.35. Increasing the number of the washing cycles to two for the sample needle reduced the above CO values to 2.1% and 6.86%, respectively. Results are shown in Figure 1. The carryover effect was completely neutralized with three washing cycles of the sample probe. *Comments.* We demonstrated a sample carryover of normal to OAT sample that significantly affected PT-INR determination for INR values >3. Although the *normal to OAT plasma* represents the worst condition in carryover phenomenon, in the routine workload of unknown samples the actual anticoagulation level might be underestimated leading to inappropriate dose adjustment thus enhancing the risk for haemorrhage complications. The sample carryover effect might be overcome by increasing the numer of washing cycles for the sample needle from one to three.

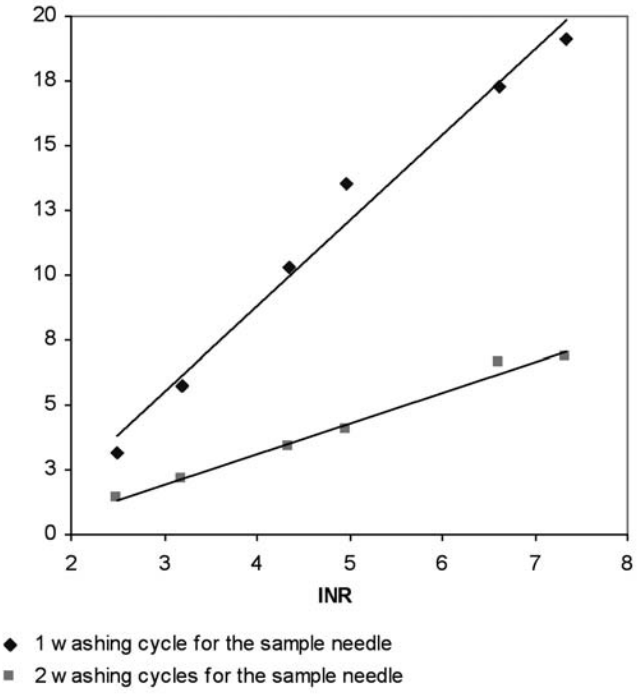

Figure 1.

Atherothrombosis and Inflammation: Clinical Aspects

C043

PERSISTENT PLATELET THROMBOXANE A2 PRODUCTION IN TYPE 1 DIABETIC PATIENTS UNDER CHRONIC ASPIRIN TREATMENT

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Background. Despite the benefit of aspirin in diabetic patients has consistently been documented, the reduction in cardiovascular events associated to aspirin use is lower in diabetic than in non diabetic patients. We have previously demonstrated a reduced platelet sensitivity to aspirin in type 2 diabetic (T2DM) patients. In order to investigate the role played by hyperglycemia in this phenomenon, we compared T2DM patients, T1DM patients and non-diabetic (ND) high risk subjects under chronic aspirin treatment. *Methods.* Agonist-induced platelet aggregation (PA), collagen-induced and serum TxA2 production, Hs-CRP levels, fasting plasma glucose and HbAlc were studied in 100 T2DM patients, in 26 T1DM patients and in 100 high risk ND patients. *Results*. T1DM patients displayed a significantly greater Mx% PA, compared to non diabetic subjects, following activation by ADP (48.0±4.8 *vs.* 33.3±1.9, respectively, $p<0.01$), by collagen (50.3±5.6 *vs.* 41.7±3.6, respectively, $p<0.02$) or by AA (13.3±2.1 *vs.*7±1.2, respectively, $p<0.01$). Collagen-induced TxA2 production was similar in T1DM and T2DM patients (845.4±381.9 pg/10^8 cells and 854.0±165.4 pg/10^8 cells, respectively) and greater than in ND patients (250.9±46.3 pg/10^8 cells). Accordingly, serum TxA2 concentration was significantly higher in T1DM and T2DM patients than in ND subjects (1512.1±377.3 pg/mL, and 1641.9±191.1 pg/mL *vs.* 977.5±132.8 pg/mL, $p<0.005$ and 0.003, respectively). No difference was found between T1DM and T2DM patients. In T2DM patients with fasting plasma glucose <126 mg/dL serum TxA2 production was 625+182 pg/mL, while in those with fasting plasma glucose >126 mg/dL serum TxA2 production was 1793+203 pg/mL. Weak correlations were observed between TxA2 production and plasma glucose (R=0.29) or HbAlc (R=0.28). By dividing T2DM patients into quartiles defined by the distribution of the fasting plasma glucose or by HbAlc values we observed higher TxA2 production levels in patients in the first quartile (698.87±145.96 pg/mL) compared to those in the last quartile (2323.76±528.85 pg/mL). However, no correlation was found between serum CRP levels and serum TxA2 production (R=0.03). *Conclusions.* This study shows similar reduced susceptibility of T1DM and T2DM patients to the beneficial effects of aspirin. These findings, as well as the observation that patients with optimal glycaemic control had lower TxA2 levels, suggest that hyperglycaemia is likely to play an important role.

C044

CARDIOVASCULAR RISK FACTORS AND OUTCOME IN PATIENTS WITH RETINAL VEIN OCCLUSION. A ROLE FOR ANTIPLATELET TREATMENT?

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We investigated 117 consecutive patients (61 M, 56 F; mean age 54±13; age at the first event 51±13) with a history of fluorangiographically confirmed retinal vein occlusion (RVO; 62 central retinal vein, CRVO; 48 branch retinal vein occlusion, BRVO, 7 both types in different eyes). RVO patients were compared to 202 age, and sex-matched (105 M, 97 F; mean age 52±12) controls, with respect to the prevalence of vascular risk factors (arterial hypertension, cigarette smoking, diabetes mellitus, obesity/overweight, dyslipidemia, abnormal hematocrit), and to a series of inherited or acquired thrombophilic abnormalities (antithrombin, Protein C, Protein S and homocysteine levels, lupus anticoagulant, anticardiolipin antibodies, FV G1691A and FII G20210A gene polymorphisms). Hypertension had a significantly higher prevalence in RVO patients than in controls (64.6% *vs.* 28.4%; OR 4.6 95%CI 2.8–7.5; $p<.0001$), as well diabetes mellitus (17.8% *vs.* 8.1%; OR 2.5 95%CI 1.1-5.5; $p<0.05$). The frequency of thrombophilic abnormalities was comparable in the two groups, nor difference were found according to RVO localization (BRVO *vs.* CRVO) or to the age at event (< or >45 yrs). BRVO patients had a significantly higher age at the event (55 *vs.* 47; $p<0.002$), and prevalence of diabetes, overweight and hypertension (29.7% *vs.* 8.7%; 83.9% *vs.* 57.8%, 78.7% *vs.* 55.9%; p always <.05). Data concerning prescribed treatment and the occurrence of further vascular (arterial, venous and retinal) events were available for 90/117 patients after a mean 7.9 yr-follow-up. Only 22 (24%) patients received antiplatelet agents (mostly aspirin 100 mg/d) and in 58 (64%) new vascular events were recorded at follow-up, in the majority of cases coronary or cerebral ischemic events (n=38). A tendency to a significantly lower prevalence of overall vascular recurrence in patients on antiplatelet treatment (45.4% *vs.* 70.6%, $p=0.06$) was shown. Our data support the role of established cardiovascular risk factors in the development of RVO, especially in BRVO and in older patients, ruling out the contribution of thrombophilia, also in younger patients. Consistent with the possible atherosclerosis-related pathogenesis, antiplatelet agents seem to be beneficial in this setting. Large-scale prospective intervention trials are needed and the correction of established risk factors (in particular hypertension) remains the main vascular prevention strategy in these patients.

C045

EARLY HEMORRHAGIC TRANSFORMATION OF BRAIN INFARCTION: RATE, PREDICTIVE FACTORS AND INFLUENCE ON CLINICAL OUTCOME. RESULTS OF A PROSPECTIVE MULTICENTER STUDY

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Background and objectives. Early hemorrhagic transformation (HT) is a complication of ischemic stroke but its effect on patient outcome is unclear. The aims of this study were to assess: 1) the rate of early HT in patients admitted for ischemic stroke 2) the correlation between early HT and functional outcome at 3 months 3) the risk factors for early HT. *Methods*. Consecutive patients with ischemic stroke were included in this prospective study in four study centers. Early HT was assessed by CT examination performed at day 5±2 after stroke onset. Study outcomes were three-month mortality or disability. Disability was assessed using a modified Rankin score (≥3 indicating disabling stroke) by neurologists unaware of the occurrence of HT in the individual cases. Outcomes in patients with and without early HT were compared by χtest. Multiple logistic regression analysis was used to identify predictors for HT. *Results*. Among 1,125 consecutive patients (median age 76.00 years), 98 (8.7%) had HT: 62 (5.5%) had hemorrhagic infarction and 36 (3.2%) parenchimal hematoma. At 3 months, 455 patients (40.7%) were disabled or died. Death or disability was seen in 33 patients with parenchimal hematoma (91.7%), in 35 patients with hemorrhagic infarction (57.4%) as compared with 387 of the 1021 patients without HT (37.9%). At logistic regression analysis, parenchimal hematoma, but not hemorrhagic infarction, was independently associated with an increased risk for death or disability (OR 15.29; 95% CI 2.35-99.35). At logistic regression analysis, parenchimal hematoma was predicted by large lesions (OR 12.20, 95% CI 5.58-26.67), stroke due to cardioembolism (OR 5.25; 95% CI 2.27-12.14) or to other causes (OR 6.77; 95% CI 1.75-26.18), high levels of blood glucose (OR 1.01; 95% CI 1.00-1.01) and thrombolytic treatment (OR 3.54, 95% CI 1.04-11.95). *Conclusions*. Early HT occurs in about 9% of patients. Parenchimal hematoma, seen in about 3% of patients, is associated with an adverse outcome. Parenchimal hematoma was predicted by large lesions due to cardioembolism or other causes, high blood glucose and treatment with thrombolysis.

C046

HIGH LIPOPROTEIN (A) LEVELS ARE ASSOCIATED WITH AN INCREASED RISK OF RETINAL VEIN OCCLUSION

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Introduction. Retinal vein occlusion (RVO) is one of the most common retinal vascular disorders affecting small, medium and large ocular vessels. Few studies, with conflicting results, and conducted in limited study populations, have hypothesised a role for high levels of lipoprotein (a) [Lp (a)] on the occurrence of RVO. Aim of this study was to investigate, in a large group of RVO patients, the role of such emerging thrombophilic parameter on the pathogenesis of RVO. *Material and Methods*. We compared 262 patients [median age: 66 years (15-88); 122 M, 140 F] with 262 age- and sex- comparable healthy subjects. Lp (a) levels was measured by an ELISA method [Mercodia Lp (a), Uppsala, Sweden]. *Results.* Circulating concentrations of Lp (a) were found to be significantly different in patients as compared to healthy subjects [189 (60-1,898) mg/L *vs.* 119.5 (6-1,216) mg/L; $p<0.0001$, respectively]. No significant differences according to the different type of occlusion (central or branch occlusion) were observed. In order to investigate the possible association between high Lp (a) levels and the disease we performed a logistic regression analysis. At the univariate analysis, Lp (a) levels > 300 mg/L were found to be associated with an increased risk of RVO (OR: 2.39, 95%CI 1.39-3.59; $p<0.0001$). Afterward, two models of multivariate analysis were performed, firstly by adjusting for age, gender, and traditional cardiovascular risk factors, and secondly also for emerging thrombophilic risk factors such as homocysteine, and B-group vitamins' levels. In both models, Lp (a) levels > 300 mg/L confirmed their role of risk factor for RVO [1st model, OR: 2.16 (1.39-3.34), $p<0.0001$; 2nd model, OR: 2.12 (1.30-3.44), $p=0.002$]. *Conclusions.* This study reports, in a large population of RVO patients, that high Lp (a) concentrations are significantly, and independently from other traditional and emerging risk factors, related to RVO, by suggesting that they may play a role in its pathogenesis.

C047

ATHEROSCLEROTIC AND THROMBOPHILIC RISK FACTORS IN PATIENTS WITH RECURRENT CENTRAL RETINAL VEIN OCCLUSION

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Background. Atherosclerotic and thrombophilic risk factors may be causes of central retinal vein occlusion (CRVO). The aim of this study was to evaluate the prevalence of the aforesaid risk factors in patients with recurrent CRVOs and patients with a single episode of CRVO. *Methods*. Seventeen patients with recurrent CRVO and 30 with a single episode of CRVO were enrolled. The atherosclerotic risk factors investigated were hypertension, diabetes, smoking, and dyslipidemia. Specific laboratory tests for the following thrombophilic markers were performed: homocystinemia (Hcy), lipoprotein (a), factor VIII, factor II G20210A and factor V G1691A polymorphisms, lupus anticoagulant, anticardiolipin antibodies, plasminogen activator inhibitor-1, and vitamins B6, B12, and folic acid deficiencies. A multivariate analysis, adjusted for age, gender, traditional and thrombophilic risk factors, was performed. Statistical significance was set at $p\leq0.05$. *Results.* Hypercholesterolemia, hypertriglyceridemia, fasting, and postmethionine hyperhomocys- teinemia (HHcy) were more prevalent in recurrent CRVO patients ($p<0.001$, $p<0.001$, $p=0.006$, and $p=0.005$, respectively). At multivariate analysis, hypercholesterolemia (OR: 5.04, 95% CI 1.39–18.17; $p=0.025$), hypertriglyceridemia (OR: 5.60, 95% CI 1.52–20.61; $p=0.017$), fasting HHcy (OR: 5.77, 95% CI 1.39–23.89; $p=0.028$), and postmethionine HHcy (OR: 10.88, 95% CI 2.50–47.42; $p=0.002$) were found to be significantly associated with recurrent CRVO. *Conclusions*. Dyslipidemia and hyperhomocysteinemia are independent risk factors for the occurrence of recurrent CRVO. A complete assessment of atherosclerotic and thrombophilic risk factors is recommended in CRVO patients. In addition, the need for a specific treatment is suggested.

C048

D-DIMER LEVELS AND RISK FACTORS FOR CARDIOVASCULAR DISEASE IN A GENERAL ADULT ITALIAN POPULATION: RESULTS FROM THE MOLI-SANI STUDY

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Introduction. Plasma D-Dimer, generated from fibrin during degradation, is a marker of thrombus formation. An association between D-dimer levels and the risk of cardiovascular disease has been proposed.*Objective*. To evaluate the association of D-Dimer levels with risk factors for cardiovascular disease in a general adult Italian population. *Methods*. The Moli-sani Study is an on-going epidemiological cohort study, on men and women, aged ≥35 years, randomly recruited from the general population of a Southern Italian region. D-dimer levels were measured on fresh citrated plasma by an automated latex enhanced immunoassay (HemosIL, IL, Milan, Italy). Until March 2008, 15,339 subjects were recruited. *Results.* After exclusion of subjects with cardiovascular and malignant disease and incomplete questionnaires or missing D-Dimer values, 11,247 subjects, 5,909 women and 5,338 men, aged 54±12 (mean±SD) years, (34-98 range) were available for the analysis. Women had higher D-Dimer levels than men (211,5 ng/mL (206,6-216,5) *vs.* 198,9 (194,3-203,6), $p<0.0001$, respectively). In women, after multivariate ANOVA adjusted for age, physical activity, social status, diabetes, menopausal status, white blood cell count, CRP levels, smoking habits, D-dimer levels were positively associated with age and CRP, and negatively with physical activity and social status; moreover non-smoker women had D-dimer levels higher than former and current smokers (Table 1). In men, multivariate ANOVA, adjusted for age, social status, diabetes, hypertension and CRP levels, showed a positive association of D-dimer levels with age and CRP levels (Table 1). The association of D-Dimer levels with age was only apparent after the age of 66 in women and 56 in men. *Conclusions*. D-Dimer levels were independently associated with risk factors for cardiovascular disease both in men and in women. Such an association might help better characterizing the significance of D-Dimer as a bio-marker of the risk of cardiovascular disease. *Supported by an unrestricted grant from Instrumentation Laboratory (Milan, Italy).*

Table 1. D-dimer levels in Multivariate ANOVA.

	Women *D-dimer levels (ng/mL)*	*Men* *D-dimer levels (ng/mL)*
Age (years)	*mean*	*mean*
35-45	193,8	183,4
46-55	190,3	184,4
56-65	193,9	192,4*
66-75	218,7*	220,7*
>76	252,4*	263,9*
CRP (mg/L)		
0-1	198,4	190,1
1-3	201,3	193,1
3-10	209,4*	204,7*
>10	226,3*	244,0*
Physical Activity		
low	218,9	
middle	207,3*	
high	199,9*	
Social Status		
low	213,3	
middle	211,9	
high	200,7*	
Smokers (cigarettes/day)		
ex	213,9	
no	221,9	
1-10	207,9*	
11-20	201,4*	
>20	198,6*	

*(*p<0.05).*

C049

IN PERIODONTAL DISEASE THE INFLAMMATORY STATE IS ASSOCIATED WITH SUB-CLINICAL ATHEROSCLEROSIS

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A significant association between the extent and severity of periodontal disease (PD) and atherothrombotic diseases has been reported in aged populations. PD is a chronic and multifactorial infectious disease of gingival tissues causing progressive bone loss around teeth. The aim of this case-control study was 1-to evaluate the role of several inflammatory markers [C-reactive protein (CRP), Interleukin-1beta (IL-1β), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necosis Factor-alpha (TNF-α), IL-1 receptor antagonist (IL-ra), Interleukin-4 (IL-4), Macrophage Inflammatory Protein-1 alpha (MIP-1α), Monocyte chemoattractant protein-1 (MCP-1), and Vascular Endothelial Growth Factor (VEGF)] in PD; 2- to investigate the relationship between inflammatory markers and intima-media thickness (IMT) in young patients (≤40 years) with no systemic signs of atherosclerosis. Carotid IMT was assessed by means of carotid ultrasonografy in 45 patients (36.4±3.6 yrs) affected by severe PD and 45 sex and age-matched healthy subjects (34.2±3.2 yrs). Cytochemokine levels were determined by using the Bio-Plex cytokine assay. CRP was assessed by a high-sensitivity assay on a BN II nephelometer. Carotid IMT values were significantly ($p<0.0001$) higher in PD (0.82±0.02 mm) than in controls (0.73±0.07 mm). In PD patients we detected higher CRP, as well as several cytochemokine levels than in controls [CRP:1.6(0.2-11.0) *vs.* 0.7(0.1-4.0) mg/L; IL-6: 84.0(5.7-663.2) *vs.* 52.9(2.9-425.8) pg/mL; IL-8: 105.1(29.4-902.6) *vs.* 59.7(16.0-233.4) pg/mL IL-4: 62.6(4.1-223.5) *vs* 54.0(20.7-128.6) pg/L; IL-1ra: 1185.3(197.4-10278.0) *vs.* 23.2(11.3-48.1) pg/mL; MCP-1: 972.5(210.5-8365.0) *vs.* 567.1(1883.5-190.6) pg/L; MIP-1alpha: 63.8(30.0-1764.7) *vs.* 48.7(26.3-138.3) pg/mL; VEGF: 1087.9(8.4-6113.9) *vs.* 434.4(0.8-2879.3) pg/m]. In PD patients, at multiple linear regression analysis, after adjusting for age, sex and cardiovascular risk factors, IL-1ra and MIP-1alpha levels were significantly and positively related to IMT. An inverse and significant relationship between VEGF levels and PD was also observed. In controls, no significant association between PD and cytochemokines was found. Severe periodontal disease in young patients is associated to sub-clinical atherosclerosis and has to be considered as an inflammation-mediated risk factor for cerebrovascular disease in young adults.

Cancer, Hemostasis and Thrombosis

C050

MEASUREMENT OF THROMBIN GENERATION REVEALS ACQUIRED ACTIVATED PROTEIN C (APC) RESISTANCE IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV)

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ET and PV are chronic myeloproliferative disorders (MPD) characterized by an increased risk of both arterial and venous thromboses. Recently, the V617F acquired mutation in the tyrosine kinase JAK2 has been described in more than 90% of patients with PV and about half of those with ET. Clinical data suggest an association between the presence of this mutation and a higher rate of thrombosis. We used the thrombin generation assay to evaluate the hypercoagulable state according to JAK2-V617F mutation status in 59 ET (30 JAK2-V617F negative and 29 JAK2-V617F heterozygous positive carriers), and 29 PV patients (3 JAK2-V617F negative and 26 JAK2-V617F positive, of whom 16 heterozygous and 10 homozygous). Thrombin generation (Calibrated Automated Thrombogram) was determined in the presence and absence of activated protein C (APC), and APC-resistance was expressed as normalized APC sensitivity ratio (nAPCsr). Tissue factor pathway inhibitor (TFPI), total and free protein S (PS), FII, FV and neutrophil elastase were measured in plasma. MPD patients showed a lower endogenous thrombin potential (ETP) in the absence of APC, but a higher ETP in the presence of APC than controls, indicating the occurrence of APC-resistance in patients (nAPCsr: ET=3.23±1.7; PV=3.66±2.4; Controls=2.11±0.9). Within ET patients the JAK2-V617F carriers had significantly higher nAPCsr compared to JAK2-V617F negative subjects, and within PV patients, the JAK2-V617F homozygous carriers had the highest nAPCsr. The measurement of plasma determinants showed a reduction of both the coagulation proteins FII and FV, and of the anticoagulants free-PS and TFPI, in MPD patients compared to controls. The reduction in these protein levels was more pronounced in the JAK2-V617F carriers. The multiple regression analysis indicates free-PS as the major plasma determinant of the nAPCsr. Neutrophil elastase levels were significantly increased in ET and PV patients compared to controls and were inversely correlated to free-PS. In conclusion, these data indicate the occurrence of an acquired APC-resistance in MPD patients, likely due to a reduction of free-PS levels. A role of neutrophil elastase in affecting the levels of free-PS is hypothesized. The APC resistant phenotype in MPD patients is influenced by the JAK2-V617F mutational load, as the homozygous status bears a more APC-resistant phenotype.

C051

ENDOTHELIAL PROTEIN C RECEPTOR GENE SILENCING IMPAIRS ACTIVATED PROTEIN C-MEDIATED PROTECTION FROM APOPTOSIS OF BREAST CANCER CELLS *IN VITRO*

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Background. The endothelial protein C receptor (EPCR) facilitates protein C activation and mediates the anti-apoptotic activity of activated protein C (APC) on endothelium. EPCR is expressed also by extra-vascular cells, including breast cancer cells. Its role in these cells is unknown. Objective. To test whether or not EPCR is involved in apoptosis of the breast cancer cell line MDA-MB-231. *Methods.* The EPCR gene of MDA-MB-231 breast cancer cells in culture was silenced by RNA interference using siRNA specific for the EPCR gene (stealth). Controls were transfected with siRNA of non specific sequence (scramble). EPCR gene silencing was obtained at 48 hours and was stable till 72 hours. Stealth- and scramble-transfected cells were analyzed for early and late apoptotic events by propidium iodide and annexin V staining by flow cytometry. Experiments were done in the presence or absence of APC (50 nmol/L) and staurosporine (10 micromol/L). Apoptosis was expressed as percent apoptotic cells over all cells analyzed. *Results.* Addition of staurosporine doubled early and late apoptotic events under all experimental conditions. In the presence of staurosporine, addition of APC inhib-

ited early and late apoptotic events in the scramble-transfected cells by 24% and 11% respectively (p<0.05 compared to no APC addition). In the stealth-transfected cells, APC did not inhibit staurosporine-induced early and late apoptosis. *Conclusions.* Silencing of the EPCR gene abolishes the protection from apoptosis conferred by APC treatment of cancer cells in the presence of the pro-apoptotic stimulus staurosporine. EPCR expression may be a means for cancer cells to prolong survival and thus escape pro-apoptotic defense stimuli of the host.

C052

THE RISK OF RECURRENT THROMBOEMBOLISM AND MAJOR BLEEDING DURING ORAL ANTICOAGULANT THERAPY IN CANCER PATIENTS WITH AND WITHOUT DISTANT METASTASES: FINDINGS FROM THE RIETE REGISTRY

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We determined the risk of recurrent venous thromboembolism (VTE) and major bleeding in 12 823 patients with symptomatic VTE who were recruited in the international RIETE registry, had an initial treatment with unfractionated or low-molecular-weight heparin (LMWH) overlapped by oral anticoagulants, and were then followed-up for three months. Of these patients, 11 365 were free from malignancy, 431 had cancer with distant metastases, and 1 027 had a more limited cancer disease. In comparison to patients without malignancy, the adjusted hazard ratio (HR) of recurrent VTE during oral anticoagulant therapy was 5.2 (95% CI, 3.5-7.9) in cancer patients with distant metastases, and 2.7 (1.8 to 3.9) in those with more limited disease; and the adjusted HR of major bleeding was 3.8 (2.4 to 6.0) and 1.2 (0.7 to 2.0), respectively. While in all cancer patients the risk of recurrent VTE during oral anticoagulation is significantly higher than that observed in patients free from malignancy, the risk of bleeding complications is increased only in the subgroup of those with distant metastases.

C053

NEW ONSET OF CANCER AND RECURRENT VENOUS THROMBOEMBOLISM AFTER DISCONTINUING ANTICOAGULATION IN PATIENTS WITH VENOUS THROMBOEMBOLIC EVENT. A PROSPECTIVE COHORT STUDY IN 1,919 PATIENTS

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Background and Objectives. The incidence of of cancer in symptomatic venous thromboembolism (VTE) has been reported to be about 15% with the highest risk of newly diagnosed cancer in the first three-six months after VTE diagnosis. The rate of cancers diagnosed after withdrawal of antithrombotic treatment is less known. The aim of the study was to investigate the long-term risk of cancer in unprovoked VTE patient and the relationship between cancer and VTE recurrence. *Design and Methods.* Prospective inception cohort of consecutive patients who concluded anticoagulant therapy after a first episode of clinically symptomatic proximal DVT and/or PE. Cancer and clinically suspected recurrent VTE were confirmed by objective diagnostic testing. VTE were classified as unprovoked and provoked. As far as cancer is concerned, patients were divided into three groups: no-cancer, early cancer (onset in the first six months), late cancer patients (onset after the first year). *Results.* 1,919 patients were enrolled , 55.4% of which had unprovoked VTE. Mean duration of prior anticoagulation therapy was 1 year (range 3-39 months) and mean duration of follow-up was 5 years. 132 patients (7.1%) had cancer diagnosis. The incidence of cancer after 1 years was 4% in unprovoked VTE and 1.2% in secondary VTE. In the following years the risk for new cancer is about 1.36% (95%CI: 1.12-1.64) per 100 person-years in unprovoked and 0.89% (95%CI 0.62-1.23) per 100 person-years in provoked VTE, uniform over time. Unprovoked VTE was a risk factor for new cancer with an hazard ratio (HR) of 2.30 (95%CI: 1.57-3.40). The main types of cancer were breast (16.8%), colon (14.5%), lung (12.2%), prostate (9.9%) and stomach (6.8%). 33 (25.0%) cancer patients had recurrent episodes of VTE compared with 341 (19.1%) no-cancer patients (HR 1.43; 95%CI: 1.01-2.05). In particular, early cancer had lower HR for recurrent VTE (HR 0.94; 95% CI: 0.46-2.00) than late cancer (HR 1.67; 95% CI: 1.12-2.48). *Conclusions.* In patients with a first VTE episode and cancer-free at withdrawal of antithrombotic treatment, the risk of cancer is about 1.36% per 100 person-years in unprovoked VTE, underlying a possible bi-directional link between prothrombotic status and cancerogenesis. If late cancer diagnosis should be considered an indication to antithrombotic prophylaxis are hypotesis generated from our data to be tested in ad-hoc interventional studies.

C054

ASSOCIATION OF V617F JAK2 MUTATION WITH THE RISK OF THROMBOSIS AMONG PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: A SYSTEMATIC REVIEW

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Background. The V617F mutation of Januse Kinase 2 (Jak2) is present in almost 100% of patients with Polycythemia Vera and in about half of patients with Essential Thrombocythemia (ET). Many studies were performed to evaluate the association between V617F Jak2 and the risk of thrombotic events (TE) in patients with ET. However, due to the heterogeneity of the studied patients and to insufficient statistical power, the results of these studies were not consistent. Therefore, we systematically reviewed all the published studies that assessed the risk of TE associated with V617F Jak2 in patients with ET. *Methods.* We searched MEDLINE and EMBASE databases (up to December 2007) and reference lists of retrieved articles, and included studies with either a cohort or case-control design, in which the incidence of clinical outcomes in carriers of V617F Jak2 was compared with that in non-carriers. Two reviewers independently selected studies and extracted study characteristics, quality and outcomes. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated for each trial and pooled. *Results.* We included 13 studies, for a total of 2556 patients. The presence of V617F Jak2 was associated with a statistically significant increased overall risk of TE (ORs 1.78, 95% CI 1.47-2.16). The risk of both venous (ORs 2.16, 95% CI 1.43-3.25) and arterial thrombosis (ORs 1.68, 95% CI 1.29-2.19) was significantly increased in patients bearing V617F Jak2. One study showed that the risk of TE in ET patients with homozygous V617F Jak2 was significantly higher than that of patients with heterozygous mutation (ORs 3.60 95% CI 1.23-10.6). *Conclusions.* Our systematic review of the published literature suggests that V617F Jak2 increases the risk of TE in ET patients by about two fold. The burden of mutant Jak2 allele also appeared to influence the TE risk in ET; however, further studies are needed to clarify this issue better.

C055

EFFICACY AND SAFETY OF PRIMARY ANTITHROMBOTIC PROPHYLAXIS WITH TICLOPIDINE AND WITH ASPIRIN IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA. RESULTS FROM A COHORT STUDY

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Background. Essential Thrombocythemia (ET) and Polycythemia Vera (PV) are two chronic myeloproliferative disorders characterized by a high rate of vascular complications, mainly arterial thrombosis (AT). While the use of aspirin for primary prophylaxis is recommended, there is no data on the efficacy and safety of tienopyridine antiplatelet drugs (ticlopidine, clopidogrel). A*ims.* To estimate the frequency of thrombotic and hemorrhagic complications, in ET and PV patients treated with ticlopidine in a single institution, prospective cohort study. *Patients and method.* Data from 246 PV (143 males, 58%), median age at diagnosis 63 years (range 20-89) and 339 ET (114 males, 33.6%) patients, median age 62 (range 20-95) consecutively diagnosed from 1985 to 2005 were analyzed. Risk factors for arterial thrombosis were present in around 30% of patients. After diagnosis, aspirin (100-300 mg daily) was given to 270 patients (155 ET, 57%), in 70 of them (25%) for secondary prophylaxis; ticlopidine (250 mg twice day) was administered to 84 patients with a previous history of gastric ulcer, gastritis or allergy to ASA (48 ET, 57%), in 19 of them (22%) for secondary prophylaxis. In 216 (137 ET, 63%) patients no antiplatelet drug was given. Cytoreductive treatment was given to 87 (32%) patients in ASA, 14 (17%) in ticlopidine and 61 (28%) in those not on antiplatelet treatment (p=0.02). A higher percentage of patients received hydroxyurea in ASA group compared with ticlopidine (19.6% *vs.* 8.6%). *Results.* After a median follow-up of 7.8 years (similar in the 3 groups of patients), 29 (14.5%) thrombotic events (5

fatal) among 200 ASA-treated patients and 18 (27.7%, 1 fatal) among 65 ticlopidine-treated patients for primary prophylaxis were recorded (p=0.016). In 216 not-treated patients, 40 (18.5%) thromboses were recorded. Major hemorrhages (need for transfusions, surgical intervention or hospital admission) were 17 (8.5%) in ASA, 8 (12.3%) in ticlopidine (p=0.299) and 25 (11.6%) in not-treated patients (p=0.392 between antiplatelet treated and not treated patients). Thrombotic rates for patients in primary prophylaxis were 0.4%, 0.8% and 2.5% in patients on ASA, ticlopidine and not-treated, respectively. *Conclusions*. ASA therapy appears more effective than ticlopidine in the primary prevention of thrombosis in ET and PV patients, without a significative increase of hemorrhagic risk compared with ticlopidine-treated or untreated patients. This increased efficacy should be further investigated by stratifying patients according to cytoreductive treatment.

C056

LEPTIN UPREGULATES TISSUE FACTOR EXPRESSION IN THE HUMAN BREAST CANCER CELL LINE MCF7

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Background. An increased risk of cancer has been associated with obesity and adipose tissue mass by epidemiological studies. Breast tumors are surrounded by adipose tissue which synthesizes adipokines. Leptin, one of the adipokines, whose levels are found elevated in obese individuals, has been shown to act as a mitogen, transforming factor and migration factor for many different cell types. Tissue factor (TF), is often expressed by tumor cells, and for its involvement in tumor growth, angiogenesis, and metastasis is considered a hallmark of cancer progression. Since leptin has been shown to induce TF expression in human monocytes, (Napoleone *et al.* J Thromb Haemost 5:1462; 2007), we decided to investigate whether leptin could modulate the constitutive expression of TF by the metastatic breast carcinoma estrogen-receptor-positive cell line MCF7. *Methods.* MCF7 cells were grown in 10% FCS DMEM until confluency. Cells were then incubated with leptin and the different reagents in various combination for different time-intervals at 37°C in 7.5% CO_2. At the end of incubation, cells were disrupted by freezing and thawing and procoagulant activity was assessed by a one-stage clotting assay and expressed in arbitrary units (U) by comparison with a standard preparation of human brain thromboplastin. TF antigen cellular expression was determined by flow-cytometry and TF mRNA levels were assessed by real time RT-PCR. *Results.* In basal conditions MCF7 cells expressed high levels of constitutive TF activity. A dose-dependent increase in TF activity was observed when MCF7 were exposed to leptin. The activity was attributable to TF, since a MoAb against TF completely blocked the shortening of the clotting time. The increase in TF activity was accompanied by an increase in TF antigen and mRNA levels. A strong inhibition of TF activity was observed when incubation was carried out in the presence of an inhibitory anti-leptin antibody. Similar results were obtained with an inhibitory anti-leptin receptor antibody, indicating that leptin exerted its effect by binding to its receptor, whose presence was detected on MCF7 membrane by flow cytometry. Experiments with specific inhibitors of MAPK signalling revealed the involvement of ERK1/2 kinase, but not p38 kinase, pathway. *Conclusions*. These data support the hypothesis that leptin, by its upregulation of TF, may contribute to processes underlying both cancer and vascular cell disorders.

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Platelets: Qualitative and Quantitative Alterations I

C057

DOMINANT INHERITANCE OF A NOVEL INTEGRIN BETA3 MUTATION ASSOCIATED WITH A HEREDITARY THROMBOCYTOPATHIC MACROTHROMBOCYTOPENIA

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We describe a family with 18 members in five generations who have moderate macrothrombocytopenia, defective platelet function and a moderate mucocutaneous bleeding diathesis, transmitted as an autosomal dominant trait and associated with a partial GPIIIa defect. Defects of GPIIb/IIIa are typical of Glanzmann's thrombasthenia (GT), an inherited bleeding disorder characterized by the failure of platelets to aggregate in response to all the physiologic agonists, but with no abnormalities in the number or size of platelets. Although a large heterogeneity has been described for GT, no family was described as having autosomal dominant GT. The main distinctive characteristics of our family are the presence of large platelets, impaired platelet aggregation to a variety of agonists, a moderate reduction of the expression of GPIIIa and of the complex GPIIb/IIIa with normal expression of other membrane glycoproteins, normal serum TxB2 production but reduced release of arachidonic acid from platelet membrane phospholipids and a reduced arachidonic acid-induced TxB2 production, impaired platelet secretion and intraplatelet calcium movements, normal platelet adhesion to fibrinogen but reduced fibrinogen-induced platelet spreading and protein tyrosine phosphorylation, indicating defective outside-in signalling. Molecular analysis revealed a D673-E712del mutation of ITGB3: a novel mutation of GPIIIa producing a mutated protein with elimination of aminoacids 657-692 located in the betaTD ectodomain. Another family with the same mutation and a similar phenotype was lately identified from a database of inherited thrombocytopenias of unknown origin and haplotype analysis indicated that the two families inherited the mutation from a common ancestral chromosome. The GPIIIa deletion of our family seems to lead to an impaired GPIIb/IIIa-mediated outside-in signalling, and indeed several of the signal transduction pathways activated by GPIIb/IIIa ligation and clustering were altered in our patient, but also to defective megakaryopoiesis, consistent with recent data showing that platelet formation and release are regulated via fibrinogen and GPIIb/IIIa. In conclusion, we have described a novel autosomal dominant thrombocytopatic macrothrombocytopenia, associated to a novel ITGB3 mutation, that adds new clues for a role of GPIIIa in platelet formation and release and in platelet function.

C058

PLATELET SIZE DIFFERENTIATES ITP FROM INHERITED MACROTHROMBOCYTOPENIAS

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Background. Distinguishing ITP from genetic thrombocytopenias is difficult whenever previous blood counts are not available and family history does not reveal affected relatives. As a consequence, misdiagnosing genetic forms with ITP is frequent and patients often receive undue therapies. Both ASH and British guidelines for ITP indicate that platelet size is an important parameter for distinguishing between these two conditions, since very large platelets are present only in genetic forms. However, this suggestion is not *evidence-based* since no study examined this matter. *Methods.* We perspectively evaluated platelet size and platelet count in 25 consecutive patients with inherited macrothrombocytopenias (MYH9-Related Disease, homozygous BSS, heterozygous BSS due to Bolzano mutation) and 63 subjects with ITP. Healthy subjects have been used as controls. Platelet size was measured by two automated

cell counters using optical (Bayer Advia 120) or impedance (Sismex XE-2100) counting methods, and by microscopy evaluation of platelet diameters on peripheral blood films by computer-assisted image analysis. Platelet count was evaluated by the counters and by manual counting upon phase contrast microscopy. *Results.* Quite similar platelet counts have been obtained by the 3 counting methods in controls and ITP patients. At the opposite, platelet counts by the manual method were significantly higher than those obtained by counters in both MYH9-RD and BSS, thus indicating that counters do not recognize giant platelets and therefore overestimate the degree of thrombocytopenia in macrothrombocytopenias. Evaluation of platelet size by the 3 methods of analysis gave consistent results in healthy subjects. However, the impedance counter did not calculate MPV in 44 of 88 patients with ITP, MYH9-RD or BSS because of platelet anisocytosis. On the contrary, the optical counter measured MPV in all patients and, in most cases, was able to distinguish the mild platelet macrocytosis of ITP (62 of 63 patients with a MPV <14 fL) from the severe macrocytosis of inherited forms (21 of 25 patients with a MPV >14 fL). Microscopy evaluation of blood smears identified very large platelets in all the 4 patients with genetic forms whose MPV was underestimated by the counter (MPV<14 fL) because of an extreme degree of platelet macrocytosis. *Conclusions.* The combined use of optical counters and blood film evaluation has 95% specificity and 100% sensitivity in distinguishing inherited macrothrombocytopenias from ITP.

C059

A NOVEL CASE OF SELECTIVE ENZYMATIC DEFECT OF CYCLOOXYGENASE-1 ASSOCIATED WITH HAEMORRHAGIC DIATHESIS

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Thromboxane (TX) A2 is synthesized in activated platelets through the sequential activity of cyclooxygenase (COX)-1 and TXA2 synthase, and it is a pro-aggregating and vasoconstrictive agent. The importance of this pathway in haemostasis is demonstrated by the efficacy of COX-1 inhibition by aspirin in the prevention of atherothrombotic disorders. We have studied a 50-year-old woman with a lifelong bleeding history: she had haemorrhagic complications following tonsillectomy, labor, knee surgery, metrorrhagias, appendicectomy and spontaneous epistaxis. Her platelet count was within the normal range (320x10(e)3/mL). She had normal platelet aggregation in response to 5 mM ADP, 4mg/ml collagen and 20 mM epinephrine. However, on two different occasions, she showed a severe defect of platelet aggregation induced by 1.3 mM arachidonic acid: 6% transmittance (N.V. 72-92%). Her bleeding time was 4.30 min (N.V. up to 8 min). COX-1 enzymatic activity, measured by serum TXB2 released during whole blood clotting *in vitro*, was repeatedly and severely reduced: 11.58 and 19 ng/mL serum on two different measurements (normal values 200-400 ng/mL serum). The characterization of COX-1 protein was performed by western blot analysis and flow cytometry. Flow cytometry detected a reduction in the mean fluorescent intensity of platelet stained for COX-1 of approx. 30% as compared to normal subjects. Western blotting showed an apparent shift of the molecular weight of COX-1 (approx. 80 kDa, controls approx. 72 kDa), and a reduced expression when normalized to beta-actin. Platelet antigenic levels of CD61 or COX-2 were normal. She referred suffering from chronic gastritis since the age of 12, and having erosions on the gastroduodenal mucosa. Her daughter showed a far less pronounced bleeding diathesis, without major bleeding episodes or methrorrhagies. She also referred dyspeptic symptoms. Her serum TXB2 levels were 126 ng/ml.

Thus, the proposita showed a combined quantitative and qualitative defect of COX-1 in platelets. Genotyping of the whole family kindred will help identifying whether the defect is transcriptional or post-transcriptional.

C060

A NEW SUBSTITUTION CAUSING GLANZMANN THROMBASTHENIA BY ELIMINATING A KEY PROLINE IN BLADE 4 OF THE αIIB

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Glanzmann thrombasthenia (GT) is a recessively inherited hemorrhagic disorder caused by the deficiency of the platelet fibrinogen receptor, αIIbβ3. We analyzed the phenotypic effects of the αIIbP258S substitution, a previously undescribed substitution identified during genetic screening of a cohort of patients with GT coming from Southern Iran. The presence of the mutation was confirmed in several family members affected. The effect of the substitution is to eliminate the key proline from αIIb propeller. The substitution was inserted by mutagenesis in pCDNA3.1 containing αIIb cDNA and HEK cells were then transfected with normal or mutated αIIb in conjunction with normal β3. By flow cytometry, the binding of 10E5-FITC (anti-human αIIbβ3) in cells expressing αIIbP258S3 was at the same level of negative controls. HEK cells transfected with either αIIbβ3 or αIIbP258S3 were analyzed by SDS-PAGE electrophoresis and immunoblotting. All the lysates were reacting at the same level 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 mutants αIIb. In reducing condition the majority of αIIb from cells transfected with αIIbP258S was in immature form. Immunoprecipitation with anti αIIb antibodies showed that αIIb-β3 complex formation was severely impaired. Immunofluorescence studies showed that the mutated protein accumulated in the endoplasmic reticulum and transferred to the Golgi, but it did not express on plasma membrane. In conclusion we report the identification of a new mutation responsible for GT located in the αIIb propeller. This mutation causes GT interfering with the formation of the αIIbβ3 complex in the endoplasmic reticulum as shown by the increase amount of pro-αIIb found in cells expressing this mutant.

C061

A PREVIOUSLY UNDESCRIBED FORM OF PLATELET STORAGE-POOL DEFICIENCY, CHARACTERIZED BY SELECTIVE DEFECT OF ADENINE NUCLEOTIDES IN PLATELET DENSE GRANULES, IS ASSOCIATED WITH SELECTIVE DEFICIENCY IN PLATELET MRP4 (ABCC4) EXPRESSION

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Background. Delta storage pool deficiency (delta-SPD), either syndromic (e.g., Hermansky-Pudlack Syndrome) or non-syndromic, is among the most frequent inherited platelet function disorders. It is characterized by selective deficiency of the content of delta-granules, including ADP, ATP and serotonin. The pathogenesis of the disease is unclear, although both defects in forming membranes of delta-granules and defects of active transport and storage of the granule content may be implicated. Recently it was shown that the MRP4 (ABCC4) transporter is highly expressed in normal human platelets and located in delta-granules and plasma membrane and *in vitro*-evidence was presented for its involvement in ADP storage (Jedlitschky et al Blood 2004). Objective. We now describe two unrelated patients with an as yet undescribed subtype of delta-SPD, with selective defect of platelet adenine nucleotides and normal serotonin concentration. *Methods.* The following parameters were studied: platelet aggregation and ATP secretion; immunofluorescence and western blot for MRP4 expression in platelets, lymphocytes, and erythrocytes; quantification of ADP, ATP, and serotonin in platelets; sequencing of the MRP4 gene. *Results.* Both patients showed easy bruising and nose bleeding and abnormalities of platelet aggregation and secretion that are typical of patients with delta-SPD. The delta granules had selectively reduced ADP and ATP content, while their serotonin concentration was normal. Expression and localization of alpha- and delta-granule marker proteins were not significantly altered. Both

patients had diminished MRP4 expression in their platelets by immunoblotting with two antibodies and by immunofluorescence microscopy. In erythrocytes and lymphocytes from these patients, however, normal MRP4 expression was detected. Sequencing of the coding region of the MRP4 gene of these patients did not show any mutation. Two patients with classical, non-syndromic delta-SPD had normal MRP4 expression in their platelets by immunoblotting; immunofluorescence microscopy showed that MRP4 was abnormally distributed in their platelet delta-granules. *Conclusions.* We describe the first two cases of a subtype of delta-SPD, characterized by selective defect of adenine nucleotides in delta granules, which is associated with selective defect of the membrane transporter MRP4 in platelets. Our results support the essential role of MRP4 in platelet adenine nucleotide storage.

C062

HUR RNA-BINDING PROTEIN IN ACUTE CORONARY SYNDROME: STABILIZATION OF PLATELET TF AND COX-1 MRNAS

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Introduction. Platelets contain megakaryocyte-derived mRNAs which may be used for de novo protein synthesis. We have evidence that platelets from ACS patients contain higher amount of TF and COX-1 specific mRNA compared to stable angina (SA) patients or healthy subjects (HS). As platelets are anucleated cells, the mRNA pattern in ACS may be due to a direct influence of the inflammatory status on megakaryocyte transcriptome and consequently on platelet mRNAs content or rather to an increased platelet mRNA stability mediated by mechanisms such as HuR RNA-binding proteins. *Aim.* To investigate whether 1) platelet TF and COX-1 mRNA are bound to HuR; 2) HuR-mediated stabilizing mechanisms are involved in the higher amount of TF and COX-1 mRNA found in platelets from ACS patients; 3) platelet stimulation by fibrinogen and thrombin results in TF and COX-1 protein synthesis. *Methods.* Platelets for RNA extraction and protein lysate were isolated from 22 ACS, 21 SA and 17 HS. HuR binding to mRNAs was assessed by immunoprecipitation (IP) with specific antibodies followed by Real Time PCR (RT-PCR) and Western Blot analysis. De novo protein synthesis was investigated in HS platelets stimulated with fibrinogen (200 microg/mL) plus thrombin (1U/mL) for 4 hours at 37°C under stirring conditions. *Results.* IP experiments showed that both TF and COX-1 mRNAs are bound to HuR. Platelets from ACS and SA patients have significantly higher amount of HuR compared to HS (2 and 1.5 fold respectively, $p<0.05$). Moreover, the amount of TF and COX-1 mRNAs found in ACS and SA is 3 and 2 fold higher respectively compared to that found in HS ($p<0.05$). *In vitro* stimulation of washed platelets from HS with fibrinogen and thrombin resulted in a 2 fold reduction in both TF and COX-1 mRNAs levels and in an increase in the protein levels compared to unstimulated samples. *Conclusions.* HuR RNA-binding proteins are involved in platelet TF and COX-1 mRNA stability. Platelets from ACS and SA patients have higher amount of HuR compared to HS and this finding may contribute to the higher steady-state levels of target mRNAs such as TF and COX-1 which can be used for proteins synthesis upon agonist stimulation.

C063

ABNORMAL LARGE PLATELETS ARE ASSOCIATED NOT ONLY WITH VON WILLEBRAND DISEASE (VWD) TYPE 2B BUT ALSO WITH SEVERE TYPE 3: THE ROLE OF VON WILLEBRAND FACTOR (VWF) DEFECTS IN THE IMPAIRED MEGAKARYOCYTOPOIESIS

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VWD type 2B results from mutations in exon 28 of the VWF gene. Gain of function of this adhesive protein results in an increased affinity for the platelet glycoprotein (GP) Ib-IX-V complex. Impaired megakaryocytopoiesis has been described in a family with the R1308P mutation (Blood 2006; 108:2587-95). *Aim of the Study.* To examine the potential consequences of VWF abnormalities on platelet production we have studied the platelets of patients with different types of VWD. *Patients and Methods.* 13 VWD patients were enrolled in the study after signing their informed consent. Diagnoses of VWD were performed according to the criteria of ISTH-SSC-SC. 8 VWD 2B from 6 families with the following mutations: R1306W (n=1), R1308C (n=1), I1309V (n=1), V1316M (n=2), R1341Q (n=2) and P1266L (n=1, 2B/1NY). 5 additional VWD cases characterized by low/absent VWF in their platelets were also studied: VWD 2M (n=1, D1277-E78delInsL), VWD 3 without inhibitors against VWF (n=2, 276delT/257delA and 6182delT/6182delT) and VWD with alloantibodies against VWF (n=2, large deletions of the VWF gene). Electron microscopy (EM) and immunolocalization of VWF were performed. *Results.* EM showed the presence of an increased population of giant platelets (15 to 40% versus <10% for controls) in all VWD 2B. Additional abnormalities were observed in the patient with the 2B/1NY, alpha-granule morphology was different with a population of enlarged granules. Immunogold staining for all type 2B patients showed that VWF was present not only inside the granules but also in the Surface-Connected Canalicular System. For 3/8 patients with VWD 2B, cleaved caspase was present in the platelets indicating abnormal caspase activity at least for R1341Q and V1316M. In VWD 2M (n=1) and 3 (n=2) with a premature stop codon, no significant modification of platelet morphology was found. In contrast, a significant number of platelets with numerous vacuoles were found larger than controls in the two VWD 3 with large deletions and alloantibodies directed against VWF. Immunogold labelling for VWF was completely negative for these two patients. *Conclusions.* patients with VWD types 2B and 3 (undetectable VWF) show platelet production defects of varying severity, suggesting a major role of VWF in the fine regulation of megakaryocytopoiesis. Up-regulation or loss of the interaction between VWF and GPIb may lead to a variable proportion of giant platelets with or without thrombocytopenia

Venous Thromboembolism: Epidemiology and Relapse

C064

ULTRASOUND FINDINGS TO GUIDE THE DURATION OF ANTICOAGULATION IN PATIENTS WITH DEEP-VEIN THROMBOSIS A RANDOMIZED STUDY

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Background. The optimal duration of oral anticoagulant therapy in patients with deep-vein thrombosis (DVT) of the lower extremities remains uncertain. *Methods.* Multicenter, randomized clinical trial in consecutive patients with acute proximal DVT who had completed an uneventful 3-month period of anticoagulation. Patients were randomized to a fixed duration of anticoagulation (i.e., no further anticoagulation for secondary DVT, an extra 3-month for idiopathic DVT) or to a flexible duration of ultrasound-guided anticoagulation (i.e., no further anticoagulation in patients with recanalized veins, continuation of anticoagulation in all other patients up to a maximum of 9 months for secondary and 21 months for idiopathic DVT). The main efficacy outcome was objectively confirmed recurrent venous thromboembolism (VTE) from randomization up to completion of 33 months of follow-up. *Results.* Recurrent VTE developed in 46 (17.2%) of the 267 patients allocated to the fixed anticoagulant duration and in 32 (11.8%) of the 271 patients randomized to the flexible duration, for an adjusted hazard ratio of 0.62 (95% CI, 0.39 to 0.97). Major bleeding occurred in 2 (0.7%) patients in the fixed duration group and 4 (1.5%) in the flexible duration group (p=0.67). In total, 14 thromboembolic events were prevented for 101 person-years of extra treatment, at the cost of 82 additional ultrasounds, and 2 additional major bleeds. In patients with idiopathic DVT, 13 thromboembolic events were prevented for 65 person-years of extra treatment, 39 additional ultrasounds, and no additional major bleeds. In patients with secondary DVT, only 1 thromboembolic event was prevented for 36 person-years of extra treatment. *Conclusions.* Tailoring the duration of anticoagulation based on ultrasound findings reduces the rate of recurrent VTE. The results suggest that limiting this strategy to patients with idiopathic DVT will provide both the best risk-benefit trade-off and be the most cost-effective.

C065

THE NATURAL HISTORY OF D-DIMER AFTER ANTICOAGULATION SUSPENSION FOR A FIRST EPISODE OF IDIOPATHIC VENOUS THROMBOEMBOLISM: THE PROLONG II STUDY

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The PROLONG study (N Engl J Med 2006;355:1780-9) showed that an abnormal D-dimer (D-d) at 1 month after vitamin K antagonists (VKAs) suspension in patients with unprovoked venous thromboembolism (VTE) is associated with a significant higher risk for recurrences as compared with normal D-d. However it is unknown whether D-d changes in the subsequent follow-up. The aim of the PROLONG II was to assess the natural history of D-d in patients with a normal D-d after VKA suspension after a first episode of unprovoked VTE. *Methods.* In a prospective multi-center (15 centres) study, patients were enrolled after at least 6 months of VKA. D-d was measured during VKAs with a qualitative method (Clearview Simplify D-dimer; Instrumentation Laboratory, Milan). If D-d was abnormal, VKAs were prolonged, while if D-d was normal anticoagulation was stopped. D-d was reassessed after one month, when patients with abnormal D-d resumed VKA, while patients with normal D-d underwent D-d measurement every 2 months over a year. Recurrent VTE was the primary outcome measure. *Results.* 284 out of 384 patients had a normal D-d at 1 month after VKA suspension. Recurrent events occurred in 8 (4.7%) of 134 (47%) with persistently normal D-d. Among the 57 patients (20%) with persistently positive D-d , 13 (22%) events were observed. Recurrent events were recorded in 3 (5%) of 61 patients (21%) with fluctuating D-d levels. *Conclusions*. recurrences are low among patients with persistently normal D-d over a year after anticoagulation suspension for a first episode

C066

D-DIMER TO DETERMINE DURATION OF ANTICOAGULATION AFTER A FIRST EPISODE OF IDIOPATHIC VENOUS THROMBOEMBOLISM: EXTENDED FOLLOW-UP OF THE PROLONG STUDY

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Background. The PROLONG study showed that D-dimer (D-d) could help tailor the individual need for prolonged anticoagulation after idiopathic venous thromboembolism (VTE). We report the results of the extended follow-up of the PROLONG study. *Methods*. D-d was performed one month after anticoagulation withdrawal in patients who had received vitamin K antagonists (VKAs) for at least 3 months for a first episode of idiopathic VTE. If D-d was normal anticoagulation was not resumed, in case of an abnormal D-d patients were randomized to either resume or not resume VKAs. The end-point was the composite of recurrent VTE and major bleeding. *Results*. D-d was abnormal in 222/608 (36.5%) patients. Total follow-up was 1548.3 patient-years (average: 2.55 years). Twenty-eight events occurred in 121 patients allocated to stop VKAs (23.1%, 9.7% person-years) and 5 in 101 patients randomized to resume VKAs (5%, 2.0% person-years) (adjusted hazard ratio- HR: 5.5; p=0.0015). Fifty-one events occurred in 386 patients with normal D-d (13.2%; 5% person-years; adjusted HR of 3.58, p=0.016, *vs.* patients who resumed VKAs). The adjusted HR of patients with abnormal D-d who stopped anticoagulation versus those with normal D-d was 1.64 (p=0.047). *Conclusions.* The extended follow-up of the PROLONG study confirms the persistent higher risk of recurrence and the benefit of VKA prolongation in patients with an abnormal D-d at one month after VKAs withdrawal for a first episode of VTE. The optimal duration of anticoagulation in subjects with normal D-d at one month after VKA withdrawal is not clearly established.

C067

THROMBOEMBOLIC RISK FACTORS IN RETINAL VEIN OCCLUSION: A COMPARISON WITH OTHER VENOUS THROMBOSIS

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Background. Retinal vein occlusion (RVO) is one of the most common retinal vascular disorders affecting both the elder and the young. The pathogenesis of RVO is poorly understood and the role of congenital and acquired risk factors has not yet been well defined, unlike other types of venous thrombosis, in which the role of risk factors is better defined. The aim of the study was to evaluate common risk factors for venous thromboembolism in patients affected with RVO compared with patients affected with other venous thrombosis (VT). *Methods.* 214 consecutive patients presenting an RVO, including central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO), 106 males and 108 females, were included in the study. 426 patients with other VT, including deep venous thrombosis (DVT), pulmonary embolism (PE), cerebral vein thrombosis (CVT) and mesenteric thrombosis (MT), 193 males and 233 females were also included. Each patient underwent complete physical examination, laboratory tests, including full coagulation screening and genetic assays. RVO patients underwent also complete ophthalmic evaluation. *Results.* RVO patients were older than VT patients (67.6±11.8 *vs.* 58.8±17.1, p<0.001). The prevalence of Factor II G20210A mutation was lower in RVO than in VT patients (3.5%

*vs.*12.5%, $p<0.004$) as was Factor V R506Q mutation (9.4% *vs.* 20.9%, $p<0.001$). No significant differences were observed between the two groups with regards to protein C, protein S, ATIII functional activity, homocysteine levels, MTHFR polymorphism and acquired risk factors. A previous episode of RVO was present in 7% of RVO patients and in 1.2% of VT patients ($p<0.001$). Previous episodes of VT were significantly less frequent in RVO than in VT patients (DVT 0.9% *vs.* 38%, PE 1.4% *vs.* 13.8%, $p<0.0001$). *Conclusions.* Our data show that RVOs are different from other types of venous thrombosis with regards to several factors (age, genetic risk factors, previous thromboembolic events), thus suggesting that the pathogenesis of RVO may depend on local damage (probably related to the anatomical conformation of the retinal vein network) not influenced by the risk factors significantly correlated to other venous thromboembolic diseases. The data also suggest that RVO could require different therapy as compared to other venous thromboembolic diseases.

C068

THE COURSE OF D-DIMER AND FACTOR VIII LEVELS DURING THE ACUTE PHASE OF LEG DEEP VENOUS THROMBOSIS AND RELATIONSHIP WITH THE THROMBOTIC BURDEN

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Few data are available on the natural course of D-dimer (D-d) and FVIII in relation to the thrombotic burden in the first months of treatment of proximal deep vein thrombosis (DVT). The aim of the study was to evaluate the course of D-d and FVIII levels in relation to the thrombotic burden in patients with a first episode of proximal DVT of the lower limbs. Subjects with a proximal DVT of the lower limbs were enrolled from the day of diagnosis with a follow-up of 180 days. All subjects received LMWH for 5-7 days, imbricated with oral anticoagulation for at least 6 months. A complete ultrasound 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) afterward. 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, n.v. <0.5 ug/mL) and FVIII (chromogenic assay). 59 patients have been enrolled so far. D-d was 5.49+0.87 ug/mLat D0; it persisted altered in 94,4%, 54,5%, 28,1% and 21,7% of cases at D7, D30, D90 and D180, respectively. D-d levels had a decay with a negative power function of time (r^2 0.566, $p<0.001$). D-d levels did not correlate with thrombotic burden at diagnosis; however, when measured at D30 they correlated with the thrombotic burden ad with the residual venous obstruction measured at 6 months (r2 0.581, $p<0.05$). In two patients who had DVT progression during follow-up D-d levels were higher than in the others. FVIII levels did not change during follow-up (D0: 2.5+0.1 IU/mL; D180 2.7+0.3 IU/mL, p=ns) and did not correlate with the thrombotic burden. D-d levels decrease according to a power decay during treatment for acute DVT. When measured at D30 but not at diagnosis they correlate to the thrombotic burden and to the residual venous obstruction at 6 months. FVIII levels are stable during the acute phase of DVT.

C069

RISK STRATIFICATION AND OUTCOMES IN HEMODYNAMICALLY STABLE PATIENTS WITH ACUTE PULMONARY EMBOLISM. A PROSPECTIVE, MULTICENTRE, COHORT STUDY WITH THREE MONTHS OF FOLLOW-UP

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The role of risk stratification in patients with hemodynamically stable acute pulmonary embolism is still unclear. We aimed to evaluate the usefulness of five prognostic markers as predictors of in-hospital pulmonary embolism-related adverse events and as generic prognostic tools in normotensive patients with acute pulmonary embolism. *Methods.* 201 consecutive patients with confirmed acute pulmonary embolism and normal blood pressure were enrolled in a multicentre, prospective, cohort study with three months of follow-up. All patients underwent a conventional anticoagulant treatment and a comprehensive risk-evaluation including echocardiographic assessment of right ventricular dysfunction (RVD) as well as determination of Troponin I, Pro-Brain Natriuretic Peptide (BNP), hypoxemia, and a clinical score. Primary end-point of the study was in-hospital clinical deterioration or death, due to pulmonary embolism. Secondary outcomes were all-cause mortality in the in-hospital phase and at three months. *Results*. One of 201 patients (0.5%) had an in-hospital pulmonary embolism-related adverse event (death). In-hospital and three-month all-cause mortality were 2% and 9% respectively. None of the prognostic markers was predictive of the primary end-point. On univariate analysis only clinical score was predictive of in-hospital all-cause mortality (HR 13.5, 95% CI 1.41-130). However this was no longer true on multivariate analysis. Clinical score (HR 4.74, CI 1.88-11.96), hypoxemia (5.83, CI 2.19-15.54) and troponin I (6.96, CI 2.29-21.16), but not BNP (4.55, CI 0.97-21.4) or RVD (1.69, C I 0.57-4.77), were predictors of all-cause mortality at three months on univariate analysis. On multivariate analysis clinical score and troponin I remained independently predictive. *Conclusions.* In this study on normotensive patients with acute pulmonary embolism prognostic markers did not predict in-hospital pulmonary-embolism related adverse events or all-cause mortality, both occurring rarely. A clinical score and troponin I were independent predictors of three-month all-cause mortality.

C070

A RISK ASSESSMENT MODEL FOR THE IDENTIFICATION OF HOSPITALIZED MEDICAL PATIENTS AT RISK OF VENOUS THROMBOEMBOLISM

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Background. Although there is an increasing awareness of the importance of medical conditions in determining venous thromboembolic (VTE) events, thromboprophylaxis in hospitalized medical patients is largely underused. We generated a simple risk assessment model (RAM) and validated it prospectively in a broad spectrum of medical patients. *Methods.* All patients admitted to a department of Internal Medicine in a 2-year period received the risk stratification adopting a predefined RAM. Active cancer, previous VTE, longstanding immobilization and already known hypercoagulability scored 3 points each; recent trauma or surgery 2 points; elderly (>70 years), heart and/or respiratory failure, acute infection, acute rheumatologic disorder, obesity, bed-rest anticipated and ongoing hormonal treatment scored 1 point each. A score > 4 was reputed to identify patients at high risk of VTE complications. While attending physicians were not informed of the VTE risk of their patients, the implementation of thromboprophylactic measures during hospitalization was recorded. All recruited patients were followed up to 90 days after admission. The development of objectively proven VTE complications and that of bleeding was recorded. *Results.* Out of 1234 patients, 434 (35.2%) reached a score >4. Of these patients, 178 (41.0%) received either pharmacological or physical thromboprophylaxis. VTE developed in 32 (12.5%) of the 256 patients who did not receive prophylaxis, and in 4 (2.2%) of the 178 who did (RR, 5.5; 95% CI, 2.0 to 15.5). VTE complications developed also in 4 of the 722 (0.6%) patients with a score <4 who did not receive prophylaxis (RR of VTE in patients at high vs low risk in the absence of prophylaxis, 22.6; 95% CI, 8.1 to 63.2). Major or clinically relevant bleeding complications developed in 3 of the 160 (1.9%, 95% CI, 0.4 to 5.4%) high risk patients who received pharmacological prophylaxis. *Conclusions.* The RAM generated at our centre appears to discriminate between hospitalized medical patients at high or low risk of VTE complications. Thromboprophylaxis is administered to a disappointingly low rate of patients at high risk of VTE. While the haemorrhagic risk of pharmacological prophylaxis is low, the frequency of VTE complications in high-risk patients who do not receive thromboprophylaxis is more than five times as high as in those who receive it.

Anticoagulant Therapy II

C071

INCIDENCE OF THROMBOEMBOLIC COMPLICATIONS IN PATIENTS WITH MECHANICAL HEART VALVES EXPERIENCING A SUBTHERAPEUTIC INR

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Introduction. Long term anticoagulation is necessary in patients with mechanical heart valves (MHV) to reduce the risk of thromboembolic complications. Subtherapeutic INRs are frequently encountered in clinical practice and MHV patients with inadequate anticoagulation may be exposed to an increased risk of thromboembolic events (TE). However, there are no data on the TE risk in these patients. We conduct a study to assess current practice patterns in the management of MHV patients presenting with subtherapeutic INR levels, and to asses the TE risk in this setting. *Methods*. The charts of all MHV patients were reviewed. Patients with stable, therapeutic anticoagulation experiencing a subtherapeutic INR were included. Patients were included if: they had at least one INR value 0.5-1 INR units below the patient-specific target INR range lower limit; the 2 INR values preceding the index INR were within or greater than the patient-specific INR target range; the time interval between the 2 INR values preceding the index INR was ≥2 weeks. Patients who underwent invasive procedures were excluded. Use and dose of low molecular weight heparin (LMWH) bridging therapy were collected. The incidence of objectively confirmed TE within 90 days after the index INR was assessed. The incidence of TE in patients with and without prophylaxis was also separately analyzed. Finally, the rate of major bleeding events was also assessed. *Results*. 294 patients were eligible for the analysis (mean age 63.3 years;139 males). 164 patients had mitral or mitro-aortic MHV (56.1.%). More than 60% of patients had at least one additional risk factor (atrial fibrillation, previous myocardial infarction, stroke or transient ischemic attack, low ejection fraction). LMWH was prescribed in 14 patients (4.8%). All patients had a follow up of at least 90 days. During the 90 days of follow up, 1 patient had a TE (0.3%; 95%CI 0, 1.9%). A stroke occurred 8 days after the index INR in a patient with bileaflet mitral and aortic MHV and concomitant atrial fibrillation. Considering only patients who did not receive bridging therapy with LMWH, the incidence of thromboembolic complication was 0.4% (95%CI 0, 2.0%). There are no major bleeding events during the period of observation. *Conclusions*. MHV patients with stable, therapeutic anticoagulation experiencing a subtherapeutic INR have a low risk of TE. Withholding LMWH bridging therapy is a reasonable therapeutic option in these patients.

C072

REINTRODUCTION OF ANTICOAGULANT THERAPY AFTER CEREBRAL HEMORRHAGE IN PATIENTS WITH PROSTHETIC HEART VALVES

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The management of patients with mechanical prosthetic heart valves (MHV) and intracranial hemorrhage (ICH) is complex because of the difficult balance between an increased risk of thromboembolic events while oral anticoagulant therapy (OAT) is withheld and an increased risk of recurrent bleeding when OAT is restarted. *Objective.* To assess the risk of thomboembolism after withholding OAT and the risk of recurrent bleeding after reintroducing OAT in patients with MHV after ICH. *Methods.* We carried out a subgroup analysis of a prospective cohort study aimed to assess the efficacy of prothrombin complex concentrates to reverse OAT in patients with ICH. Data from 16 patients with MHV and 1 patient with a recent biological prosthesis and atrial fibrillation were ascertained. Information on when OAT was stopped and when anticoagulant treatment was restarted and on the rate of haemorrhagic and thromboembolic events were collected. *Results.* Mean age was 71 years, 9 were females. Valves in the aortic and in the mitral position were equally distributed; 3 patients had a mitro-aortic MHV, 8 patients had concomitant atrial fibrillation. Three patients were on acenocoumarol, 14 on warfarin; the mean INR at the time of ICH was 3.2. At the end of follow-up, 90 days after ICH, 2 patients died: one on the day of the event and one 37 days after ICH because of an ischemic stroke. This was the only thromboembolic event recorded (5.88%, 95% CI 0.3 to 30.8), no recurrent ICH was documented (0%, 95% CI 0 to 22.9%). Mean time of anticoagulant treatment withholding was 18 days (range 2 to 60); in most cases (8 patients) anticoagulant therapy was restarted with LMWH. The patient with a fatal ischemic stroke had anticoagulant treatment restarted 5 days after ICH. In patients with spontaneous haemorrhage anticoagulant therapy was restarted later than in patients with post-traumatic hemorrhage (21.2 *vs.* 14 days, $p<0.03$). There were no significant differences in the timing of restarting anticoagulant treatment beetwen patients who underwent neurosurgery and patients who did not (17.9 days and 18.8 days, respectively, $p=0.293$). *Conclusions.* The risk of thromboembolic complications when withholding OAT for a mean of 18 days after ICH and the risk of recurrent bleeding when anticoagulant treatment is resumed appear to be low, but larger studies are needed to produce a decision analysis in order individualize the optimal timing to restart anticoagulant therapy.

C073

GENDER DIFFERENCES FOR BLEEDING AND THROMBOTIC RISK IN ATRIAL FIBRILLATION (AF) PATIENTS ON ORAL ANTICOAGULANT TREATMENT

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Systemic embolism is the most serious complication of AF. Adjusted-dose OAT (target INR 2.5) is recommended for patients with AF at moderate-high risk of stroke. However, ischemic stroke can occur in AF patients despite well conducted OAT. To evaluate the risk of adverse events of OAT in relation to gender, we performed a prospective study on 662 AF patients [423 males, 239 females; median age 75 (49-94) years, total follow-up period 2365 patient years (pt-yrs)]. The quality of anticoagulation levels and the occurrence of bleeding and thrombotic events were recorded. Patients spent 14%, 71% and 14% of time below, within and above the intended therapeutic range respectively. In relation to the quality of anticoagulation, no difference was observed either between genders or among patients with and without bleeding and thrombotic events. During follow-up 32 patients, rate 1.35x100 pt/yrs (22 males, 10 females) had major bleeds: 17 cerebral, 13 gastrointestinal, 1 muscular haematoma, 1 haematuria. Eight haemorrhages were fatal (7 males, 1 female; $p=0.015$), of which 7 were cerebral. Thirty nine ischemic events (rate 1.6x100 pt/yrs) were recorded: 22 were stroke (1 fatal), 13 were TIAs, 4 were peripheral emboli. The events were more frequent among females with respect to males (rate 2.34 vs 1.25x100 pt/yrs; $p=0.042$) after correction for age. In addition strokes occurring among females were more disabling, and RR for severe and fatal stroke, defined according to Modified Rankin scale, of females vs males was 2.4 (95% CI 1.0-5.1; $p=0.034$). In conclusion AF males on OAT seem to be at higher bleeding risk with respect to females. On the contrary females show a high risk for thrombotic events respect to males, despite well conducted therapy.

C074

WARFARIN MAINTENANCE DOSE : INFLUENCE OF AGE AND GENDER

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The optimal dose of warfarin varies among individuals, and the prediction of a maintenance dose is difficult. The use of anticoagulant therapy is expanding among the elderly population. We investigated the

influence of age and gender on warfarin dose requirement.. Because warfarin therapy is often initiated in the outpatient setting, a better understanding of the factors that predict lower dose requirements may reduce the risk of unanticipated over-anticoagulation and hemorrhage. The study was carried out in 5 anticoagulation italian clinic to evaluate the influence of age and gender on warfarin dose requirement. We collected clinical and demographic data including age, gender, disease states, concomitant medications, indication, weekly warfarin dosage. A total of 21,177 (M=11,001, F=10,073) patients were investigated. The warfarin dose was inversely related to age and was strongly associated with gender. The median weekly dose to maintain a therapeutic range between INR 2-3 varied from 37.7 mg, for men <47 years of age, to 24.8 mg, for men >80 years of age, and respectively from 35.1 mg, for women <47 years of age, to 22.8 mg for women >80 years old. Women required on average 2.14 mg less per week than men. Among patients assigned to a therapeutic range between 2.5-3.5, the warfarin weekly dose varied from 39.6 mg, for men who were <47 years of age, to 27.1 mg for men >80 years of age, and respectively, from 33.7 mg, for women who were <47 years of age, to 24.9 mg for women >80 years old. Women required on average 2.44 mg less per week than men. Warfarin dose requirements decrease greatly with age and gender. These results suggest that, when warfarin is initiated, lower induction doses than those usually used in younger patients should be considered for the elderly population, also taking into account the patient gender.

C075

ORAL ANTICOAGULANT THERAPY IN PATIENTS WITH MECHANICAL HEART VALVE AND INTRACRANIAL HAEMORRHAGE. A SYSTEMATIC REVIEW

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Objective and Background. Optimal timing for restarting anticoagulant therapy after intracranial bleeding is a critical issue. The purpose of this systematic review is to summarize published studies on the management of oral anticoagulant therapy after intracranial bleeding secondary to the use of vitamin K antagonists in patients with a mechanical heart valve. *Methods.* A computer-assisted search of the MEDLINE and EMBASE electronic databases till January 2008 was performed. Two investigators independently performed study selection and completed a predefined quality assessment and data extraction form. Main inclusion criterion was the enrolment of patients with a mechanical heart valve and intracranial haemorrhage during oral anticoagulant treatment. Any randomised controlled trial, observational cohort study, case series and reports was included. *Results*. No randomised controlled trials were identified. Six observational cohort studies were included in the final analysis. All studies were of low quality. A total of 120 patients were enrolled. Anticoagulation was restarted within a broad time range (2 days - 3 months). Four ischemic strokes and two recurrent cerebral haemorrhages occurred after anticoagulation was restarted after a mean follow-up of 7.9 months. Eighteen patients were described in the selected case reports. Anticoagulant therapy was restarted within 4 days to 8 weeks. Two patients had a recurrent haemorrhagic event and no ischemic events were reported. *Conclusions*. Stopping oral anticoagulant therapy for few days (even for 7-14 days) after the occurrence of cerebral haemorrhage in patients with a mechanical heart valve is apparently safe. However, well-designed studies are strongly recommended to provide better evidence

C076

ANTICOAGULATION MANAGEMENT BY POINT OF CARE DEVICES AND COMPUTER MONITORING BY GENERAL PRACTITIONERS

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Background. It has been shown that point of care testing (POC) is appropriate for the management of oral anticoagulant therapy (OAT); the use a computerized decision support system is able to improve therapeutic effectiveness; quality of OAT management is similar in general practitioner (GP) and anticoagulation clinic (ACC) systems. The aim of this study was to evaluate the feasibility and effectiveness of POC based computer assisted OAT monitoring by GP. *Methods.* A selected group of GP was asked to take part in the study on a voluntary basis. Participating GP underwent a training course about OAT, POC and software use. Unselected consecutive patients referring to ACC of Perugia were switched to GP management, after giving informed consent. The POC for the capillary determination of INR was the Coagucheck (Roche Diagnostics, Milan, Italy), and the software used to manage OAT was Anthema 5.3 (NTE, Barcelona, Spain). POC devices used in the study underwent a quality control scheme four times a year. Outcome of the study was the comparison of therapeutic quality control indexes and clinical event rates among cohorts of patients followed by GP, ACC and a primary care POC-based remote Computer OAT management Network (PCN) active in Umbria since 2001. The project was co-funded by a research grant of the Region Umbria. *Results.* Main results are shown in the Table 1. *Conclusions.* The results of our study showed that the therapeutic and clinical quality obtained in the GP model were the same as that measured in the analysis of the ACC and PCN group, confirming the feasibility and effectiveness of this model of management of OAT.

Table 1.

Therapeutic quality control indexes			
	GP	*ACC*	*PCN*
Patients (N)	207	695	794
Mean INR (DS)	2.54 (0.81)	2.43 (0.93)	2.53 (0.79)
Monthly accesses/patient	1.05	1.31	1.29
INR in range % (below range %)	60.3 (20.6)	55.2 (29.0)	59.1 (24.7)
Time in range % (below range %)	67.1 (17.4)	66.2 (18.8)	70.5 (17.8)
Clinical event rates			
	GP	*ACC*	*PCN*
Patient/years (N)	130	512	720
Bleedings, N (%; 95% CI)	0	7 (1.4; 0.4-2.4)	8 (1.1; 0.3-1.9)
Thromboembolic events, N (%; 95% CI)	2 (1.5; 0-3.7)	4 (0.8; 0-1.5)	9 (1.3; 0.4-2.1)
Total deaths N (%; 95% CI)*	0	5 (1.0; 0.1-1.8)	6 (0.8; 0.2-1.5)

** Accounting for fatal bleedings and thromboembolic events and unknown deaths*

C077

PROSPECTIVE EVALUATION OF THE CLINICAL QUALITY OF ORAL ANTICOAGULANT TREATMENT IN A PRIMARY CARE COMPUTER ASSISTED MANAGEMENT MODEL

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Background. Management of oral anticoagulant therapy (OAT) has become a burden for specialized anticoagulation clinics (ACC). Anticoagulation management services have been implemented as alternative to ACC. The validation of these models has usually been granted through assessment of therapeutic quality indexes. Actually the gold standard index of effectiveness is the rate of failures and complications of OAT. The reported rates of clinical adverse events in the literature are: major bleedings 0.4-2.6%/year, thromboembolic events 0-3%, cardiovascular deaths 1.8%/year (ACCP 2004, Njaastad 2006, AMADEUS 2008). The aim of this study is to report the results of a clinical events monitoring scheme in primary care (PC) computer assisted OAT management setting. *Methods.* Setting. This prospective observational study was run in a network of computer assisted services comprehensive of ACC, remote computer distributed (RC) or general practitioner (GP) OAT management. INR determination was performed by venous blood or POC device. The POC for the capillary determination of INR was the Coagucheck (Roche Diagnostics, Milan, Italy) or Pro-Time (IL, Milan, Italy), and the software used to manage OAT was Anthema 5.3 (NTE, Barcelona, Spain). Every three months POC devices were centrally checked and a therapeutic quality assessment scheme was run. Study protocol. Since January 2007, a computer assisted routine to identify and record data about patients lost at follow-up for 6 weeks or more was monthly run in each units of the network. Details on clinical events (major bleeding, thromboembolic event, death) and adverse non-clinical events (temporary or definitive withdrawal, change of site of monitoring) were collected through direct visits or phone calls. *Results.* 1622 outpatients on OAT were followed-up for 12 months. Of these patients, 512 were managed by AC through venous blood INR laboratory determination, 260 by RC monitoring through INR laboratory determination, 720 by RC monitoring and 130 patients by GP through INR POC determination. Major results are shown in the Table 1. *Conclusions*. We showed

that in our primary care computer assisted management model the rate of clinical events is comparable to that obtained from available prospective studies. Particularly, we found a low incidence of thromboembolic events, if compared with prospective interventional clinical trials.

Table 1.

	Number	*% (95% CI)*
Adverse clinical events (ace)	55	3.4 (2.5-4.3)
Major bleedings [of which fatal]	15 (1)	0.9 [0.06] (0.5-1.4)
Thrombotic events [of which fatal]	15 (4)	0.9 [0.2] (0.5-1.4)
Unknown deaths	6	0.4 (0.1-0.7)
Deaths non oat related	19	1.2 (0.6-1.7)
Adverse non clinical events (ance)	105	6.5 (5.3-7.7)
End of oat therapy	38	2.3 (1.6-3.1)
Interruption for surgery (>6 wks)	5	0.3 (0.0-0.6)
Switch to asa/lmwh	18	1.1 (0.6-1.6)
Switch to other monitoring site	17	1.0 (0.6-1.5)
Interruption oat for other reason	27	1.7 (1.0-2.3)
Lost at follow-up	8	0.5 (0.2-0.8)
Total	168	10.4(8.9-11.8)

Hemorrhagic Diseases: Diagnosis and Clinical Aspects

C078

PROSPECTIVE ASSESSMENT OF BLEEDING SEVERITY IN CONSECUTIVE PATIENTS REFERRED FOR HEMOSTATIC EVALUATION

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Background. Quantitative assessment of bleeding symptoms has been recently described as a promising tool for the evaluation of patients with a possible hemorrhagic diathesis. *Aim of the study.* To assess the diagnostic role of a quantitative bleeding questionnaire in a prospective series of patients referred to second level Hemostasis Centers. *Methods.* Patients consecutively referred for hemostatic evaluation to two Centers (Vicenza and Leiden) from October 2004 to December 2006 were enrolled. A standardized bleeding questionnaire was administered to all patients by a physician; patients were subsequently managed by another physician, blinded to the questionnaire results, who requested further laboratory evaluation as deemed necessary and gave the final diagnosis. The performance of the bleeding questionnaire was therefore assessed by considering either the number of bleeding symptoms and their severity, this latter expressed as a bleeding score (BS). An abnormal bleeding history was defined has having at least 3 bleeding symptoms or a BS higher than 3. *Results.* After the exclusion of subjects aged < 4 and > 80 years, taking anticoagulant or antiplatelet drugs or having LAC, 215 subjects were available for analysis (126 females, 89 males; median age 35 years). 71 subjects were referred for abnormal hemostatic tests (ABN group), 105 for bleeding symptoms (BLEED group) and 39 for family investigation (FAM group). Patients were classified as being: 148 subjects normals or carriers of an asymptomatic clotting defect; 18 VWD; 6 mild haemophilia, 14 platelet function defect. The specificity and sensitivity of the *more than 3 bleeding symptoms* criterium was 94.7 and 25% in the ABN group, 89.9% and 23.1% in the BLEED group and 91% and 0% in the FAM group. The specificity and sensitivity of the *BS higher than 3* criterium was 98.2% and 25% in the ABN group, 84.1% and 38.5% in the BLEED group and 86.4% and 0% in the FAM group. The overall ROC area was slightly greater for the *BS higher than 3* criterium (0.60 *vs.* 0.56, p=0.26). *Conclusions.* We confirm that a positive bleeding history, collected using a standardized tool, could be considered as a sufficient specific test that always warrants further investigation. A negative bleeding history does not satisfactorily exclude the presence of a (mild) bleeding disorder, and laboratory evaluation is always required to fully exclude it.

C079

PROTHROMBIN COMPLEX CONCENTRATES FOR URGENT ANTICOAGULATION REVERSAL IN PATIENTS WITH INTRACRANIAL HAEMORRHAGE

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Background. Intracranial haemorrhage (ICH) is the most serious and potentially fatal complication of oral anticoagulant therapy (OAT). When this complication occurs, timely and complete reversal of anticoagulant effect becomes imperative. Prothrombin complex concentrates (PCCs) produce a rapid and adequate action and substantially shorten the time needed to reverse OAT effects. *Aim of the study.* To evaluate the efficacy and safety of emergency OAT reversal in patients with ICH by a balanced PCC containing coagulation factors II, IX and X. *Patient and methods.* Patients suffering from acute ICH while receiving OAT were eligible for this prospective study if their International Normalized Ratio (INR) was higher or equal to 2. Stratified 35-50 UI/kg PCC doses were infused based on initial INR and an intravenous dose of Vitamin K (10 mg) was given to all the patients. *Results.* A total of 92 patients (50 males; mean age: 74 years, range 34-92 years) were included in the study in 10 Italian centers. Indications for OAT were atrial fibrillation (50 patients), mechanical heart valves (22), severe cardiomyopathy (2), venous thromboembolism (18). Intracranial bleedings were intracerebral in 74 patients

(80%), subdural in 12 patients (13%), and subarachnoidal in 6 patients (7%); 42 patients (45%) underwent urgent neurosurgery intervention. The median INR at presentation was 3.3 (range 2-9). At 30 minutes after PCC administration the median INR was 1.4 (range: 0.9-3.1), declining to lower or equal to 1.5 in 75% of patients; only 5 patients (5.4%) with INR exceeding 2 received a second infusion of PCC. The benefit of PCC was maintained for a long time, since in 98% of all post-infusion time points through 48 h median INR remained lower or equal to 1.5 (median:1.19; range:0.9-2.3). During hospitalization only one patient suffered from non-fatal ICH recurrence, while neither thrombotic complications nor significant adverse events were observed; no case of peri-operative bleeding in patients undergoing surgery occurred. Eleven patients died (11.9%), although none of the deaths was judged to be related to PCC administration. *Conclusions.* PCC administration is an effective, rapid ad safe treatment for the urgent reversal of OAT in patients with intracranial haemorrhage. Broader use of PCC in this clinical setting appears to be appropriated.

C080

PATIENTS WITH MILD HAEMOPHILIA A AND NO DETECTABLE FVIII MUTATION HAVE POORER RESPONSE TO DESMOPRESSIN

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Background. Desmopressin is the treatment of choice for patients with mild haemophilia A (FVIII:C ≥5%) since a median 2 to 6-folds FVIII:C increase is usually observed post-infusion. However, the range of response is wide and patients with similar basal FVIII:C may respond in a significantly different way. Despite that several clinical studies have demonstrated the clinical efficacy and safety of desmopressin, few data are available on the relationship of biological response with a given FVI-II mutation. *Aim of the study.* To evaluate the relationship between type of FVIII mutation and response to desmopressin. *Methods.* We prospectively evaluated the biological response to subcutaneous desmopressin and FVIII genotype in a group of 51 patients with FVIII:C ≥5%. Desmopressin was injected subcutaneously and blood samples taken at 0, 30, 60, 120 240, 480 minutes and 24 hours after injection. FVIII mutations search strategy was based on DHPLC and gene sequencing. *Results*. Mean basal FVIII:C was 18±9 U/dL (range 5-37) and the median increase at peak was 2.5-fold (range 1.1-7.1). Twelve patients with low FVIII:C and normal or increased FVIII:Ag (type II defect) had similar basal and folds increase compared to the remaining patients. At multivariate regression, the peak VWF:Ag concentration reached after desmopressin infusion and patient age were positively related with the FVIII:C half-life (p=0.003 and p=0.002 respectively), but not with FVIII:C peak or AUC. A total of 28 different gene mutations were identified (10 novel) in 42 patients, while 9 had no identifiable gene mutations even after two different complete gene sequencing with 2 different sets of primers. No mutations in exons 18-22 and 24 of VWF were identified. Patients with no mutations had similar basal FVIII:C (16.9±8.5 *vs.* 18.7±9.4 U/dL) and VWF:Ag levels (109.1±31.7 vs 133.1±57.5 U/dL) compared with patients with a mutation. However, the magnitude of FVIII:C increase was significantly different (1.8±0.6 vs 2.9±0.9; p=0.002 by ANOVA). *Conclusions.* A poor biological response to desmopressin was associated with the absence of evident FVIII mutation.

C081

SAFETY OF LONG-TERM USE OF ARTERIOVENOUS FISTULA (AVF) AS VENOUS ACCESS IN HEMOPHILIC CHILDREN

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Background. Hemophilic children, undergoing regular prophylaxis or ITI, need a long-lasting uncomplicated venous access. *Methods*. Children lacking a venous access suitable for frequent infusions were eligible for AVF creation. AVF were accessed at home by parents. Doppler ultrasound of the limb and echocardiography were regularly performed. *Results.* Between 1999 and 2007, 43 AVF were created in 38 patients (FVI-II/FIX <2%; median age 2.6 years, range: 0.9-11.9; 23 with inhibitors). AVF did not mature in 6 children (16%) and 5 of them underwent a second procedure that was successful in 4. Overall successful maturation was obtained in 36 AVF (84%), that were first accessed after a median of 56 days (21-135) and used for a median of 5.1 years (0.7-7.9) for ITI (20), prophylaxis (11) and on-demand treatment (5). Complications not preventing AVF use were: thrombosis of a venous branch (1, 3%) and transient distal ischaemia (4, 11%). Other complications were: aneurysmatic dilatation (4, 11%) observed after a median of 5.4 years (3.5-7.7), limb hypertrophy (1, 3%) after 5.4 years and AVF overflow (1, 3%) after 6.9 years. These complications were reason for surgical dismantlement and transition to peripheral veins after a median of 6.6 years (range: 3.5-7.1). Uncomplicated AVF were dismantled after 4-7.4 years in 2 children who developed adequate peripheral veins. Conclusions: AVF were satisfactorily safe in hemophilic children, allowing long-term home treatment in 36/38 (95%). Regular follow-up allows early remedial intervention in case of complications, however, transition to peripheral veins should be implemented as soon as possible.

C082

THREE NOVEL DYSFIBRINOGENEMIAS ASSOCIATED WITH MUTATIONS IN BETA AND GAMMA CHAINS: FIBRINOGEN VICENZA II, CESENA I AND II

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Fibrinogen is a complex glycoprotein involved in the final step of the coagulation cascade as the thrombin substrate for fibrin generation and it is required for platelet aggregation to occur by binding to glycoprotein IIb/IIIa exposed on platelet membrane after platelet activation. Fibrinogen is synthesized in hepatocytes as a hexamer composed of two sets of three polypeptide chains (Alfa, Beta, and gamma). Each chain is encoded by a separate gene, FGA, FGB, and FGG, which are clustered in a 50-kb region on chromosome 4q32.1. Inherited disorders of fibrinogen are classified according to a complete lack of fibrinogen in plasma (afibrinogenemia), a partial deficiency (hypofibrinogenemia) or evidence of an abnormal circulating molecule (dysfibrinogenemia). We report three novel mutations associated with inherited dysfibrinogenemia. The first patient presented mild bleeding symptoms (menorrhagia and prolonged oozing after dental extraction). Thrombin and Reptilase times were markedly prolonged, while PT and PTT were normal. PT-derived fibrinogen was around 130 mg/dL, while Clauss was 60 mg/dL. Purified fibrinogen showed an isolated abnormality of fibrin polymerization. A novel heterozygous Ter462Gln mutation in Beta gene (Fibrinogen Vicenza II) was present, predicting a beta chain 12 amino acid longer than normal. A similar mutation (Ter462Lys) was previously considered responsible for fibrinogen Osaka VI, associated with massive post-partum bleeding. The second asymptomatic patient showed reduced functional fibrinogen level (around 80 mg/dL). A novel heterozygous T>A mutation in exon 8 of fibrinogen gamma chain gene was identified, predicting a Cys339Ser mutation (Fibrinogen Cesena I). The third mutation was identified in a woman with deep vein thrombosis post-partum at age 26 and in his father with idiopathic vein thrombosis at age 40. All clotting tests were prolonged and a functional fibrinogen of 69 mg/dL was measured. A heterozygous T>C mutation in exon 7 of fibrinogen gamma chain was identified, predicting a Trp208Arg mutation (Firbinogen Cesena II). The molecular basis of inherited disorders of fibrinogen are still worthy to be explored to better elucidate structure-function relationships which can explain the different clinical phenotypes.

C083

EARLY ONSET OF BLEEDING IN HEMOPHILIACS WITH NULL MUTATIONS IN THE FVIII GENE

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Objective. Large heterogeneity of clinical phenotype in patients with severe hemophilia A has been observed. The aim of this study was to investigate the effect of the type of FVIII gene mutation on the clinical phenotype in a cohort of severe hemophilia A children. *Methods.* We used data from a multicentre retrospective cohort study (Canal study). Patient characteristics, data on bleeding episodes and treatment regimens during the first 50 EDs were collected. We analysed 276 patients with complete treatment data and known gene defect. *Results.* The median age at first bleeding was 10.1 months (IQR 6.5-13.8) in patients with null mutations (large deletions, inversions and nonsense mutations) and

12 months (IQR 8.3-17.5) in those without null mutations ($p<0.05$). Also the median age at first joint bleeding was significantly lower in patients with null mutations, 15.3 months (IQR 10.1-24.7), compared to 23.8 months (IQR 16.7-35.7) in patients without null mutations, $p<0.01$. The frequency and site of bleeding episodes, as well as the dosage of concentrate required for on-demand treatment and prophylactic regimens were not significantly different in patients with null mutations compared to those without. *Conclusions.* Our results indicate that patients with null mutations in the FVIII gene have an earlier bleeding onset including joint bleed and suggest the need for early prophylaxis in these children.

C084

CLINICAL-MOLECULAR PREDICTORS OF THROMBOCYTOPENIA AND RISK OF BLEEDING IN PATIENTS WITH VON WILLEBRAND DISEASE TYPE 2B: A PROSPECTIVE STUDY IN A COHORT OF 67 PATIENTS

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Background. Type 2B von Willebrand disease (VWD2B) is caused by an abnormal von Willebrand factor (VWF) with increased affinity for the platelet receptor glycoprotein 1b-alpha. This usually results in moderate-severe thrombocytopenia. *Aims, Design of the study, Patients and Methods*. To determine the prevalence and clinical-molecular predictors of thrombocytopenia as well as the risk of bleeding associated with VWD2B we have enrolled 67 VWD2B patients from 38 unrelated families in a 2-year prospective study. At enrolment, patients with phenotypic diagnosis of VWD2B and identified mutation in exon 28 of the VWF gene were also exposed to detailed questionnaire useful to calculate the bleeding severity score (BSS). Platelet counts with mean platelet volume and morphologic evaluations of blood smear were associated with the occurrence of physiological (pregnancy) or pathological (infections, surgeries) stress situations or DDAVP administration. Active VWF was tested in plasma using the AuVWFa11-based immunoadsorbent assay, which allows quantification of the VWF-GpIbα-binding conformation *in vivo*. Bleeding-free survival according to BSS [<4 (reference), 4–8, >8] and to different platelets levels [$\geq 140\times10^9$/L (reference), $<140\times10^9$/L] was calculated with the Kaplan-Meier method. *Results.* Thrombocytopenia was found in 20 patients (30%) at baseline and in 38 (57%) after stress situations. Platelet counts were always normal in 16 (24%) patients from 5 families with P1266L/Q or R1308L mutations and normal multimers in plasma. The activated VWF measured by AuVWFa11 nanobody was higher than normal in all but those 16 cases with values 2 to 6-fold higher than controls: values >1 correlated always with thrombocytopenia. Bleeding-free survival calculated with the Kaplan-Meier method was significantly different in the three groups of patients with BSS <4, 4-8 and >8 (log rank test: $p=0.003$) and in the two groups with platelet counts higher or lower than 140,000//microliters (log rank test: $p<0.0001$). The adjusted hazard ratio (HR, 95% CI) was about four times higher in patient who had BSS >8 [HR=3.78 (1.00-14.79)] and platelet counts <140,000/microliters [HR=3.65 (1.53-8.70)], compared to the reference group. *Conclusions.* Not all VWD2B patients show thrombocytopenia at baseline and platelet counts can decrease only after physiological and pathological stress situations. Activated VWF as tested by nanobody is useful to predict thrombocytopenia in VWD2B.

Nutrition and Thrombosis

C085

ALCOHOL CONSUMPTION AND ALL CAUSE MORTALITY RISK AMONG PATIENTS WITH PREVIOUS CARDIOVASCULAR DISEASE: A META-ANALYSIS OF 15 PROSPECTIVE STUDIES

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Introduction. Many epidemiological studies have consistently suggested that moderate alcohol consumption in apparently healthy people is associated with a lower cardiovascular disease (CVD) morbidity and mortality. At present, data on the relation of moderate alcohol intake with mortality in patients with previous CVD, are sparse and controversial. *Aims.* We performed a meta-analysis of the available prospective studies, with the aim to assess the relationship of alcohol doses with mortality for any cause in subjects with a history of CVD. *Methods.* Articles were retrieved from those listed until March 2008 by searches in PUBMED (www.pubmed.gov), and EMBASE (http://www.embase.com) with the Medical Subject Heading (MeSH) terms cardiovascular disease, diabetes, and patients in combination with the MeSH terms or text words alcohol drinking, wine, beer, spirits, mortality, morbidity, survival, and death, supplemented by references of the selected articles. Two independent reviewers selected 15 prospective studies on subjects with a history of CVD (including type II diabetic patients), for a total of 175,983 patients and more than 26,000 deaths. Data were pooled with a weighed, least-squares regression analysis of second-order fractional polynomial models. Random model was chosen to describe the pooled effects, while sensitivity analysis were performed by fixed models. *Results.* A J-shaped relationship between increasing amounts of alcohol intake and total mortality was observed in adjusted studies, among patients with previous CVD (Figure 1). Low to moderate consumption of alcohol (up to an average of 28.5 grams/day) significantly reduced total mortality, while higher doses increased it. Maximal protection (21%, 95%CI:9-32%), was obtained at an average alcohol intake of 7 grams/day. Analysing only those studies that reported data on patients with coronary or cerebrovascular disease (7 studies, 16,412 patients), the J-shaped curve was confirmed (protection was maximal at alcohol intake of 2,5-5 grams/day (24%, 99%CI: 12-33%), and persisted up to an average alcohol intake of 12.5 grams/day). *Conclusions.* Low alcohol consumption is significantly associated with a reduced total mortality in patients with previous CVD. 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, not only in the general population, but in patients with cardiovascular disease too.

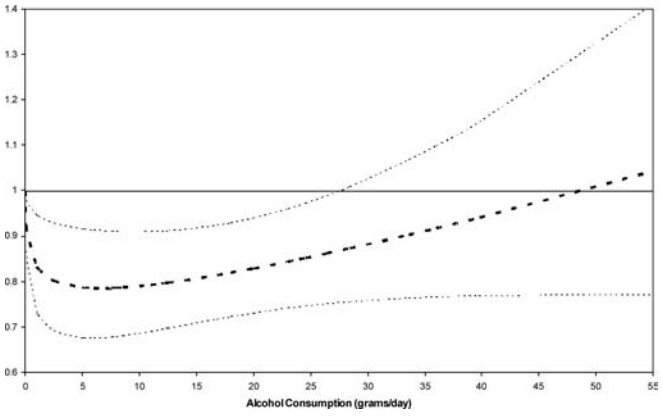

Figure 1.

C086

THE OLIVE OIL PHENOLIC ANTIOXIDANT HYDROXYTYROSOL SUPPRESSES PHORBOL ESTER-INDUCED MATRIX METALLOPROTEINASE-9 EXPRESSION BY INHIBITING PROSTAGLANDIN E2 PRODUCTION AND PKCALPHA ACTIVATION IN HUMAN MONOCYTOID CELLS

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Objectives. Mediterranean diets, of which olive oil is an important component, are associated with low prevalence of cardiovascular diseases, but active dietary components and their mechanisms of action are incompletely understood. The local production of matrix metalloproteinase(MMP)-9 by macrophages contributes to matrix degradation and plaque instability. Since MMP-9 production by macrophages could occur through a prostaglandin (PG)E2 dependent pathway, we sought to examine the effect of hydroxytyrosol (HT) on MMP-9 expression and activity and to explore the underlying mechanisms of action. *Methods.* U937 human monocytoid cells were treated with HT (1-50 micromol/L) for 60 min before stimulation with 30 nmol/L phorbol myristate acetate (PMA) for 24 h or alternatively with selective inhibitors of both PKC iso-enzymes and cyclooxygenase(COX)-2. Cell supernatants were tested for gelatinase activity by zymography and the release of MMP-9 protein, PGE2 and tissue inhibitor of metalloproteinases (TIMP)-1 and -2 were assayed by ELISA. Finally, total cell and membrane enriched extracts were analysed for COX-2 expression and PKC iso-enzymes translocation respectively by Western analysis. *Results.* HT (1-50 micromol/L) reduced PMA-induced MMP-9 activity at zymography in a concentration-dependent manner, with 50% inhibitory concentration (IC50) at 10 micromol/L ($p<0.01$). 10 micromol/L HT as well as the specific COX-2 inhibitor NS-398 (5 micromol/L) reduced MMP-9 protein release by about 50% without significantly affecting TIMP-1 and -2 release. Correspondently, 10 micromol/L HT inhibited the PMA-induced PGE2 production (by 54±7%) and the expression of the rate limiting enzyme COX-2 (by 43±5%) without affecting COX-1. Since PKC signalling is involved in COX-2 expression, the HT effect on PKC activation was also investigated. We found that HT (1-50 micromol/L) reduced in a concentration-dependent manner, the activation of PKC alpha iso-enzyme. *Conclusions*. HT, the major olive and olive oil phenolic antioxidant, inhibits MMP-9 expression and release, interfering with PGE2 production and COX-2 expression in human monocytes. This effect seems to be, at least in part, mediated by the HT interference with PKC alpha membrane translocation and activation. Overall these results help to understand the plaque stabilization effect showed by specific components of Mediterranean diets.

C087

ADHERENCE TO MEDITERRANEAN DIET AND HEALTH STATUS – A META-ANALYSIS

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Background. Mediterranean diet (MD) has been extensively showed to have beneficial effects on the health status. Over the last years, studies evaluating the adherence to such dietary pattern through operationalised scores in relation to the occurrence of disease and/or mortality have been obtained. In this report, we quantitatively reviewed the prospective cohort studies that have analyzed the relation between adherence to MD and a health outcome in primary prevention. *Methods*. We conducted electronic search of MEDLINE, EMBASE, Web of Science, and the Cochrane Library till the end of January 2008. Studies were selected if they analyzed prospectively the association of MD and health status in an otherwise healthy population. Eligible outcomes were overall mortality, mortality from cardiovascular diseases, mortality from and/or incidence of cancer, as well as incidence of degenerative diseases. *Results*. Eleven prospective studies were included in the meta-analysis, including a total of 1,570,719 subjects followed for a time ranging from 3 to 18 years. The cumulative analysis among 8 studies (514,816 subjects and 33,576 deaths) that evaluated overall mortality in relation to the adherence to MD reported that a greater adherence to MD was significantly associated with a reduced risk of mortality (HR: 0.83, 95%CI 0.77-0.89). Likewise, a significant reduction of cardiovascular mortality (HR: 0.77, 95%CI 0.70-0.84), incidence and/or mortality from cancer (HR: 0.86, 95%CI 0.82-0.91) as well as incidence of Parkinson's and Alzheimer's diseases (HR: 0.88, 95%CI 0.78-0.99) was showed. *Conclusions.* A greater adherence to the traditional MD is associated with a significant improvement of health status, as seen by a significant reduction of overall mortality (-17%), mortality from cardiovascular diseases (-23%) and neoplasm (-14%) as well as of incidence of degenerative diseases (-12%).

C088

ISCHEMIC STROKE IN YOUNG ADULTS: GENETIC ANALYSIS OF 60 POLYMORPHISMS IN 17 GENES INVOLVED IN METHIONINE METABOLISM

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Previous studies have suggested an association between ischemic stroke and hyperhomocysteinemia, a complex trait determined by environmental and genetic factors. Our hypothesis was that variations in genes directly or indirectly involved in methionine metabolism may contribute to genetic susceptibility for stroke. We investigated 60 polymorphisms in AHCY, BHMT1, BHMT2, CBS, ENOSF1, FOLH1, MTHFD1, MTHFR, MTR, MTRR, NNMT, PON1, PON2, SLC19A1, SHMT1, TCN2, TYMS genes according to their demonstrated/putative function on the basis of literature data, localization in promoter or regulatory and coding regions and/or heterozygosity values>0.300. On genomic DNA of 381 patients and 762 sex and age matched controls, we evaluated the 60 polymorphisms by using a primer extension based microarray technology (GenomeLab SNP Stream Technology, Beckman Coulter). All polymorphisms resulted in Hardy-Weinberg equilibrium in patients and controls. Genotype distribution resulted significantly different between patients and controls for the following SNPs: rs819146 AHCY, rs651852 rs567754 rs 3733890 rs10037045 BHMT1, rs682985 BHMT2, rs202680 FOLH1, rs2357481 MTHFD1, rs1801133 rs1801131 MTHFR, rs3819100 NNMT, and rs20721958 rs10418 TCN2. At the multiple logistic regression analysis with stroke as dependent variable and hypertension, diabetes mellitus, dyslipidemia, smoking habit and the single polymorphisms as independent variable, the rs819146 AHCY, rs651852 BHMT1, rs567754 BHMT1, and rs1801131 MTHFR, gene polymorphisms resulted independent protective factor for stroke; whereas, rs10037045 BHMT1, rs202680 FOLH1, and rs1801133 MTHFR, gene polymorphisms resulted independent risk factor for stroke. After haplotype reconstruction in each gene investigated, generalized linear model analyses adjusted for traditional risk factors (hypertension, smoking habit, dyslipidemia, diabetes) showed a significant association among stroke and BHMT1, BHMT2, FOLH1, and MTHFR haplotypes. This study identifies significant genetic associations between stroke and haplotypes in BHMT1, BHMT2, FOLH1, and MTHFR genes offering new insights in the pathogenesis of stroke.

C089

GENETIC ANALYSIS OF 56 POLYMORPHISMS IN 17 GENES INVOLVED IN METHIONINE METABOLISM IN PATIENTS WITH ABDOMINAL AORTIC ANEURYSM

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Previous studies suggested an association between abdominal aortic aneurysm (AAA) and hyperhomocysteinemia, a complex trait determined by genetic and environmental factors. Our hypothesis was that polymorphisms in genes directly or indirectly involved in methionine metabolism may contribute to AAA susceptibility. We studied 56 polymorphisms in MTHFR, MTR, MTRR, CBS, MTHFD1, SLC19A1, NNMT, TCN2,

AHCY, BHMT, BHMT2, FOLH1, TYMS, ENOSF1, SHMT1, PON1, PON2 genes according to their demonstrated/putative function, localization in promoter or regulatory and coding regions and/or heterozygosity values>0.300. Polymorphisms were evaluated by using a primer extension based microarray technology in 423 AAA patients and 423 matched controls. All polymorphisms resulted in Hardy-Weinberg equilibrium in patients and controls. Genotype distribution resulted significantly different between patients and controls for the following SNPs: rs819146 AHCY, rs202680 FOLH1, rs8003379 MTHFD1, rs2853523 MTR, rs326118 MTRR, rs3788205 SCL19A1 and rs16430 TYMS. At the multiple logistic regression analysis adjusted for traditional cardiovascular risk factors (sex, age, hypertension, smoking habit, dyslipidemia, diabetes) and chronic obstructive pulmonary disease (COPD), rs8003379 MTHFD1 (OR=0.41, 95%CI 0.26-0.65) and rs326118 MTRR (OR=0.47, 95%CI 0.29-0.77) polymorphisms resulted independent susceptibility factor for AAA. After haplotype reconstruction, logistic regression analyses adjusted for traditional risk factors and COPD showed a significant association among AAA and AHCY, FOLH1, MTHFD1, MTR, NNMT, PON1 and TYMS haplotypes. This study identifies significant genetic associations between AAA and haplotypes in AHCY, FOLH1, MTHFD1, MTR, NNMT, PON1 and TYMS genes. These association are independent from the role of these genes in modulating Hcy levels. This study offers new insights in the pathogenesis of AAA.

C090

HOMOCYSTEINE, B-GROUP VITAMINS (VITAMIN B6 AND FOLIC ACID) AND RHEOLOGICAL PARAMETERS AS INDEPENDENT RISK FACTORS FOR RETINAL VEIN OCCLUSION

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Introduction. Retinal vein occlusion (RVO) is one of the most common retinal vascular disorders affecting small, medium and large ocular vessels. Over the last years, an association between some emerging thrombophilic risk factors and RVO has been reported. Aim of this study is to thoroughly evaluate the emerging risk pattern of a large group of patients with RVO. *Material and Methods.* In 430 RVO patients [median age: 66 years (15-88); 202 M, 228 F] and in a similar group of age- and sex- comparable healthy subjects, we measured homocysteine (Hcy) levels by FPIA method (Abbott, Norway), serum vitamin B6 by HPLC (Immundiagnostik, Germany), serum folic acid and vitamin B12 by radioimmunoassay (ICN Pharmaceuticals, USA) as well as hemorheological variables [whole blood viscosity (WBV) at high and low shear rates, erythrocytes' filtration (EF), plasma viscosity (PV)] by using a Rotational Viscosimeter (Contraves, Switzerland), and a microcomputer-assisted filtrometer (Myrenne, Germany). *Results.* Hcy, vitamin B6, and folate levels among thrombophilic parameters, as well as WBV at high shear rate and EF among hemorheological parameters were found to be significantly different in patients as compared to healthy subjects. In order to investigate the possible association between these parameters and the disease we divided the study population into tertiles of their distributions among healthy control group, and we performed a logistic regression analysis. According to the univariate analysis, the highest tertile of Hcy (OR: 5.3 95%CI 3.6-10.3; $p<0.0001$) the lowest tertiles of vitamin B6 (OR: 3.1, 95%CI 2.1-5.5; $p<0.0001$) and folic acid (OR: 4.1, 95%CI 2.8-7.8; $p<0.0001$), and the highest tertiles of WBV at shear rate of 94.5 cycles s-1 (OR: 2.78, 95%CI 1.82-4.24; $p<0.0001$), and EF (OR: 0.42, 95%CI 0.26-0.68; $p<0.0001$) were found to be significantly associated with RVO. At multivariate analysis, after adjustment for possible confounders in three different models, obtained by entering covariates simultaneously or added separately, the highest tertile of Hcy (OR: 5.4, 95%CI 2.5-8.8; $p<0.0001$) as well as the lowest tertiles of vitamin B6 (OR: 3.2, 95%CI 2.6-6.9; $p<0.0001$) and folate (OR: 4.2, 95%CI 4.8-13.9; $p<0.0001$), and the highest tertiles of WBV at shear rate 94.5 cycles s-1 (OR: 2.75, 95%CI 1.66-4.54; $p<0.0001$) and EF (OR: 0.40, 95%CI 0.23-0.71, $p<0.0001$), maintained their significant independent associations with RVO. *Conclusions.* The present findings, obtained in a large group of patients with RVO, report a significant and independent role for thrombophilic and hemorheological variables on the occurrence of RVO. These data could help physicians to better optimize the therapeutic strategies in these patients.

C091

THROMBOPHILIC RISK FACTORS IN PATIENTS WITH NON ARTERITIC ISCHAEMIC OPTIC NEUROPATHY

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Background. Non arteritic anterior ischaemic optic neuropathy (NAION) is a multifactorial disease leading to severe visual impairment which is caused by an infarction of the vessels supplying the optic nerve head. *Aim.* to evaluate whether thrombophilia may be a risk factor for development of NAION. *Materials and methods.* Sixty-five newly diagnosed NAION patients and sixty-five age- and gender matched healthy controls were studied. All participants underwent specific blood testing for homocysteine (FPIA method, Abbott, Norway), and lipoprotein (a) (ELISA method, Mercodia Lp(a), Uppsala, Sweden). Plasma levels of vitamin B6, measured by HPLC (Immundiagnostik, Germany), folic acid, and B12 evaluated by radioimmunoassay (ICN Pharmaceuticals, USA) were also determined. In addition, traditional cardiovascular risk factors were considered. Odds ratio (OR) and 95% confidence intervals (CI) were presented. Statistical significance was set at $p\leq0.05$. *Results.* at univariate analysis, the highest tertile of homocysteine (OR 2.91; 95% CI 1.43-5.92; $p=0.001$) and the lowest tertile of vitamin B6 (OR 2.66; 95% CI 1.30-5.45; $p=0.002$) were significantly associated with NAION. At multivariate analysis, adjusted for age, gender, smoking habit, hypertension, dyslipidaemia, and thrombophilic risk factors, the highest tertile of homocysteine (OR 7.09; 95% CI 2.64-19.10; $p<0.0001$) and the lowest tertile of vitamin B6 (OR 2.46; 95% CI 1.07-5.64; $p<0.0001$) maintained their significant relationships with NAION. Moreover, elevated plasma levels of lipoprotein (a) were found to be correlated with NAION both at univariate analysis (OR 6.61; 95% CI 2.60-16.76, $p<0.0001$) and at multivariate analysis (OR 4.91; 95% CI 1.78-13.51, $p<0.0001$). *Conclusions*. this study demonstrated that elevated plasma homocysteine and lipoprotein (a) levels, as well as low vitamin B6 levels, may increase the risk of developing NAION. A screening for thrombophilic markers should be recommended in all subjects experiencing NAION.

Venous Thromboembolism: Prophylaxis and Therapy

C092

GRADUATED COMPRESSION STOCKINGS VS. LOW-MOLECULAR-WEIGHT HEPARIN FOR PREVENTION OF VENOUS THROMBOEMBOLISM AFTER KNEE ARTRHOSCOPY. A PROSPECTIVE RANDOMIZED STUDY (KANT STUDY)

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Introduction. In absence of prophylaxis, the incidence of venographically proven DVT after KA is reported to be as high as 18%. Clear indication for antithrombotic prophylaxis after KA is lacking. *Methods*. We randomised patients undergoing KA to wear GCS or to receive once-daily sq LMWH, for 7 days. All patients underwent bilateral whole-leg ultrasonography at day 8±1, or earlier if clinically indicated. Suspected symptomatic pulmonary embolism (PE) was ruled-out according to accepted standards. Patients with a normal diagnostic work-up were followed-up clinically for 3 months. As primary efficacy outcome we chose the combined incidence of symptomatic PE, symptomatic and asymptomatic proximal DVT and symptomatic isolated calf DVT. The primary safety outcome was the incidence of major and clinically relevant bleeding. Secondary outcome were the overall incidence of proximal and distal DVT and of symptomatic PE and the overall incidence of bleeding. Data were analysed with the χ^2 test. *Results*. Overall 1317 patients were randomised (GCS, n=660; LMWH, n=657). The incidence of the primary efficacy outcome was 3.18% in the GCS group and 0.91% in the LMWH group (difference 2.3%, 95%CI 0.6 to 3.9%; p=0.005 2tailed); the incidence of primary safety outcome was 0.30% in the GCS group and 0.91% in the LMWH group (difference -0.6%, 95%CI -1.6 to 0.4%; p=0.178 2tailed). The incidence of the secondary efficacy outcome was 3.3% (43 out of 1317; 95%CI 2.4-4.3%; 1.1% proximal). The incidence of the secondary safety outcome was 3.9% (51 out of 1317; 95%CI 2.9-5.0%). There was no significant statistical difference in bleeding complications between the two treatment groups (3.3% of patients in GCS group and 4.4% of patients in LMWH group; absolute difference -1.1%;95%CI -3.3% to 1.2%; p=0.322, two-tailed). All patients with a normal diagnostic work-up experienced a totally uneventful follow-up. *Conclusions*. One-week fixed-dose sq LMWH is more effective than GCS for prevention of VTE after KA, without increased bleeding risk.

C093

ABSENCE OF RESIDUAL VEIN THROMBOSIS AFTER AN EPISODE OF IDIOPATHIC DEEP VEIN THROMBOSIS: SHORT-TERM ANTICOAGULATION IS SAFE. THE EXTENDED DACUS STUDY

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Background. The optimal duration of Oral Anticoagulant Therapy (OAT) for Deep Vein Thrombosis (DVT) can be tailored by Residual Vein Thrombosis (RVT) (Siragusa S *et al.* Blood 2003;102(11):OC183), a marker able to assess the individual risk for recurrent thrombosis. However, in patients with idiopathic DVT the safety of early interruption of OAT, because of absence of RVT, is still debated. Objective of the study. In the present study, we evaluated the safety of withholding OAT, in patients with idiopathic DVT and without RVT, three months after the index thrombotic episode. Study design. Prospective controlled study with two groups: patients without RVT stopped OAT after 3 months while those with RVT continued for additional 3 months. Materials and Methods. Consecutive patients with a first episode of idiopathic DVT of the lower limbs; patients with cancer or known thrombophilia were excluded. At the third months of OAT, RVT was assessed as previously described; briefly, RVT was considered absent when a clot occupying less than 40% of the vein lumen was detected by compression ultrasonography. Events, classified as recurrent DVT and/or Pulmonary Embolism (PE) and/or major and minor bleeding were evaluated; all patients were followed-up for at least 12 months after OAT discontinuation. *Results.* During the period 1999-2006, 518 patients were included in the study. In 206 (39.7%) RVT was considered absent (RVT negative group) and they stopped OAT; the remaining 312 patients continued anticoagulants for additional 3 months (RVT positive group). Total duration of follow-up (FU) was 184.7 years for RVT negative group (with a mean FU of 3.0+0.83 years) and 191.3 years for RVT positive group (with a mean FU of 3.1+0.89 years). The rate and type of events during FU is reported in Table 1. *Conclusions.* This investigation shows that in patients without RVT, three months of OAT are safe even after an episode of idiopathic DVT. This hold for at least 30% of the entire DVT population and has an important clinical impact; in fact, it is possible to select a group of patients with a very low risk for recurrences over a period of 3 years. This approach carries also a negligible risk for bleeding.

Table 1. Events between RVT Negative and Positive Groups.

Outcomes	*RVT Neg. group (206)*	*RVT Pos. group (312)*	*p value*
Recurrences, n/total (%)*	2/206 (0.9)	63/312 (20.2)	<0.0005
Recurrences, n/100 person-year (%)*	2/184.7 (1.1)	63/191.3 (32.9)	<0.0005
Type of recurrent VTE			
DVT	1	43	
DVT + PE	0	6	
Isolated PE	0	3	
Controlateral	1	11	
Major bleeding, n/total (%)**	0/206	3/312 (0.9)	
Major bleeding, n/100 person-Yr (%)**	0/184.7	3/191.3 (1.5)	

*After OAT discontinuation, **During OAT

C094

NATURAL HISTORY OF MESENTERIC VENOUS THROMBOSIS: A LARGE MULTI-CENTRE STUDY

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Introduction. Mesenteric venous thrombosis (MVT) is an uncommon but potentially life-threatening disease, accounting for 5-15% of mesenteric ischemic events. Most cases of MVT are either identified at laparotomy or at autopsy, with a mortality rate of approximately 50% in old series. Advances in imaging techniques have facilitated the early diagnosis of MVT and, thus, have contributed to a decrease in mortality in the more recent series. Information on the natural history of MVT and on efficacy and safety of long term treatment with oral anticoagulant is based on small uncontrolled series of patients with a limited follow up. Therefore, the aim of our study is to clarify the natural history of this disease in a large cohort of patients recently diagnosed with MVT. *Methods.* The charts of all patients with splancnic vein thrombosis who are currently attending or who have attended four anticoagulation clinics (Denver, Albuquerque, Varese and Palermo) were reviewed. At these centres, patients are regularly followed up for the monitoring of oral anticoagulant therapy (OAT) and information on clinical events is documented and registered in a computerized database. All patients with objectively diagnosed MVT, were selected. The charts of eligible patients were reviewed for baseline clinical characteristics including sex, age, prior history of cardiovascular disease and use and type of anticoagulant therapy. Information on mortality and all objectively confirmed recurrent venous thromboembolic events were noted. *Results*. Seventy seven patients (mean age 49.2 years; 45 males) were included. Thirteen patients had a previous thromboembolic event. Thirty two were idiopathic. Forty six patients were on long term anticoagulant therapy. Median follow up was 36 months (Range 2-204 months). Seven patients had a recurrent thromboembolic event (5 splancnic vein thromboses and two PEs) for an incidence rate of 23.4 events /1000 year patient. Five patients had a recur-

rent thromboembolic event when suspended oral anticoagulant therapy for an incidence rate of 45.9 events /1000 year patient. Two patients (2.6%) had a major bleeding event (one subdural haematoma and one gastroenteric bleeding). Seven patients (9.1%) died during follow up (4 were related to cancer). *Conclusions.* patients with a previous episode of MVT had a low risk of recurrent thromboembolic events. In these patients long term anticoagulant therapy appeared to be safe and effective.

C095

BOLUS TENECTEPLASE FOR RIGHT VENTRICLE DYSFUNCTION IN HEMODYNAMICALLY STABLE PATIENTS WITH PULMONARY EMBOLISM

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Background. The clinical benefit of thrombolytic treatment over heparin in patients with pulmonary embolism (PE) without hemodynamic compromise remains controversial. In these patients bolus tenecteplase (TNK) has the potential to provide an effective and safe thrombolysis. *Methods and results*. We evaluated the effect of TNK on right ventricle dysfunction (RVD) assessed by echocardiography in hemodynamically stable patients with PE in a multicenter, randomized, double-blind, placebo-controlled study. RVD was defined as right/left ventricle (RV/LV) end-diastolic dimension (EDD) ratio >1 in the apical 4-chamber view. Patients were randomized to receive weight-adjusted single-bolus TNK, 30 to 50 mg, or placebo within 6 hours from baseline echocardiography. All patients received unfractionated heparin at dose adjusted by a standardized nomogram. Reduction of RV/LV-EDD at 24 hours was the primary outcome measure and was evaluated by an independent committee unaware of treatment allocation. Overall, 58 patients were randomized. Echocardiograms were adequate for efficacy analysis in 51 patients, 23 randomized to TNK and 28 to placebo. Baseline mean RV/LV-EDD was 1.36±0.27 and 1.32±0.26 in the TNK and placebo group, respectively. The mean reduction of RV/LV-EDD at 24 hours was 0.31±0.38 mm and 0.10±0.35mm in patients randomized to TNK or placebo, respectively (p<0.05). One patient randomized to TNK suffered a clinical event (recurrent PE) in comparison to three patients randomized to placebo (1 recurrent PE; 1 clinical deterioration and 1 non PE-related death). Two non fatal major bleedings occurred with TNK (1 intracranial) and one with placebo. *Conclusions*. In hemodynamically stable patients with PE, single bolus TNK is associated with reduction of RVD at 24 hours. Whether this benefit is associated with an improved clinical outcome without excessive bleeding is currently explored in a large clinical trial.

C096

A PROSPECTIVE BLIND STUDY ON DIAGNOSIS AND NATURAL HISTORY OF ISOLATED CALF DEEP VEIN THROMBOSIS IN SYMPTOMATIC OUTPATIENTS (THE CALTHRO STUDY)

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An accepted diagnostic procedure in outpatients symptomatic for leg deep vein thrombosis (DVT) is to exclude a proximal DVT by a compression ultrasound (CUS) limited to proximal veins (LCUS), repeated after 5-7 days if pre-test clinical probability (PCP) is likely and/or D-dimer assay (Dd) altered. This is based on the premise that isolated calf DVT (ICDVT) do not need to be diagnosed and treated. Conversely, some authors consider ICDVTs at risk of complications and recommend to perform a single complete CUS examination of all deep veins (CCUS) to diagnose and treat also ICDVTs. However, no adequate information is available on natural history of ICDVTs and on the clinical risk associated with untreated ICDVTs. We aimed to asses the rate and clinical consequences of untreated ICDVTs in symptomatic outpatients. Outpatients symptomatic for suspected leg DVT referring to the two participant Clinical centers were eligible. The study was not funded. It was not possible to enroll all consecutive eligible patients (pts), that were included based on the availability of expert medical staff in the day of their presentation. All pts were treated in line with the LCUS strategy: those without proximal DVT and likely PCP and/or altered Dd were eligible for the study and received a blind CUS of the calf deep veins (CDV) by a second operator. The results of this investigation were open only after 3 months. All pts received a call phone by a doctor 3 m after inclusion. We investigated 337 (M/F 166/171) subjects, symptomatic for leg DVT (R: 149; L: 169; bil: 19). The CUS investigation of CDV was inconclusive in 5 subjects (1.5%), negative in 285 (84.6%) and positive for thrombosis in at least one CDV in 47 (13.9%); The veins involved were: Muscle 27, Anterior Tibial 2 Posterior Tibial 13, Peroneal 11. CUS was considered inconclusive in many single vein tracts. At 3 m 45/47 subjects with ICDVT declared a complete remission or improvement of their problems. Two subjects had complications: one was admitted to an hospital before the 7th day visit with a PE diagnosis; the other had a proximal DVT diagnosis after 2 m. The LCUS strategy failed in 2/337 (0.6%) cases examined and in 2/47 with ICDVT diagnosis. All the remaining cases with diagnosed ICDVT improved spontaneously without treatment except elastic stockings. These results seem to be against the need for a CCUS in symptomatic outpatients.

C097

MULTIDETECTOR COMPUTED TOMOGRAPHY TO ASSESS RIGHT VENTRICULAR DYSFUNCTION IN PATIENTS WITH ACUTE PULMONARY EMBOLISM

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Background. In patients with acute pulmonary embolism (PE) right ventricle dysfunction (RVD) assessed by echocardiography has been consistently shown to be associated with an adverse in-hospital outcome. The aim of this study was to evaluate the accuracy of multidetector computed tomography (MDCT) in assessing RVD using echocardiography as the reference standard. The secondary aim was to prospectively assess the prognostic value of MDCT-detected RVD. *Methods.* Consecutive patients were included in this study in the 8 participating study centers if they had a) symptomatic acute PE diagnosed by MDCT and b) echocardiography done and serum troponin assessed within 6 hours from the diagnostic MDCT. A ratio of right to left ventricle short-axis diameters (R/LV) >1 at the valvular plane in their maximum dimension was the criterion for MDCT-detected RVD. Eight or 16 slice MDCT were used and all were centrally evaluated for RVD. Criteria for RVD at echocardiography were 1) end-diastolic R/LV >0.7 in parasternal long axis and/or subcostal views, and/or >1 in 4 chamber view. Troponin was categorized as positive or negative according to the locally used cut-off value. *Results*. Overall, 168 patients were included in the study (males 76, median age 66.8 years): 88 patients (52.3%) had RVD at echocardiography, 56 patients (34.6%) elevated troponin and 54 patients (32%) both. Troponin levels were more commonly elevated in patients with than without RVD at echocardiography (57.5 versus 9.1%; p<0.001). RVD at MDCT was seen in 96 patients (57.1%). R/LV at MDCT was higher in patients with echocardiographic RVD (1.24 versus 0.92; p<0.001), and in patients with elevated troponin (1.25 versus 1.02; p <0.001), in comparison to patients without RVD or normal troponin. Sensitivity and specificity of MDCT for RVD were 82.8% and 66.2% with reference to RVD at echocardiography and 84.9% and 50% to elevated troponin. Eight patients died: 7 with RVD at MDCT (7.3%) and 1 without (1.3%). Overall, 12 patients (7.7%) died or had cardiogenic shock, 10 with RVD at multidetector CT. RVD at MDCT was an independent predictor for in-hospital death or cardiogenic shock at logistic regression analysis. Conclusions Our results indicate that MDCT may be used to assess RVD and to predict clinical outcome in patients with acute PE.

C098

PARNAPARIN VERSUS ASPIRIN IN THE TREATMENT OF RETINAL VEIN OCCLUSION. A RANDOMIZED, DOUBLE BLIND, CONTROLLED STUDY

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Background. Retinal vein occlusion (RVO) is a common cause of unilateral visual loss. Evidence based treatment recommendations for patients with RVO cannot be made since there is a lack of adequate clinical trials. *Methods.* We have carried out a multicenter, randomized, double blind, controlled trial to compare the efficacy and safety of aspirin and of a low molecular weight heparin, parnaparin, in the treatment of RVO. Patients were eligible if delay between symptoms onset and objective diagnosis was less than 15 days and in the absence of predefined ophthalmologic criteria, previous RVO, contraindications to study treatments or ongoing anticoagulant or aspirin therapy at the time of the event. Patients were randomized to aspirin 100 mg/day for 3 months or to a fixed daily dose of parnaparin, 12.800 IU for 7 days followed by 6.400 IU for a total of 3 months. The primary end-point of the study was the incidence of functional worsening of the eye with RVO at 6 months, as assessed by fluorescein angiography, visual acuity, and visual field. Study end-points were adjudicated by an independent committee. *Results*. Sixty-seven patients were enrolled in the study and 58 of them (28 treated with parnaparin, 30 with aspirin) were evaluable for the analysis. Baseline characteristics were well balanced between groups. Functional worsening was adjudicated in 20.7% of patients treated with parnaparin and in 59.4% of patients treated with ASA (p=0.002). Worsened fluorescein angiography was adjudicated in 17.9% and in 63.3% of patients, respectively (p=0.002). Mean visual acuity improved from 0.37 at baseline to 0.69 at 6 months in the group treated with parnaparin (p=0.010) and from 0.36 at baseline to 0.42 at 6 months in the group treated with aspirin (p=0.032). The difference in the variation between the two groups was statistically significant (p<0.05). No differences between groups were documented in the visual field scores. Recurrent RVO was diagnosed in 3 patients, all treated with ASA (p=n.s.). Bleeding rates were similar between the two groups. *Conclusions*. Parnaparin appears to be more effective than aspirin in preventing functional worsening in patients with RVO. The results of this study need to be confirmed in a larger clinical trial.

Hemophilia

C099

U1-SNRNA-MEDIATED RESCUE OF MRNA PROCESSING IN SEVERE 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 FVIIa. Studies in animal and cellular models of human diseases showed that modified small nuclear RNAs (snRNAs) can promote changes in mRNA splicing and thus in gene expression. Splicing 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 snRNA-mediated rescue of coagulation factor VII (FVII) expression impaired by the IVS7+5 g/a mutation, which is associated to life-threatening bleeding in homozygous patients. This change occurs in the first of six homologous 37bp repeats containing cryptic donor splice site (5'ss) identical to the normal one. Expression of extended FVII minigenes in human hepatoma cells (Hep3B) and studies at the mRNA level (RT-PCR, fluorescent labeling and capillary electrophoresis) indicated that the IVS7+5g/a induces exon 7 skipping and activation of the first downstream cryptic 5'ss, thus generating frameshifts. Levels of normal transcripts were barely detectable (<0.2%). To restore correct mRNA processing we engineered the U1-snRNA, the spliceosome components selectively recognizing 5'ss. Vectors for three U1-snRNAs, complementary to the mutated 5'ss (U1+5a) or to neighbouring sequences, were created and co-expressed with FVII minigenes in Hep3B. The U1-snRNAs reduced from 80-40% the exon 7 skipping, thus increasing exon definition. The U1+5a construct also dramatically increased recognition of the correct 5'ss over the 37bp-downstream cryptic site preferentially activated by the mutation, thus inducing appreciable synthesis of normal transcripts (from barely detectable to 50%). This effect, which was dose-dependent, clearly demonstrated that impaired recognition by the U1-snRNA was the mechanism responsible for FVII deficiency. These findings suggest compensatory U1-snRNAs as therapeutic tools in coagulation factor deficiencies caused by mutations at 5'ss, a frequent cause of severe defects.

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C100

THE ITALIAN REGISTRY OF IMMUNE TOLERANCE INDUCTION IN HAEMOPHILIA A WITH INHIBITORS: 2-YEAR UPDATE (PROGNOSTIC FACTORS IN IMMUNE TOLERANCE, THE PROFIT STUDY)

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Induction of immune tolerance (ITI) is presently the only therapeutic approach able to eradicate or reduce the inhibitor production in haemophiliacs. The optimal dose regimen and type of FVIII product for ITI, however, are still unknown and the cost-benefit ratios of such treatment are unclear, since treated patients show heterogeneous clinical features. National registries are useful to monitor the practice of ITI treatment and to identify predictors of response helpful to optimise the selection of ITI candidates. The AICE established a retrospective-prospective registry (the PROFIT Study) to collect clinical, laboratory and genetic data on ITI in haemophilia A. Outcome was centrally reviewed according to the current definitions of success (undetectable inhibitor and normalised FVIII pharmacokinetic parameters, PK), partial response (inhibitor titer <5 BU/mL and/or abnormal PK) and failure. At April 2008, data on 110 ITI courses (1996-2007) in 102 haemophiliacs (98 severe, 99 high-responders) were provided by 24 Centres. Patients underwent the first ITI at a median age of 4.8 yrs (0.3-52.5; 56% <8yrs) with a median

pre-ITI titer of 4.1 BU/mL (<0.5-200; 72% <10) and a median of 21 mo. (0-332; 59% <24) from the diagnosis. Median historical peak was 64 BU/mL (1.5-800; 82% <200). FVIII/VWF products were used in 28% of courses and recombinant FVIII in the remaining at doses ≥100IU/Kg/d in 35% and 75%, respectively (range:25 IU/Kg/qod-220 IU/Kg/d). The outcome of first courses completed in 87 patients was success in 45 (52%), partial response in 14 (16%) and failure in 28 (32%). Median time to achieve success was 6 mo. (1.5-40). The median success follow-up was 3.4 yrs (0.3-10.4); a relapse occurred after 7 yrs in one patient. Median pre-ITI titer, historical peak, ITI-peak and daily FVIII dose in patients who achieved the success compared to those who failed were: 2.4 *vs.* 7.2, 42 *vs.* 128, 20 *vs.* 400 BU/mL and 100 *vs.* 170 IU/Kg/d, respectively. The PROFIT Registry is providing a detailed picture of ITI practice in Italy over the last decade. Despite the presence of ≥1 known negative predictors of response in about 2/3 of patients, 68% achieved complete or partial response, allowing bleeding control with FVIII treatment or prophylaxis. Regimens with intermediate-high FVIII doses and recombinant products are more frequently used. Inhibitor-related variables (historical peak, titer at ITI start, peak on ITI) are likely to be the most important predictors of outcome.

C101

NON-INVASIVE TOOL FOR EARLY DETECTION OF X-LINKED DISORDERS

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The discovery in 1997 that free fetal DNA (ffDNA) is present in the plasma of pregnant women has opened an alternative to existing methods for conventional prenatal diagnosis. The fetal DNA in maternal plasma represents a source of genetic material which can be obtained non-invasively and reduced the procedure related risks of an invasive prenatal diagnosis. Technical advances, such as the development of quantitative real-time PCR, have allowed to obtain an high sensitivity and specificity in the detection of fetal sex. Therefore the availability of early fetal gender could have an important role for the assessment of pregnancies at risk of X-linked genetic disorders, as haemophilia. We examined maternal plasma from 140 pregnant women at the different gestational ages from 4 to 18 weeks to identify the optimal period to obtain an adequate amount of fetal DNA for diagnosis, to define the distribution of fetal DNA concentration at different gestational ages and to evaluate accuracy of this approach. Fetal DNA quantification in maternal plasma was carried out by real-time PCR on the SRY gene. In all cases, the fetal gender was also assessed by first trimester ultrasound analysis. Detection of fetal DNA in maternal plasma displayed a low sensitivity of SRY gene at 4 to 7 weeks (72%), increasing significantly after 8th weeks of gestation (86%). The latter sensitivity combined with a high specificity (95%) allowed us to achieve an accurate method for fetal sex analysis. In conclusion, fetal DNA from maternal plasma seems to be an adequate source of genetic material for non-invasive prenatal diagnosis. However an early prenatal diagnosis should be avoided at 4-7th weeks of gestation since the fetal DNA concentration, the sensitivity and accuracy of this diagnostic test are too low. Therefore this method could be used in pregnancies at risk of X-linked disorders, as haemophilia after 8th week of gestation in association with ultrasound results in order to increase the reliability of the technique.

C102

THE HAEMOPHILIA REGISTRY OF THE ITALIAN ASSOCIATION OF HAEMOPHILIA CENTRES

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Introduction and aim. Since 2003 the Italian Association of Hemophilia Centres (AICE) run a new program aimed at building up the Italian Registry of Hemophilia and Allied Disorders. *Materials and Methods*. The AICE identified an expert panel to steer the registry. A computer software (Emocard) to assist patient management was developed and all the AICE-affiliated hemophilia treatment centres (HTC) were prompted to adopt it. Twice a year a predefined set of anonymized data is centralized and merged into a national database. Duplicated entries are managed through a confidentiality sparing mechanism. The database covers socio-demographic, clinical, laboratory and treatment data. A subset of data is shared with the Ministry of Health (Istituto Superiore di Sanità, ISS). *Results*. Database growth and coverage: Since December 2004 five data extractions were carried out. The database contains 7827 unique records, 492 of them referring to dead patients. The number of active HTC involved in the registry project did progressively increase to 48/49 on December 2007. Database growth showed a constantly positive trend over time, with a mean increase rate of approximately 12%. The coverage by the registry of the Italian hemophilia population can be estimated at >90%. The database has collected records of the following alive patients: Hemophilia A (HA): 1519 severe, 460 moderate and 1109 mild; Hemophilia B (HB): 249 severe, 153 moderate and 236 mild; von Willebrand disease: 1502 type 1, 396 type 2 and 106 type 3. Inhibitor patients were 309, of which 198 high responders and 72 low responders; 163 actual and 146 past, of which 42 transient. Genetic analysis for mutation screening was reported for 785 (52%) of severe HA and for 143 (57%) of severe HB patients. *Age at diagnosis*. Median (interquartile range) age at diagnosis was 1.0 (0.6-6.0) for severe, 4.0 (1.1-16.0) for moderate and 14.0 (4.2-30.0) for mild HA patients and 1.5 (1-10.0) for severe, 8.0 (2.0-18.7) for moderate and 20.0 (6.2-39.5) years for mild HB patients. In the last 11 years (1996-2006) the median annual number of newly diagnosed patients has been 18 (range 6-26) for severe HA and 4 (range 0-7) for severe HB. Further data about inhibitors and HCV/HIV infections are shown in the Table 1. *Conclusions.* The Italian registry run by AICE adds to the list of the available national haemophilia registries and is intended to inform treatment policies and foster research projects in Italy.

Table 1.

	% of Inhibitors	% of HIV infected patients	% of HCV infected patients
HA sev	19.2%	13.5% (95% CI 11.5-15.5)	56.0% (95% CI 53.2-58.9)
HA mod	4.3%	3.6 (95% CI 1.6-5.7)	51.1 (95% CI 45.5-56.7)
HA mild	1.1%	1.7 (95% CI 0.7-2.7)	42.4 (95% CI 37.9-46.9)
HB sev	3.0%	19.1% (95% CI 13.4-24.8)	60.4% (95% CI 52.3-68.6)
HB mod	0.7%	6.9 (95% CI 2.3-11.5)	51.1 (95% CI 40.8-61.4)
HB mild	0	3.1 (95% CI 0.1-6.1)	32.6 (95% CI 23.0-42.2)

C103

IDENTIFICATION OF FVIII GENE MUTATIONS IN PATIENTS WITH HAEMOPHILIA A USING COMBINATORIAL SEQUENCING BY HYBRIDIZATION

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Haemophilia A is a common inherited recessive X-linked disorder of blood clotting caused by deficiency of factor VIII in the coagulation cascade and affects approximately 1 in 5,000 males world-wide. The factor VIII gene, comprises 26 exons ranging from 69 bp (exon 5) to 3.1 kb (exon 14) in size, spans 186 kb of genomic DNA and produces a 9030 nt mRNA. The severity of haemophilia A in the pedigree should be determined first as this will influence the diagnostic strategy to be employed. Mutations have been found in nearly all 26 exons of the factor VIII gene, over 400 mutations have been identified and de novo mutations represent approximately 30% of all cases. The most common detection methods include DNA sequence analysis which requires numerous reactions and individual analysis of each exon or alternative screening methods such as SSCP, DGGE and dHPLC. We applied the new combinatorial sequencing-by-bybridization (cSBH) as an alternative method to the traditional Sanger dideoxy chain termination approach. Previous work have shown that cSBH is an attractive alternative method for point mutation detection. We increased the quality of results with a new cSBH method that use two different colors (TAMRA and QUASAR). The platform is an indirect method which uses standard chemistry of base-specific hybridization of complementary nucleic acids to indirectly assemble the order of bases in a target DNA. Short oligonucleotide probes are arrayed in the form of high-density arrays of universal sequence and hybridized to sample DNA molecules. The resulting hybridization pattern is used to generate the target sequence using com-

puter algorithms. We report development of a strategy to implement 2-color cSBH to screen a range of mutations within the FVIII gene. We demonstrated that using only one HyChip array large regions of genomic, DNA can be sequenced with excellent readability and we confirmed known FVIII gene mutations. We sequenced PCR product of 1.2 kb in size; in this way long exons such as exons 14 and 26 had to be divided in three and two fragments, respectively. The other smaller exons 1-25 could be pooled together in four different groups and sequenced completely on four chips, with base readability of 100% and an accuracy of 100%. cSBH sequence data showed 100% accuracy with only 0.2% of bases not called.

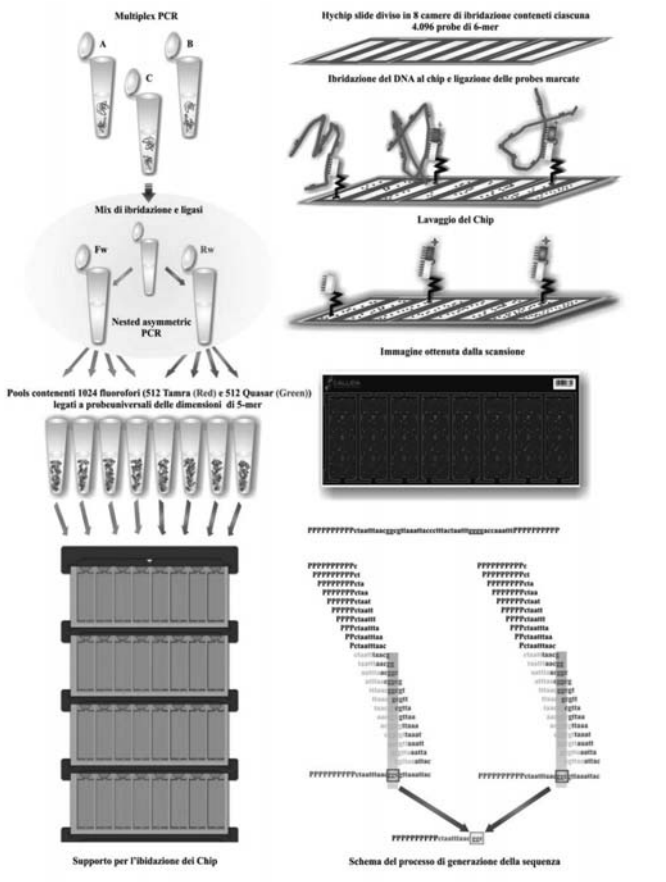

Figure 1.

C104

HOW TO TREAT ACUTE CORONARY SYNDROMES IN HAEMOPHILIACS? OPEN ISSUES BETWEEN HAEMORRHAGIC AND THROMBOTIC RISK FROM A CASE REPORT AND REVIEW OF THE LITERATURE

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Haemophiliacs are thought to be protected from cardiovascular disease. The advances in global care with the prolonged patients' life expectancy and several case-reports, however, raise questions on the management of acute coronary syndromes in this setting, in particular with respect to percutaneous coronary interventions (PCI, requiring combination of GpIIb/IIIa antagonists, heparin and dual antiplatelet agents) and long-term treatment. Recently, a 63 yr-old severe haemophiliac was admitted to our Centre because of persistent dyspnoea. He suffered from arterial hypertension, diabetes mellitus and chronic kidney disease. Signs of myocardial ischaemia were revealed by electrocardiography, with diffuse hypokinesia at two-dimensional echocardiography. Nitrates, diuretics and low-dose unfractionated heparin (UFH) were started. As dyspnoea and atypical chest pain at rest recurred, after 25 IU/Kg followed by 1 IU/Kg/hr continuous infusion of recombinant FVIII (maintaining FVIII levels about 25%) and 75 mg clopidogrel, the patient underwent PCI. Severe multi-vessel coronary disease was shown, hampering angioplasty and stenting, and the patient was candidate to bypass-grafting intervention. FVIII infusion was continued for 72 hrs and clopidogrel substituted for UFH. The patient suddenly died 2 days before undergoing surgery. Few data are available concerning management of haemophiliacs with acute coronary syndromes, both in the acute phase, when PCI is usually performed, and, in particular, for secondary prevention with long-term antiplatelet agents. A review of literature enabled to identify only eleven PCIs in haemophilic patients (five severe), in the majority of cases without administrating GpIIb/IIIa antagonists and reducing heparin (often unfractionated) and antiplatelet doses. In three procedures bivalirudin was substituted for heparin. Bar-metal stents have been placed in some cases with single/dual antiplatelet treatment, in association with FVIII prophylaxis in a severe patient. Balancing thrombotic and haemorrhagic risk in this clinical setting remains an open issue, in particular for the management of long-term antiplatelet treatment in severe patients.

C105

PLATELET FACTOR V ANTIGEN LEVELS IN 6 HOMOZYGOUS AND IN 19 HETEROZYGOUS FACTOR V DEFICIENT PATIENTS

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Background. In humans, approximately 80% of the coagulation factor V (FV) circulates in plasma and the remaining 20% is contained within the alfa-granules of platelets (Plts), in a partially activated form. The role of intra-Plts FV is not clear. It is released upon Plts activation, potentially providing an increase of local concentration of FV at sites of vascular injury. This mechanism facilitates site-specific haemostasis. *Aims.* To evaluate the relationship between plasma and intra-Plts FV antigen levels in heterozygous and homozygous FV deficient subjects. *Patients and methods.* After informed consent blood was drawn from an antecubital vein and collected in 3.8% sodium citrate solution (1:9 vol/vol) from 19 subjects with heterozygous FV defect, 6 homozygous subjects and 42 relatives. In the all study population we evaluated plasma and intra-Plts FV antigen levels. Carriers of FV Leiden mutation or HR2 haplotype were excluded. *Results.* FV plasma levels (mean±SD) in heterozygous FV deficient subjects and in their normal family members were 56.28±13.20% and 93.28±17.50%, respectively. Intra-Plts FV levels (mean±SD) in heterozygous FV deficient subjects and in their non deficient relatives were 42.60±13.99% and 73.80±22.27%, respectively. The differences between heterozygous and normal family members were highly statistical significant so for plasma as for intra-Plts FV levels (t-Student test p value, <0.0001). In homozygous subjects both plasma and intra-Plts FV levels (mean±SD) were below 3±1%.*Conclusions.* In our study we have found a strict correlation between plasma and intra-Plts FV antigen levels. Both in heterozygous FV deficient subjects and in normal relatives we have found a plasma/intra-Plts ratio of about 1.3. This is an indirect evidence of the relationship between these two FV pools, i.e., the levels of intra-Plts FV depend on those of plasma FV. Accordingly, it has been shown that human megakaryocytes are able either to synthesize FV or to endocytose FV from plasma, the latter mechanism being the more relevant.

Heart and Thrombosis

C106

IMPACT OF RESIDUAL PLATELET REACTIVITY ON THE OCCURRENCE OF MACE IN ACUTE CORONARY SYNDROME PATIENTS ON DUAL ANTIPLATELET THERAPY

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A dual antiplatelet regimen of aspirin plus clopidogrel is the standard treatment of patients with acute coronary syndromes (ACS) undergoing percutaneous coronary revascularization (PCI) with stent implantation. A growing body of evidence, obtained in chronic cardiovascular patients as well as in ACS, is demonstrating that the biological entity of the residual platelet reactivity (RPR) despite dual antiplatelet treatment is associated with an increased risk of adverse cardiovascular events. This is the largest prospective study planned to demonstrate the clinical impact of RPR by different agonists (arachidonic acid - AA, ADP and collagen) on the occurrence of major adverse coronary events in the setting of ACS. We have enrolled 1112 ACS patients (961 M/151 F; age: 69 (39-94) yrs) undergoing PCI on dual antiplatelet therapy. RPR has been defined as maximal platelet aggregation by 1 mM arachidonic acid ≥20%, 10 μM ADP ≥70% and 2 microg/mL collagen ≥56% on venus blood samples obtained within 24 hrs from PCI. At a median follow-up of 8 months (1-48), MACE, including cardiac death, myocardial infarction (MI) and target lesion revascularization (TLR) for symptomatic restenosis, were recorded in 202 patients (18.1%): 24 (2.1%) cardiac deaths, 55 (4.9%) MI and 147 (13.2%) TLR. At univariate analysis, RPR by ADP was significantly associated with cardiac death [OR 4.09 (1.7-9.5), $p<0.001$] and MI [OR 1.98 (1.01-3.8), $p<0.05$, whereas RPR by AA and collagen were significantly associated with MI [OR 2.6 (1.5-4.6) $p<0.001$ and OR 2.09 (1.05-4.1) $p<0.05$, respectively]. At multivariate analysis adjusted for age, sex, hypertension, diabetes, smoking habit, dyslipidemia, BMI, systolic left ventricular function and renal function, RPR by ADP and collagen were independent predictors of MACE [OR 1.6 (1.03-2.7), $p<0.05$ and OR 1.6 (1.01-2.6), $p<0.05$, respectively]. These results, obtained in a large number of patients, demonstrate that RPR is a clinical entity associated with the future occurrence of ischemic cardiac events. In particular, we found that RPR by ADP is a predictor of both cardiac deaths and MI and RPR by AA and collagen are predictors of MI. These data pave the way to future studies addressed to evaluate the possible clinical benefits of a tailored antiplatelet therapy in the setting of ACS.

C107

INCIDENCE AND CLINICAL IMPACT OF DUAL NONRESPONSIVENESS TO ASPIRIN AND CLOPIDOGREL IN PATIENTS WITH DRUG ELUTING STENTS

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No prospective data exist about the possible association of dual clopidogrel and aspirin nonresponsiveness with thrombotic events in patients with DES. We sought to determine whether the dual clopidogrel and aspirin nonresponsiveness identifies patients at increased the risk of drug-eluting stent (DES) thrombosis as compared to isolated clopidogrel nonresponsivenss. Platelet function was assessed after a loading dose of 600 mg of clopidogrel in 746 consecutive patients who had successful DES implantation and who were compliant to 6-month dual antiplatelet treatment. Clopidogrel and aspirin nonresponsiveness was defined as platelet aggregation by adenosine 5'-diphosphate (ADP) ≥70% and by arachidonic acid ≥20% respectively. The primary and secondary end points was defined as definite/probable DES thrombosis and the composite of cardiac mortality and definite or probable stent thrombosis at 6-month follow-up respectively. The percentage of definite or probable stent thrombosis was significantly higher in dual nonresponders (11.1%) than in dual responders (2.1%), as well as than in isolated clopidogrel (2.2%) or aspirin nonresponders (2.3%). The incidence of the composite end point was 4.4% in isolated clopidogrel nonresponders, 2.3% in isolated aspirin nonresponders and 13.3% in dual aspirin and clopidogrel nonresponders. Dual clopidogrel and aspirin nonresponsiveness was an independent predictor of DES thrombosis (HR 3.65,95% CI 1.06-12.63, $p=0.041$) and the composite of cardiac mortality and DES thrombosis (HR 3.51,95%CI 1.09-11.25, $p=0.035$). Dual nonresponsiveness to aspirin and clopidogrel is a relatively infrequent condition that identifies patients at very high risk of DES thrombosis.

C108

CYTOCHROME P450 2C19 LOSS-OF-FUNCTION POLYMORPHISM IS ASSOCIATED WITH THE OCCURRENCE OF DRUG-ELUTING STENT THROMBOSIS

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Background. Non-responsiveness to clopidogrel, identified by a residual platelet reactivity (RPR) to ADP is an independent predictor of stent thrombosis (ST) in patients receiving drug-eluting stents (DES). Recently, we demonstrated that CYP2C19*2 allele is associated with RPR in high risk vascular patients on dual antiplatelet treatment. *Aim.* Aim of this study was to evaluate the role of CYP2C19*2 polymorphism in the occurrence of DES thrombosis within 6-month follow-up in patients undergoing percutaneous coronary interventions (PCI) with DES implantation on dual antiplatelet treatment. *Methods and Results,* 772 patients who had successful DES implantation were studied for CYP2C19*2 polymorphism and RPR (by 10 μM ADP-induced platelet-rich-plasma aggregation), and followed-up for 6 months. Patients with ST or composite of ST+cardiac mortality (ST+CM) showed a higher prevalence of carriers (*2/*2+*1/*2) of the rare allele (54.1% *vs.* 31.3%, $p=0.025$ and 51.7% *vs.* 31.2%, $p=0.020$, respectively). At the multivariate logistic regression analysis with ST or ST+CM as dependent variable, and CYP2C19*2 polymorphism, ADP RPR, as well as further previously demonstrated clinical and procedural risk factors for ST as independent variables, CYP2C19*2 allele [ST OR 3.43(1.01-12.78), $p=0.047$; ST+CM OR 3.08(1.23-7.72), $p=0.016$] and ADP RPR [ST OR 2.70(1.00-8.42), $p=0.049$; ST+CM OR 2.98(1.08-12.98), $p=0.019$] resulted independent risk factors. Subjects with the contemporary presence of CYP2C19*2 allele and ADP RPR showed a strong risk of ST or ST+CM (OR=5.79(1.04-39.01), $p=0.033$ and OR=11.45(1.84-71.27), $p=0.009$]. *Conclusions.* This study demonstrates that CYP2C19*2 allele is associated with the occurrence of ST or ST+CM in high risk vascular patients on dual antiplatelet treatment. These findings could have significant impact on the future design of pharmacogenetic antiaggregant strategies.

C109

GENETIC ANALYSIS OF PPARGAMMA: ACUTE CORONARY SYNDROME PATIENTS VERSUS CONTROLS

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PPARγ is a nuclear transcription factor involved in the control of energy, lipid and glucose homeostasis. The gene is located on chromosome 3 (3p25). PPARγ 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). Aim of this study, was to evaluate the role of 25 single nucleotide polymorphisms (SNPs) in PPARγ gene and the relative PPARγ gene haplotypes in determining genetic susceptibility to ACS in an Italian population. We studied 749 patients affected by ACS (median age 66 ys, 384 males) compared with 749 age- and sex-matched control subjects. We developed a multiplex PCR-oligonucledotide extension approach by GenomeLab platform to detect SNPs. The SNPs selection was performed on their putative function and frequency in PPARγ gene. Only one SNP, the common polymorphism C161T, was investigated by RFLP analysis. The SNPs were used for haplotype reconstruction by using Phase v2.1 software. Among the 25 selected SNPs, 21 passed the quality assessment for being analyzed. All the frequency distributions are in Hardy-Weinberg equilibrium. Among the investigated PPARγ variants, only three (rs3112394, rs11715073,

rs10510417) showed a statistically significant difference in genotype distribution between controls and ACS patients. At the logistic regression analysis, one (rs3112394 G/A) out of 3 SNPs represented a significant increased risk factor for ACS (OR:1.75, 95% CI: 1.12-2.73, p=0.015) but, when the analysis was adjusted for traditional cardiovascular risk factors (gender, age, hypertension, smoking habit, diabetes, dyslipidemia, family history of ACS), the rs3112394 SNP did not remain an independent risk factor. We evaluated the pairwaise LD between all pairs of SNPs markers of the gene; among SNPs there were polymorphisms with D' values greater than 0.95 and r2 value greater than 0.86. Our data suggest that these sequence variants within PPARγ gene are in strong linkage disequilibrium; therefore only 15 SNPs were used to perform haplotype analysis. No difference in haplotype distribution between patients and controls, was found. In conclusion, our data on a large population of ACS patients indicate that PPARγ gene does not represent a susceptibility factor for ACS.

C110

THROMBOCYTOPENIA IN PATIENTS WITH AN ACUTE CORONARY SYNDROME. THE GRACE REGISTRY

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Objective. We examined the incidence of thrombocytopenia after hospital admission, associated patient and treatment characteristics and outcomes in patients enrolled in the Global Registry of Acute Coronary Events (GRACE). *Background.* Heparin (unfractionated or low-molecular weight) and glycoprotein IIb/IIIa inhibition have become standards of care for treatment of acute coronary syndromes (ACS). Both therapies can be associated with an immune-mediated thrombocytopenia of clinical importance. The prevalence of thrombocytopenia in patients with ACS in general, and specifically related to these therapies, and the associated outcomes have little study outside of clinical trials. *Methods.* Patients were stratified into four groups: those with suspected heparin-induced thrombocytopenia (HIT), those with glycoprotein IIb/IIIa inhibitor-associated thrombocytopenia (GAT), those with *other* thrombocytopenia (not diagnosed as HIT or associated with glycoprotein inhibitors), and those with no thrombocytopenia. *Results.* Between June 2000 and September 2007, 52,647 patients with ACS and information on thrombocytopenia were enrolled in GRACE. Of these, 152 (0.3%) were reported to develop clinically recognized HIT, 324 (0.6%) developed GAT, and 368 (0.7%) developed other thrombocytopenia. Patients with HIT, GAT, or other thrombocytopenia were significantly more likely to die in hospital *vs.* those without: adjusted odds ratios (95% confidence intervals) 1.94 (1.07-3.53), 3.45 (2.35-5.05), and 2.83 (1.97-4.06), respectively. They were also more likely to suffer major bleeding, (re)infarction, or stroke. *Conclusions.* Less than 2% of ACS patients in this large, multinational registry were reported to develop thrombocytopenia. Regardless of whether patients had clinically recognized HIT, GAT, or other thrombocytopenia, all three groups had significantly higher rates of major bleeding, recurrent infarction, stroke, and death.

C111

PULMONARY FUNCTION, C-REACTIVE PROTEIN AND CARDIOVASCULAR RISK IN THE MOLI-SANI POPULATION

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Background. The relation between cardiovascular disease and pulmonary function, in particular in obstructive disorders, is complex and increasingly appreciated. Inflammation could be an important bridge between these two pathological entities. *Aim.* To evaluate the association of pulmonary function, the inflammatory marker C-reactive protein (CRP), and cardiovascular risk estimate. *Methods.* The Moli-sani Project is an on-going cohort study of men and women aged ≥35, randomly recruited from a Southern Italy general population. Until march 2008, 15,339 subjects had been enrolled. We measured Forced Vital Capacity (FVC) and Forced Expiratory Volume in the first second (FEV1) (VMAX, Sensor Medics, Milan, Italy) and predicted values were derived from European Respiratory Society guidelines (ERS93). High sensitivity CRP levels were measured by immunoturbidimetric method (IL, Milan, Italy) on fresh plasma samples. Blood pressure was measured according to European Guidelines with an automatic device (Omron-705IT). Cardiovascular risk was calculated applying the equation of the Cuore Project.Subjects with CRP levels ≥10 mg/L (n=622), history of cardiovascular (n=743) or pulmonary disease (n=1,252) or malignancy (n=476) and with insufficient technical quality spirometric tests (n=4,892) were excluded. *Results.* Finally, 4,312 men and 4,373 women aged 52.5±10.8 and 51.8±10.4 years respectively were analyzed. CRP levels were inversely associated with Forced Vital Capacity (FVC) and Forced Expiratory Volume in the first second (FEV1) in men (p<0.0001 for both) and in women (p<0.0001 for both, Table 1). These associations were independent of age, height, CVD risk score, obesity (BMI or Waist to Hip ratio), hypertension, diabetes, metabolic syndrome, social status, physical activity, smoking habits and work exposures. When FVC percent predicted and FEV1 percent predicted were used, the magnitude of associations was similar. Only in men CRP was also associated with FEV1/FVC ratio (p=0.003 in multivariate analyses). Decreased pulmonary function was associated with increased cardiovascular risk score both in men and women (p<0.0001 for FVC and FEV1 in men, p<0.0006 for both in women) in multivariate analysis (Table 1). *Conclusions.* Among participants in the Moli-sani study, lower pulmonary function was associated with systemic inflammation (as measured by increased CRP levels), and increased risk of CVD. These results indicate that further research of the relation between pulmonary function, systemic inflammation and CVD is warranted.

Table 1.

	CVD Cuore risk score					*hs_CRP (mg/L)*				
	0-4	*5-19*	*> 20*	*p*	*R2*	*0-0.9*	*1-2.9*	*> 3*	*p*	*R2*
MEN										
FVC (L)	4.88	4.82	4.72	<0.0001	0.6%	4.91	4.79	4.71	<0.0001	2%
FEV1 (L)	3.48	3.42	3.35	<0.0001	0.7%	3.51	3.41	3.34	<0.0001	2.8%
FEV1_FVC	76.82	76.52	75.95	<0.0001	0.8%	76.63	76.65	57.02	0.003	0.3%
WOMEN										
FVC (L)	3.50	3.43	3.53	<0.0001	0.2%	3.57	3.49	3.40	<0.0001	1.1%
FEV1 (L)	2.69	2.66	2.74	0.0006	0.1%	2.76	2.69	2.63	<0.0001	0.9%
FEV1_FVC	77.96	77.94	77.58	0.02	0.1%	77.86	77.78	77.83	-	-

p and R2 for Multivariate analyses

C112

INFLUENCE OF SELECTIVITY OF BETA-BLOCKERS ON VASCULAR EVENTS IN PATIENTS WITH HEART FAILURE OR ACUTE CORONARY SYNDROME: A SYSTEMATIC REVIEW.

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Background. Beta-blockers are widely used in patients with acute coronary syndrome (ACS) or heart failure (HF). A large study in HF patients suggested that carvedilol, a non-selective beta-blocker, is more effective in reducing vascular events than the selective beta-blocker metoprolol. In addition, selective beta-blockers may have a lesser effect on the prothrombotic state in these patients. We conducted a systematic review to assess the efficacy of selective and non-selective beta-blockers in affecting vascular events in ACS or HF patients. *Methods*. Medline, EMBASE and Cochrane Library (1981 to March 2007) and selected reference lists were searched. Randomized trials comparing selective or non-selective beta-blockers with placebo, or directly comparing the two beta-blockers were selected. Reports were not restricted to English language. Studies had a minimum treatment period of three months and had total mortality or vascular events as their primary or secondary outcome. Data abstraction, checking, and quality assessment were completed in duplicate. *Results.* Of 33 included studies, 28 compared beta-blocker with placebo (30,889 patients) and 5 directly compared beta-blockers (3,733 patients). In ACS patients, selective beta-blockers in placebo controlled trials had no statistically significant effect on total mortality (RR 0.82, 95% confidence interval 0.67-1.01) or vascular events (RR 0.68, 0.42-1.11). Non-selective beta-blockers were associated with a significant decrease in total mortality (RR 0.73, 0.64-0.82), and vascular events (RR 0.71, 0.59-0.84). In HF patients, selective and non-selective beta-blockers reduced total mortality, while only non-selective beta-blockers decreased vascular events (RR 0.80, 0.64-1.00). One study directly compared different beta-blockers in ACS, with no clear differences, while in HF non-selective beta-blockers significantly decreased mortality (RR 0.86, 0.78-0.94). *Conclusions.* Non-selective beta-blockers seem more effective than selective compounds in preventing all cause mortality and vascular events in patients with ACS, and to a lesser extent, in HF. These results should be interpreted with caution, since they are partially based on indirect comparison between different trials. therefore, the potentially different antithrombotic effects of beta-blockers require further study.

Hemostasis and Vascular Biology: Basic Aspects

C113

GLYCOPROTEINS (GP)IB-IX-V, GPVI AND INTEGRIN ALPHA2BETA1 DEPENDENT CALCIUM SIGNALS COOPERATIVELY REGULATE PLATELET ADHESION TO COLLAGEN UNDER FLOW

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We have investigated the calcium signaling relationship between GPIb-IX-V,GPVI and $\alpha 2\beta 1$ in regulating platelet adhesion to collagen in the presence of WVF domains under flow conditions. Platelets were labeled with FLUO3-AM and perfused onto a surface of fibrillar type I collagen at the shear rates of 600 and 3000 s(-1). We analyzed concurrently platelet adhesion, translocation and calcium transients in single platelets interacting with the surface using a videoimaging method. Platelet adhesion and platelet activation at 600 s(-1) were similar in the absence or presence of VWF domains. Blockade of $\alpha 2\beta 1$ or GPVI with monoclonal antibodies caused a 50% inhibition of adherent platelets and 20-30% inhibition of activated platelets. The concomitant addition of both antibody inhibited platelet adhesion by >90%. Both $\alpha 2\beta 1$ and GPVI partecipate in the initial stage of platelet adhesion and generate intracellular calcium signals. At high shear rate platelet adhesion and activation were markedly diminished compared to the low shear rate, but upon the addition of VWF domain we observed a 4-5 times-enhancement in adhesion and activation. Calcium elevations in single activated platelets demonstrated a series of calcium transients consisting of short lasting peaks and longer sustained waves reaching an intracellular calcium concentration as high as 2-3 μM. Blockade of GP Ib-IX-V markedly inhibited both platelet adhesion and activation (90%). The blockade of $\alpha 2\beta 1$ markedly reduced the number of adhering platelets and most of the platelets were translocating ones (>90%). Blockade of GPVI greatly decreased the number of adhering platelets and the percentage of translocating platelets was about 15%, a figure similar to the one observed in the absence of blocking antibodies; calcium transients consisted of short lasting and rare long lasting waves reaching a concentration lower than 1.5-2 μM. These results suggest that $\alpha 2\beta 1$ plays a pivotal role in platelet arrest following platelet tethering through A1 domain-GPIb-IX-V interaction and that amplification and temporal modification of calcium signals are obtained by the concerted action of the two receptors subsequently reinforced by the activation through GPVI. We therefore suggest that the adhesion potential of platelets results from the increase and duration of intracellular calcium triggered by the different ligand -receptor interactions varying with different shear conditions.

C114

TAFI-DEPENDENT INHIBITION OF FIBRINOLYSIS BY PLATELETS EVALUATED IN WHOLE BLOOD BY THROMBOELASTOGRAPHY

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Platelet-rich thrombi are known to be resistant to fibrinolysis, a phenomenon generally attributed to the release of platelet PAI-1. TAFI is a plasma proenzyme that, upon activation by thrombin or plasmin, is converted to a carboxypeptidase (TAFIa) that inhibits plasminogen activation on the fibrin surface. In view of the role of platelets in thrombin formation, we investigated the contribution of TAFI to the antifibrinolytic effect of platelets in whole blood. Platelet-poor (PWB, <40,000/uL) and platelet-rich (RWB, >400,000/uL) blood samples were obtained from normal blood (NWB, ca. 200,000/uL) by removal or addition of autologous platelets. Clot lysis (time to 50% lysis) was monitored by thromboelastography (Haemoscope), using recalcified human blood supplemented with t-PA (100 ng/mL) and tissue factor (1/1000 diluted Recombiplastin). Platelets, besides the obvious effect on clot time and clot firmness, dramatically inhibited fibrinolysis, lysis time being 12.2±4.1, 32.3±5.8, and 48.0±8.2 min in PWB, NWB, and RWB, respectively. On addition of the TAFIa inhibitor, PTCI, lysis time shortened proportionally to platelet number (by 7.2, 44.6, and 58.3%, in PWB, NWB, and RWB, respectively), indicating that the greater the platelet concentration the greater the TAFI-mediated inhibition of fibrinolysis.

Similar results were obtained with an anti-TAFI monoclonal Ab (MAB) that prevents the activation of TAFI by thrombin (but not by plasmin), underscoring the pivotal role of thrombin-induced TAFI activation. Addition of the glycoprotein IIb/IIIa inhibitor, Abciximab, attenuated the differences in lysis time between PWB and RWB only moderately (by about 20%), and had little effect on the shortening of lysis time by PTCI. Moreover, a marked TAFI-mediated inhibition of fibrinolysis was seen when PWB was enriched with platelet membranes, indicating that a major part of the antifibrinolytic activity of platelets is independent on platelet aggregation, clot retraction and PAI-1 release. The assay of thrombin and TAFIa in blood confirmed that the generation of these enzymes was stimulated by platelets in a concentration-dependent fashion. Our data indicate that TAFI activation is one the major mechanisms by which platelets make clots resistant to fibrinolysis and suggest, on the one hand, the potential of TAFI inhibitors as antithrombotic agents, and, on the other hand, the suitability of thromboelastography to investigate blood fibrinolytic potential.

C115

LPS-STIMULATED MONOCYTES INHIBIT FIBRINOLYSIS THROUGH A TISSUE FACTOR (TF)- AND TAFI-MEDIATED MECHANISM AND ATTENUATE THE PROFIBRINOLYTIC ACTIVITY OF HEPARINS

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TAFI is the precursor of a carboxypeptidase (TAFIa) that inhibits fibrinolysis by removing the plasminogen and t-PA binding sites from fibrin, thereby reducing plasmin formation. Because thrombin is the main activator of TAFI, heightened clotting activation is expected to impair fibrinolysis. This view, however, is challenged by the observation that soluble tissue factor (TF) preparations did not influence fibrinolysis when added to plasma. In order to assess whether cell-associated TF is capable of inhibiting fibrinolysis we tested TF-expressing monocytes in a plasma clot lysis model of physiological relevance. LPS-stimulated (TF^+) and unstimulated (TF^-) human mononuclear cells (MNC, 3×10^6/mL) were added to wells of a microtiter plate along with plasma, t-PA (20 ng/mL) and calcium chloride (20 mM), and clot time and fibrinolysis time were measured spectrophotometrically. In normal plasma, TF^+MNC shortened clot time but had no effect on fibrinolysis time. When factor XII-deficient (FXII-d) or contact-inhibited plasma was used, TF^+MNC, besides accelerating clot formation, prolonged fibrinolysis time as compared to TF^-MNC (64±11 *vs.* 39±8 min). Testing mixtures of stimulated and unstimulated MNC, a significant prolongation of lysis time was observed with as little as 3% TF^+MNC. Fibrinolysis was impaired also when FXII-d clots were generated onto adherent TF^+monocytes. The antifibrinolytic effect of TF^+cells was abolished by an anti-TF antibody, by an antibody preventing thrombin-induced (but not plasmin-induced) TAFI activation, and by a TAFIa inhibitor (PTCI). Assay of thrombin and TAFIa in FXII-d plasma revealed that TF^+MNC shortened the lag phase and enhanced both the generation rate and peak concentration of the enzymes. In normal plasma, instead, TF^+MNC produced only a shortening of the lag phase without changes in total thrombin and TAFIa formation, which explains why these cells did not inhibit fibrinolysis under this condition. Finally, the profibrinolytic effect of unfractionated heparin and enoxaparin was markedly lower (~50%) in the presence of TF^+MNC than in the presence of a thromboplastin preparation displaying identical TF activity. In conclusion, LPS-stimulated monocytes, either adherent or in suspension, inhibit fibrinolysis through a TF-mediated enhancement of TAFI activation, and make clots resistant to the profibrinolytic activity of heparins. These findings may have both pathophysiological and clinical relevance.

C116

COAGULATION FACTOR VII LEVELS ARE MODULATED BY THE BIOLOGICAL CLOCK

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Introduction. Daily fluctuations in the levels of a number of clotting factors have been reported in humans, which might contribute to temporal variations in the frequency of cardiovascular and haemorrhagic events. We characterized the temporal variations of factor VII (FVII) levels, the protease triggering blood coagulation. The human and mouse FVII gene promoters contain E-boxes, putative DNA-binding sites for CLOCK:BMAL1 and NPAS2:BMAL1 heterodimers and hallmarks of circadian regulation. *Methods.* Temporal variations of FVII levels were investigated in humans and in wild-type and transgenic C57BL/6J mice (4 time points/day). Mice were subjected to different lighting or feeding conditions. Plasma FVII activity and mRNA were evaluated by fluorogenic assays and real-time PCR. ResuHUMANS- FVII activity levels in 13 healthy men were significantly higher in the morning (+6%). *Mouse Model.* a) Circadian nature- FVII activity as well as liver mRNA levels in wild-type mice showed significant daily variations with a peak of activity (+16%) at the light/dark transition. Peaks of mRNA levels in liver preceded those in plasma, to indicate a transcriptional regulation. Rhythms were maintained in constant darkness, to indicate a circadian control. b) Clock-/-; Npas2-/- double mutant mice- Rhythms of FVII expression in liver of these mice were abolished, to indicate a role for CLOCK and NPAS2 circadian transcription factors. c) Clockdelta19/delta19 and Npas2-/- mice- Rhythms of FVII expression in these mice, expressing functionally defective heterodimers, were maintained, to demonstrate *in vivo* the overlapping role of CLOCK and NPAS2 in the control of FVII transcription. d) Transcriptional control- Luciferase reporter assays with the 5'regulatory region (1kb) of mouse FVII gene showed a 4-fold transactivation by both circadian transcription factor BMAL1/CLOCK or BMAL1/NPAS2 heterodimers. e) Influence of light- In conditions mimicking summer or winter photoperiods, FVII activity showed daily rhythms, with mean daily FVII levels significantly reduced in summer-like photoperiods. In addition, exposition to chronic jet-lag, mimicked through continuous abrupt shifts in the lighting schedule, suppressed FVII rhythms and dampened FVII levels. f) Influence of feeding- Restricted feeding and fasting abolished FVII activity rhythms. *Conclusions.* Our findings provide novel insights into the modulation of FVII activity levels by the biological clock, which might have implication in human pathophysiology.

C117

THE FIBRINOGEN ELONGATED GAMMA-CHAIN INHIBITS THROMBIN-INDUCED PLATELET RESPONSE, HINDERING THE INTERACTION WITH DIFFERENT RECEPTORS

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Objective. The expression of the elongated fibrinogen γ chain, termed gamma', derived from alternative splicing of mRNA, is inversely correlated with the risk of venous thromboembolism. The inserted sequence of 20 amino acids interacts with the anion binding exosite (ABE)-II of thrombin. This study investigated whether and how γ binding to ABE-II affects thrombin interaction with its platelet receptors, i.e. glycoprotein Ibα (GpIb-alfa), protease-activated receptor (PAR)-1 and PAR–4. *Methods and Results.* Both synthetic γ peptide and fibrinogen fragment D containing one elongated γ and one normal gamma chain (Fragment D*), inhibited thrombin-induced platelet aggregation, up to 70%, with IC50 values of 42±3.5 μM and 0.47±0.03 μM, respectively. Likewise, Fragment D* and the synthetic gamma' peptide, competitively inhibited the thrombin binding to GpIb-alpha with a Ki ≈40 μM and ≈0.5 μM, respectively. Both these γ chain-containing ligands non-competitively inhibited the thrombin cleavage of a synthetic PAR-1 peptide, of native PAR-1 molecules on intact platelets, and of the synthetic chromogenic peptide D-Phe-Pip-Arg-pNA, while PAR-4 cleavage was unaffected. *Conclusions.* Fibrinogen γ chain binds with high affinity to thrombin and inhibits with cooperative mechanisms the platelet response to thrombin. Thus, its variations *in vivo* may affect both haemostasis and thrombosis in arterial circulation.

C118

NA+/H+ EXCHANGER 1- AND AQUAPORIN-1-DEPENDENT HYPEROSMOLARY CHANGES DECREASE NITRIC OXIDE PRODUCTION AND INDUCE VCAM-1 EXPRESSION IN ENDOTHELIAL CELLS EXPOSED TO HIGH GLUCOSE

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Introduction. Previous research has suggested that high glucose is per se sufficient to induce endothelial dysfunction in terms of decreased nitric oxide (NO) availability and increased endothelial vascular cell adhesion molecule (VCAM)-1 expression. We here investigated: 1) the contribution of hyperosmolarity in the regulation of endothelial nitric oxide synthase and VCAM-1 expression in human endothelial cells exposed to high glucose; 2) the involvement of membrane associated water channels aquaporin-1 (AQP1) and Na^+/H^+ exchange 1 in the regulation of VCAM-1 and nitric oxide by hyperosmolarity. *Methods.* Human aortic endothelial cells (HAEC) were exposed to 5.5 mmol/L glucose (normoglycemia, basal), high glucose (25 and 45 mmol/L, HG), and a control with the same hyperosmolarity as high glucose (mannitol 25 and 45 mmol/L, HM), in the presence or absence of Na^+/H^+ exchange 1 cariporide (1 micromol/L) or AQP1- inihibitor dimethylsulfoxide (1% DMSO). Analyses of VCAM-1 expression, of the water channel AQP1, of the active phosphorylated form of endothelial nitric oxide synthase (Ser1146-eNOS), and adhesion of U937 monocytoid cells to the endothelium were performed after either short-term (1-3 days) or long-term (1-2 weeks) exposures. NO production was measured by the Griess assay. *Results*. Both short- and long-term exposure to high glucose and the hyperosmolar control decreased the expression of Ser1146-eNOS and, in parallel, increased total VCAM-1 protein at immunoblotting (Table 1). This last however occurred without induction of VCAM-1 surface expression and monocytoid cell adhesion. After 24 hours incubation with hyperosmolar stimuli, there was a significant enhancement of AQP1 expression in a concentration-dependent manner. The protein kinase C (PKC) inihibitor calphostin C and the PKCbeta isoform inhibitor LY379196 (LY) blunted both high glucose- and high mannitol-induced VCAM-1, while increasing the expression of Ser1146-eNOS. 1% DMSO and cariporide inhibited hyperosmolarity-induced APQ-1 and total VCAM-1 expressions, while increasing nitrite levels and Ser1146-eNOS expression. *Conclusions. H*igh glucose decreases eNOS activation and increases total VCAM-1 expression in HAEC through a hyperosmolar mechanism. These effects are mediated by activation of membrane associated water channels aquaporin-1 (AQP1) and Na^+/H^+ exchange 1 and PKCbeta-mediated intracellular signaling pathway.

Table 1.

	HG, 25 mmol/L	*HM, 25 mmol/L*	*HG, 25 mmol/L + LY*	*HM, 25 mmol/L+ LY*	*HG, 25 mmol/L + cariporide*	*HM, 25 mmol/L + cariporide*	*HG, 25 mmol/L + 1%DMSO*	*HM, 25 mmol/L+ 1%DMSO*
Total VCAM (% control)	275±5*	200±10*	175±8**	163±7**	140±10**	130±13**	233±10**	180±7**
Nitrite (% control)	63±1*	53±3*	79±2**	84±1**	110±5**	122±12**	115±13**	132±10**
Ser^{1146}-eNOS (% control)	27±5*	32±4*	109±5**	73±4**	67±7**	83±10**	118±20**	127±16**

**, p<.05 vs untreated; **, p<.05 vs HG or HM*

C119

VASCULAR FIBROSIS IN STROKE–PRONE RATS (SHRSP) IS PREVENTED BY DRUGS ACTIVE ON INFLAMMATORY PATHWAYS

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Background and Aims. Salt-loading accelerates hypertension, proteinuria and the development of cerebrovascular lesions in SHRSP. We investigated the effects of salt-loading on vascular fibrosis and collagen deposition in the thoracic aorta of SHRSP. The effects of drugs acting on different aspects of the inflammatory pathways, rosuvastatin (RSV) and aspirin (ASA), were evaluated. *Methods.* SHRSP,fed a high-salt diet, were daily treated with vehicle or RSV 10 mg/kg or ASA 60 mg/kg. A group of SHRSP fed standard chow represents the basal group. Rats were weekly housed in metabolic cages for urine collection and proteinuria determination. Analyses of excreted proteins were performed by 1-dimensional electrophoresis. After proteinuria reached the value of 100 mg/day in vehicle-treated group, all the rats underwent daily magnetic resonance imaging interrogations until brain damage was identified. At appearance of brain damage in the vehicle-treated group, all the animals belonging to the different experimental groups were euthanized simultaneously and the aortas collected for examinations. *Results.* The delay, between the start of dietary treatment and the time when proteinuria reached >100 mg/day, was 4.75±0.45 weeks in vehicle treated rats but it increased to 6.25±0.41 in ASA group and 9.13±0.67 ($p<0.01$ vs vehicle) in RSV group. Accumulation of inflammatory markers were observed in urine of vehicle-treated rats after 4 weeks of dietary treatments, whereas ASA and RSV treatments delayed the appearance of inflammatory markers after 7 and 9 weeks of treatment. While the aorta wall thickness was significantly increased in the vehicle group respect the basal group ($p<0.005$), the cross-sectional area of the media remained unchanged. Furthermore, as result of salt-loading, interstitial ($p<0.005$) and fibrillar ($p<0.01$) collagen increased. The wall thickness decreased in both RSV- ($p<0.001$ vs vehicle) and ASA -treated rats ($p<0.05$ vs vehicle). The drug treatments were effective also in reducing interstitial ($p<0.005$ and $p<0.001$ vs vehicle for RSV and ASA, respectively) and fibrillar ($p<0.01$ and $p< 0.001$ vs vehicle for RSV and ASA, respectively) collagen accumulation. *Conclusions.* Rosuvastatin and aspirin treatments of salt-loaded SHRSP prevented inward eutrophic arterial remodeling, the increase of wall thickness and the accumulation of collagen. These protective effetcs are associated to a delay of proteinuria and systemic inflammation development.

Platelets: Qualitative and Quantitative Alterations II

C120

THE D1424N AND R1933X MUTATIONS OF MYH9 CAUSE THROMBOCYTOPENIA THROUGH LOSS OF REGULATION OF PROPLATELET FORMATION BY TYPE I COLLAGEN

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Background. MYH9-related disease (MYH9-RD) is an inherited macrothrombocytopenia caused by mutations in the gene for the heavy chain of myosin IIA. The pathogenethic mechanisms of thrombocytopenia of these patients are largely unknown. It was recently shown that myosin IIA is a negative regulator of proplatelet formation (PPF) by murine megakaryocytes (Mks). Other studies demonstrated that Mk adhesion to type I collagen strongly inhibits PPF by both murine and human Mks. We recently found that suppression of PPF by type I collagen is mediated by myosin IIA (Balduini A *et al.*, submitted). Since in bone marrow type I collagen is selectively located in the osteoblastic niche, this inhibitory pathway could contribute to prevent PPF until Mks move to the vascular niche, where platelet release is permitted by the absence of collagen I. Purpose. To test the hypothesis that MYH9 mutations affect PPF by human Mks. *Methods.* We studied 2 patients carrying the D1424N or the R1933X mutation and 5 healthy individuals. Mks were cultured from peripheral blood mononuclear cells. For each subject, at day 12 of culture, 50% of Mks was plated on type I collagen-coated coverslips, and 50% was plated on bovine serum albumin (BSA) as a control substrate. The percentage of PPF (%PPF), as well as cell morphology, were assessed by both phase-contrast and fluorescence microscopy after 24 hours. Results. No differences in Mks recovery or maturation were observed at day 12 between patients and controls. In control subjects, the %PPF of Mks plated on BSA was variable from 2.0 to 7.0, while no proplatelets were ever identified when the same cells were plated on type I collagen. Thus, we confirmed that collagen I strongly inhibits PPF by normal human Mks. On the contrary, in the MYH9-RD patient with D1424N, the %PPF in Mks plated on collagen I was comparable to that of Mks on BSA (3.6 *vs.* 2.4, respectively). Similar results were obtained in the patient with R1933X. Moreover, on both substrates, proplatelets formed by MYH9-RD Mks had altered morphology and alpha-tubulin organization. Conclusions. The D1424N and R1933X mutations cause the loss of inhibition of PPF exerted by type I collagen on human Mks. This mechanism could determine *in vivo* a premature, ectopic platelet release in the osteoblastic niche, thus resulting in the ineffective thrombopoiesis of MYH9-RD. The observed defects of alpha-tubulin re-organization in proplatelets could further contribute to the pathogenesis of macrothrombocytopenia.

C121

AN UPDATE FROM THE ITALIAN REGISTRY FOR MYH9-RELATED DISEASE: FROM PROGNOSTIC ASSESSMENT TO NOVEL THERAPEUTIC APPROACHES

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On behalf of the Investigators of The Italian Registry for MYH9-related disease

MYH9-related disease (MYH9-RD) is an autosomal-dominant thrombocytopenia characterized by the association with nephropathy, sensorineural deafness, and/or cataracts. The Italian Registry for MYH9-RD (http://www.registromyh9.org/) was created in 2006 to promote the study of this disorder. To date, 142 Italian centers join the Registry. By the screening of more than 400 macrothrombocytopenic subjects, the database included 148 patients belonging to 82 pedigrees, thus suggesting that MYH9-RD is less rare than previously thought. Based on this case series, novel clinical aspects of the disorder have been identified and statistically significant genotype-phenotype correlations have been described for the most frequent mutations (Hum Mutat 2008;29:409). Analysis of Registry patients suggested that MYH9 mutations could result also in a liver damage. More detailed studies on this matter are ongoing. Moreover, a better characterization of the disorder allowed us to propose novel therapeutic options. To date, no treatment is available for thrombocytopenia of MYH9-RD and patients receive platelet transfusions to stop bleeding episodes or before undergoing invasive procedures. Recent clinical trials showed that the thrombopoietin analogue Eltrombopag increases platelet count of patients with ITP or HCV-related thrombocytopenia. Since *in vitro* studies demonstrated that thrombopoietin is able to stimulate differentiation and maturation of megakaryocytes in MYH9-RD, we hypothesized that Eltrombopag could increase platelet count also in this condition. The Registry is now starting a phase II clinical trial to test the efficacy and safety of this drug in MYH9-RD. The design of the study will be presented and discussed. The most severe complication of MYH9-RD is a progressive nephropathy that usually evolves to end-stage renal failure. We treated for the first time 4 patients with MYH9-RD and nephropathy by pharmacological blockade of the renin-angiotensin system (RAS). In all cases we observed a normalization of proteinuria, which, in the two subjects with a longer follow-up, was still maintained after 68 and 40 months, respectively, without worsening of kidney function. No spontaneous improvement of MYH9 nephropathy was ever observed in the 26 untreated patients we have followed-up so far. We suggest that this therapeutic approach, together with an early recognition of MYH9-RD patients, could actually modify the natural history of the disease. The Registry is promoting a larger clinical trial to better define the advantages of RAS blockade in MYH9-RD.

C122

MORE PLATELETS, LESS THROMBOSIS: THE PARADOX OF ESSENTIAL THROMBOCYTHEMIA

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To investigate the role of thrombocytosis, alone or in combination with standard (age, previous cardiovascular events) and novel (leukocytosis, JAK2V617F mutational status) risk factors, in the cardiovascular events of essential thrombocythemia (ET) we analyzed a cohort of 1,063 patients prospectively followed in three Italian institutions. There were 709 females and 354 males and median age at diagnosis was 55 years (range 8 to 93 years). Median platelet and leukocyte counts were 806 (376-3,000) and 8.8 (3.3-35)×10^9/L, respectively. JAK2V617F mutation was found in 465 of 860 patients valuable (51%). During up to 38 years of follow up (median 4.8 years), 118 major thrombosis (2.3% patients/year) were diagnosed and included 48 ischemic strokes or TIA, 25 myocardial infarction, 11 peripheral arterial thrombosis and 34 venous thromboembolism. Severe bleeding episodes (gastrointestinal in 80%) were 39 (0.76% patients/year). Multivariable analysis (including center, gender, standard risk factors, hemoglobin level, leukocyte and platelet values, antiplatelet drugs and chemotherapy) confirmed that age and previous thrombosis were independent factors for occlusive events (HR=1.7, 95% CI=1.1-2.6, p=0.01). None of the variables influenced the risk for major bleeding. Platelet count at diagnosis above 1,000×10^9/L was significantly associated with a lower rate of thrombosis. Compared to a reference platelet count below 650×10^9/L, multivariable risk estimates in patients presenting with platelet counts ranging from 650 to 1,000×10^9/L or above 1,000×10^9/L were 0.6 (95% CI=0.4-1.0, p=0.1) and 0.5 (95% CI=0.3-0.8, p=0.01), respectively. Thrombocytosis above 1,000 ×10^9/L, combined with leukocytes less than 11×10^9/L, individuates a *low-risk* category with a rate of thrombosis of 1.59% patients/year. On the contrary, the highest risk category (thrombosis rate, 2.95% patients/year, RR 2.43, p=0.017) was constituted of patients with leukocytosis (>11×10^9/L), lower platelet count (<1,000×10^9/L) and a JAK2V617F mutated genotype in most cases (77% *vs.* 26% in the low-risk group). These data challenge the theory that elevated platelet count increases thrombosis risk in ET, and suggest that global myeloproliferation rather than thrombocytosis alone should be the target of therapy.

C123

HETEROZYGOUS ALA156VAL MUTATION IN THE GPIB ALPHA (HETEROZYGOUS BERNARD-SOLIER SYNDROME BOLZANO TYPE) INDUCES MACROTHROMBOCYTOPENIA BY HAMPERING PROPLATELET FORMATION

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Background. Pathogenesis of macrothrombocytopenia in Bernard-Soulier syndrome (BSS) is still obscure, although the normal amount of bone

marrow megakaryocytes (MKs) and the normal platelet survival point towards defective platelet formation as the causative defect. *Patients and Methods*. We analyzed *in vitro* megakaryocyte differentiation and maturation, as well as proplatelet formation (PPF) in 4 patients heterozygous for the Ala156Val mutation in the GPIb alpha (Bolzano mutation). All of them had mild to moderate thrombocytopenia and enlarged platelets. MKs were differentiated from cord blood $CD34^+$ cells (1 patient) and peripheral blood mononuclear cells (3 patients) for 12 days. Mature MKs were grown in suspension or plated onto glass coverslips coated with collagen I, III or IV, fibrinogen (FNG), or von Willebrand Factor (VWF). MK differentiation-maturation and PPF were evaluated by phase contrast and fluorescence microscopy upon cell staining with anti-tubulin and CD41 antibodies. Controls were analyzed in parallel with each patient sample. *Results.* The MK differentiation-maturation from peripheral and cord blood progenitors was comparable to controls, while both quantitative and qualitative defects of PPF were observed. In suspension, PPF was observed in 7% and 15% of MKs derived from patient and control cord blood, respectively. In patients as in controls, Mk adhesion to FNG or VWF reduced PPF, while adhesion to type I collagen, but not to type III and IV, totally inhibited PPF. Similarly to cord blood experiments, PPF by MKs in suspension derived from patients' peripheral blood was 50% of controls, and the inhibitory effect of FBN and VWF on PPF was similar in patients and controls. In all experimental conditions and in all patients, proplatelets extended by MKs had a defective alpha-tubulin organization in the peripheral microtubule coil, which was evident both at the level of proplatelet tips and bodies. Impaired separation of tips from the proplatelet body was also observed. Moreover, proplatelet tips showed an increased size that was consistent with the increased diameters of peripheral blood platelets measured in the same patients. *Conclusions.* MKs from patients carrying the Bolzano mutation present both quantitative and qualitative abnormalities of *in vitro* PPF. This indicates a key role for GPIb in PPF and confirms the hypothesis that macrothrombocytopenia of BSS derives from defective platelet formation.

C124

CYCLIC EDTA-INDUCED PSEUDOTHROMBOCYTOPENIA (PTCP): A NEW CONDITION

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PTCP is an artifactual thrombocytopenia deriving from *in vitro* clumping of platelets (plts) in blood samples anticoagulated with strong calcium chelating agents, such as EDTA. Most frequently, EDTA changes the conformation of GPIIb/IIIa of plt surface and exposes a neoepitope recognized by natural antibodies (Abs) with agglutinating activity. PTCP has been associated with several disorders, but it can be observed also in healthy subjects. Cyclic thrombocytopenia is a rare condition characterized by periodic fluctuations in plt number leading to symptomatic bleeding at the time of plt nadirs. The pathogenesis of cyclic thrombocytopenia remains elusive, although fluctuating auto-Ab production has been observed in few cases. We describe here the first patient with cyclic PTCP. A 36-year-old woman was referred to our institution because of a fluctuating thrombocytopenia identified by automated counters in EDTA blood samples. History revealed 5 episodes of *thrombocytopenia* during the previous 6 months (plt counts from 34 to 251×10^9/L), without any bleeding tendency. All other routine laboratory tests were normal and the *patient* was completely well. Laboratory investigation during 3 different *thrombocytopenic* phases revealed that: a) plt count was normal by both contrast phase microscopy in native blood and by counters in citrated or heparinized blood; b) plasma from EDTA-anticoagulated blood of patient agglutinated plts from controls. This phenomenon was prevented by preincubation of control plts with the GRGDR peptide or mAbs against GPIIb-IIIa; c) although SDS-PAGE of patient's plts demonstrated normal content of GPIIb-IIIa, flow cytometry revealed a severely reduced binding of mAbs to these glycoproteins in EDTA samples. Thus, we concluded that the patient was affected by PTCP and that this phenomenon derived from the presence of auto-Abs recognizing GPIIb-IIIa in the presence of EDTA. When the experiments described above were performed during the remission phases of *thrombocytopenia*, the effect of patient plasma described in b) was no longer observed, and the binding of mAbs to GPIIb-IIIa by flow cytometry was within the normal range. This suggests that the anti GPIIb-IIIa auto-Abs were absent or their concentration was too low to exert any visible effect. In conclusion, cyclic EDTA-dependent PTCP is a new condition that has to be considered in the differential diagnosis for thrombocytopenic patients with no bleeding tendency and highly variable plt counts.

C125

THROMBIN GENERATION IN PLATELET RICH PLASMA OF PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV)

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Thrombosis is a major cause of morbidity and mortality in patients with ET and PV. It is therefore important to identify patients at greater risk in order to prevent these complications. Thrombin Generation (TG) assay is a promising recent method for determining hyper- or hypocoagulable states. Compared to standard coagulation tests, it reflects closely the *in vivo* hemostasis by giving a global information of the coagulation potential. No studies have explored so far the TG potential of platelets in ET and PV patients. To address this issue we studied the TG potential of 20 consecutive patients with ET [males/females: 5/15; mean age (range): 55.3 (22-77) years] and 5 with PV [males/females: 3/2; mean age (range): 55.6 (44-72) years]. For comparison 11 healthy controls were enrolled into the study. TG was assessed by the Calibrated Automated Thrombogram (CAT) in platelet rich plasma (PRP) adjusted to 150,000 platelets/µl with autologous platelet poor plasma (PPP). TG was induced by 1 pM tissue factor (TF) in the absence of phospholipids. A flow cytometric analysis was performed to measure the levels of platelet surface TF from the same subjects. The results of CAT assay showed an increase in the TG potential of PRP from ET and PV subjects compared to controls. Both ET and PV patients showed significantly ($p<0.05$) higher levels of thrombin peak (ET: 147±48 nM thrombin; PV: 160±39 nM thrombin) compared to controls (C) (115±22 nM thrombin; $p<0.05$), and significantly shorter time to peak (ET: 12.5±2.8 min; PV: 10.3±2.2 min; C: 14.5±2 min). The slope of the thrombin generation curve was significantly higher in ET (26±18 nM/min; $p<0.05$) and PV patients (34±15 nM/min; $p<0.01$) versus controls (14±5 nM/min; $p<0.01$). Only the endogenous thrombin potential (ETP) was significantly ($p<0.05$) lower in the PV group (1354±237 nM*min) compared to both ET (1,654±255 nM*min) and control groups (1,600±186 nM*min). Flow cytometry analysis showed an increase in TF antigen expression (as % positive platelets) on the surface of platelets from both ET and PV patients compared to controls. In conclusion, our data demonstrate an increased coagulation potential of PRP from patients with ET and PV compared to controls, as shown by the maximum concentration of thrombin generated and the rapid propagation and clot formation (i.e. increased slope). A contribution of platelet TF is also suggested.

C126

GENETIC ANALYSIS OF THE SLC35D3 GENE IN PATIENTS WITH INHERITED, NON-SYNDROMIC DELTA-PLATELET STORAGE POOL DEFICIENCY

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Background. Delta storage pool deficiency (delta-SPD), either syndromic (e.g., Hermansky-Pudlack Syndrome) or non-syndromic, is among the most frequent inherited platelet function disorders. It is characterized by selective deficiency of the content of delta-granules, including ADP, ATP and serotonin. The pathogenesis of the disease is unclear, although both defects in forming membranes of delta-granules and defects of active transport and storage of the granule content may be implicated. It has been recently reported that the product of the Slc35d3 gene, an orphan transporter with significant sequence homology to sugar nucleotide transporters, plays a key role in the regulation of the content of murine platelet delta granules. *Objective*. We performed a genetic analysis of the Slc35d3 gene in a cohort of patients with inherited, non-syndromic delta-SPD. Patients and Methods. 13 patients with inherited delta-SPD were selected for genetic analysis. All these patients had typical abnormalities of platelet function that are associated with delta-SPD: platelet aggregation was impaired; the median (range) platelet ADP concentration was 0.65 (0.19-1.16) nmoles/108 platelets (normal range 1.30-2.88); the median (range) ratio between platelet ATP and ADP concentrations was 6.5 (3.35-33.4) (normal range 1.55-3.42); the median (range) platelet serotonin concentration was 0.1 (0.025-0.17) nmoles/108 platelets (normal range 0.19-0.40), while the concentration of platelet fibrinogen (which is stored in the platelet alpha granules) was normal in all. Genomic DNA was extracted, and the 2 exons of the Slc35d3 gene were amplified by PCR, using 5 couples of primers. The sequence of each PCR product was analysed with the BLAST program, using the normal human Slc35d3 gene sequence as a template. *Results.* No mutations of the Slc35d3 coding region were found in our 13 patients with inherited, non-syndromic delta-SPD. *Conclusions.* Our findings on a relatively large series of patients with inherited, non-syndromic delta-SPD suggest that this platelet function disorder is unlikely associated with abnormalities of the Slc35d3 coding region.

POSTERS

Atherothrombosis

P001

LP(A): A POSSIBLE LINK WITH MIGRAINE

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Background. Migraine, a common multifactorial neurovascular disorder, has been suggested to be an independent risk factor for stroke and data from literature evidenced that elevated lipoprotein(a) [Lp(a)] concentrations represent a risk factor for stroke. Aim of our study was to evaluate the role of Lp(a) in affecting migraine, so possibly contributing to identify a biological marker of predisposition to the disease. *Materials and Methods*. Lp(a) levels have been detected in 138 migraine patients (110 females and 28 males), among which 90 with aura, and 120 healthy subjects (87 females and 25 males), comparable for age and gender. Plasma levels of Lp(a) have been determined by an ELISA method. Median value of Lp(a) was 104 (1-2110) mg/L in migraine patients and 103 (9-695) mg/L in the control group (p=0.8). A significant difference among tertiles of Lp(a) concentrations between patients and controls was found (p=0.04). In particular, a significant difference in the high tertile of Lp(a) between patients and controls was observed (p=0.001). Moreover, abnormal Lp(a) levels, defined as >300 mg/L, have been observed to influence significantly the predisposition to migraine [OR 3.4 95%CI(1.57-7.55) 0, p=0.002], after adjustment for age, gender and traditional risk factors. No difference in Lp(a) concentrations was observed between patients with aura and without aura, and no relationship was found between abnormal Lp(a) concentrations and headache intensity. *Conclusions*. The present study evidences a role for Lp(a) in affecting the risk of migraine, so providing information on a novel possible mechanism involved in the predisposition to the disease.

P002

SYMPTOMATIC PERIPHERAL ARTERIAL DISEASE AND THROMBOPHILIC RISK FACTORS

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Few data are available on thrombophilic alterations and progression of peripheral arterial disease (PAD). The aim of our study was to assess the association of thrombophilic alterations in patients with symptomatic PAD. We studied 282 patients (male/female 181/101) with PAD (Fontaine's stage: II n= 176, III n=41, and IV n=65) consecutively referred to our Unit. As control group we studied 209 apparently healthy subjects (male/female 122/87). The following thrombophilic risk factors were evaluated: platelet count, PT, aPTT, fibrinogen, D-dimer, homocysteine, Factor VIII (FVIII), lupus anticoagulant (LAC) , G20210A prothrombin, and R506Q FV Leiden mutations. Patients with PAD had higher plasma levels of homocysteine, fibrinogen, D-dimer, and FVIII in comparison with control subjects. Platelet count and aPTT were similar in the PAD and control group. LAC was more frequent in the PAD group compared with the control group. Plasma levels of homocysteine (r=0.210, p<0.001), fibrinogen (r=0.275, p<0.001), FVIII (r=0.327, p<0.001) were positively and significantly correlated to Fontaine's stage. The prevalence of LAC increased from 4.8% in the control group to 25.0% in patients with Fontaine's stage IV (p<0.001). Considering as dichotomous the following variables: homocysteine, FVIII, presence of LAC, G20210A prothrombin, and R506Q FV Leiden mutations, a significant correlation between the number of altered thrombophilic parameters and the Fontaine's stage was observed (r=0.388, p<0.001). The frequency of patients with at least two thrombophilic alterations was 5.3% in the control group, and it increased to 33.9% in patients with stage IV. Whereas, the frequency of patients without thrombophilic alteration was 69.4% in the control group, 47.3% in the stage II, 34.6% in the stage III, and 18.5% in the stage IV group (p<0.001 for trend). Altered levels of several important thrombophilic risk factors are independently associated with PAD symptoms. Moreover, the presence of several thrombophilic alterations raised the likelihood of PAD severity. The clinical significance of this association needs to be tested in prospective population-based trials.

P003

EVALUATION OF CARDIOVASCULAR RISK IN RETINAL VEIN OCCLUSION

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Background. The pathogenesis of retinal vein occlusion (RVO) is unclear, but thrombophilia and cardiovascular risk factors have been shown to play a role. The occurrence of RVO has been surmised as a predictor of a subsequent cardiovascular event. In the present study, we aimed at evaluating the relationship between RVO and either cardiovascular risk factors or thrombophilia, and at estimating the prevalence of cardiovascular events after a first episode of RVO. *Methods.* We studied 132 patients with RVO, involving both central retinal vein (CRVO, N=100) and its branches(BRVO, N=32), confirmed by fluorescein angiography. Congenital and acquired thrombophilia, and cardiovascular risk factors (hypertension, diabetes mellitus, dyslipidaemia, BMI >25, high Lp(a) levels, smoking) were evaluated. Patients were followed for vascular events after RVO. *Results.* Five (8.3%) patients younger than 50 years and four (5.5%) over 50 years had an hereditary thrombophilia, including factor V Leiden, protein C or S deficiency, prothrombin G20210A polymorphisms. Antiphospholipid antibodies, hyperhomocysteinemia, factor VIII or PAI-1 levels increase were present in 28% of patients. Hyperhomocysteinemia was more frequent in patients with BRVO than in those with CRVO (25% *vs.* 7%, p 0.005). One or more cardiovascular risk factors were found in 35 (58%) patients of the younger group, and in 66 (91%) of the older group (p<0.001). Hypertension and BMI >25 were more frequent in patients with BRVO than in those with CRVO (65% and 33%, p 0.001; 62.5% and 29%, p 0.001, respectively). Ninety-one patients (68.9%) received anticoagulant therapy for at least three months, and 28 (21.2%) antiplatelet agents. RVO improvement or solved in 71.8% of patients. Participants were followed for a mean period of 5.9±4.2 years; one patient was lost during the follow up. Vascular events after RVO occurred in 17/131 patients(12.9%), and the prevalence was significantly higher in the older than in the younger population (19.7% vs 5%, p 0.01) (odds ratio [OR] 4.67, 95% CI 1.2-17.1). A similar figure was seen in the CRVO subgroup, with a higher prevalence in older than in younger subjects (21.5% *vs.* 4%, p 0.009; OR 6.46, 95% CI 1.3-30.8), but not in patients with BRVO (9% *vs.* 15%). *Conclusions.* Besides thrombophilic conditions, cardiovascular risk factors constitute frequent findings in RVO. Moreover, patients with a first RVO are likely at risk of a subsequent systemic vascular event.

P004

DETERMINANTS OF SOLUBLE CD40 LIGAND CIRCULATING LEVELS IN PATIENTS WITH ATRIAL FIBRILLATION

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Previous studies showed elevated markers of platelet activation in patients with atrial fibrillation (AF). However it unclear if this is related to AF per se or to other mechanisms. To this purpose, we studied 20 persistent AF (11 males, 9 females, age 69±9.2 years) who underwent elective *DC* cardioversion; sCD40L (R&D Systems, Minneapolis, MN, USA) was evaluated prior to cardioversion and 1 month afterwards. One month after conversion there was no significant change in sCD40L levels compared to baseline values (5.02±1.9 *vs.*4.89±2.1 ng/mL respectively; p>0.05), despite successful DC cardioversion and maintenance of sinus rhytm . Then, we analyzed sCD40L in 269 patients (males 136, females 133, age 72.6±10.3 years) affected by AF (53 paroxismal, 34 persistent and 182 permanent). Multivariate logistic regression analysis including as independent variables age, sex, type of atrial fibrillation, statin use , anticoagulants, aspirin, previous stroke, previous myocardial infarction, hypertension, diabetes and hypercholesterolemia, identified

hypertension and diabetes as independent predictor of high levels of sCD40L (O.R.: 2.498; C.I.:1.175-5.311 respectively; p=0.01). The study shows that AF *per se* does not induce sCD40L increase and suggests that in AF patients platelet activation could depend on the coexistence of atherosclerotic risk factors, such as hypertension and diabetes.

P005

RETINAL VEIN OCCLUSION: RISK FACTORS AND TREATMENT

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Introduction. Retinal vein occlusion (RVO) etiopathogenesis and treatment remain matter of study and debate. Retinal vessels anatomy and studies on risk factors suggest that RVO may be a venous complication of atherosclerosis. METHODS. Anamnestic, clinical and haematologic data about 50 patients with RVO were collected; 31 were men (mean age at exordium 54.3, range 18-77) and 19 were female (mean age at exordium 57.5, range 29-76). Diagnosis of RVO was confirmed by fundus oculi examination and fluorangiography. Patients were treated with subcutaneous calcic Nadroparin 200 IU/kg/die for 30 days and 100 IU/kg/die during the following 60 days if diagnosis occurred in the first 30 days from onset of RVO; patients were treated with Calcic Nadroparin 100 IU/kg/die if diagnosed more than 30 days from onset. Patients were strictly monitored with hemocromocytometric exam for the associated heparin induced thrombocytopenia (HIT) risk for the first two weeks of heparin treatment. Secondary thromboprophylaxis was performed with acetylsalicylic acid (ASA) 100 mg/die, if no retinal haemorrhagic episodes occurred up to 90 days of treatment with LMWH or if diagnosed more than 90 days after the exordium of RVO. Anticoagulant oral therapy (OAT) was started in case of previous venous thromboembolism before RVO. Anti thrombotic treatment with low dose LMWH was performed at dosages of 80-100 IU/kg/die if retinal haemorrhages persist. Haemostatic parameters were analized. *Results.* Central retinal vein occlusion (CRVO) occurred in 30 patients whereas branch retinal vein occlusion (BRVO) occurred in 20 patients; 63.3% of patients with CRVO and 80% of patients with BRVO were older than 50 years. Following systemic risk factors were found: hypercolesterolemy (71.4% of patients), arterial hypertension (62%), diabetes mellitus (24%), smoke (18%). Hyperhomocisteinemia was found in 58% of patients, and 20% of patients presented altered levels of coagulation factors. No cases of Antithrombin III, Protein C and Protein S deficiency were found. The other results are reported in the allegated Table 1. ACE Del mutation (homozygous or heterozygous) was found in 77.6% of patients, whereas PAI-1 4G mutation (homozygous or heterozygous) was found in 83.7%. *Conclusions.* None of the 37 patients treated with LMWH developed HIT nor haemorrhagic comlications. Our study demonstrate safety and efficacy of LMWH treatment in RVO (21.6% of re-canalizations up to now).

Table 1.

Haemostasis-related risk factors	*Patients %*
Hyperhomocysteinemia	58
Elevated levels of PAI-1 Ag	17
Elevated levels of F VIII	14
Elevated levels of Lp(a)	12,2
Elevated levels of F XI	10
APC-R 8,3	
Factor V Leiden (heterozigousity)	8,2
F XII deficiency	8,1
Elevated levels of F IX	4,2
Prothrombin gene G20210A mutation (heterozigousity)	4,1
Antiphospholipid antibodies	4
Deficiences of AT III, Prot. C, Prot. S	0

Haemostasis-related risk factors in RVO Patients

P006

THROMBOPHILIA IN YOUNG PATIENTS WITH ACUTE CORONARY HEART DISEASE

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Thrombophilia is a prominent risk factor for venous thromboembolism. The role of thrombophilia in determining the risk of arterial thrombotic events is less well defined. We have screened for inherited and acquired thrombophilia (MTHFR C677T mutation, Factor V Leiden, Factor II G20210A mutation, antithrombin, protein C and protein S deficiencies, lupus anticoagulant, anti-cardiolipin antibodies, hyperhomocysteinemia) and conventional cardiovascular risk factors (hyperlipidaemia, arterial hypertension, cigarette smoking, impaired fasting glucose (IFG), diabetes mellitus, and overweight), 137 consecutive patients (99 M and 38 F; mean age 44.27±10.5 yrs) with a first episode of acute coronary heart disease occurred in young age (≤50 years). As many as 203 age- and sex-matched apparently healthy subjects (131 M and 72 F; mean age 42.68±6.09 yrs), from the same ethnic background, served as controls. MTHFR homozygous mutation was found in 22.1% of patients and in 16.4% of control subjects, this difference was not statistically significant. Similarly, Factor V Leiden and Factor II G20210A mutation prevalences were not statistically different between patients and controls (7.4% *vs.* 8.5%; 8.1% *vs.* 9.5%). There was no difference between patients and controls as to the protein C, S and antithrombin deficiency. Among conventional cardiovascular risk factors, arterial hypertension was found in 60/136 (44.1%) patients and in 39/200 (19.5%) controls (p<0.0001; OR:3.26; 95% CI 2.00-5.3; χ^2 test). A significant difference was also found in cigarette smoking (80.9% *vs.* 41.0%; p<0.0001; OR:6.00, CI 3.65-10.16) and diabetes mellitus (9.7% *vs.* 0.8%; p=0.002; OR:13.8, CI 1.72-111.10). There was no statistical difference in prevalence of hypercholesterolemia (46.4% *vs.* 40.5%), IFG (19.2% *vs.* 11.6%), overweight (55.6% *vs.* 53.1%) and hyperhomocysteinemia (38.8% *vs.* 39.1%). *Conclusions.* We found that conventional cardiovascular risk factors, except for high cholesterol levels, increase the risk of coronary artery disease in our young population, whereas prothrombin G20210A mutation, FV Leiden, MTHFR C677T mutation, protein C, S and antithrombin deficiencies and hyperhomocysteinemia did not increase the risk.

P007

G20210A PROTHROMBIN MUTATION IS ASSOCIATED WITH MORE SEVERE STAGES OF PERIPHERAL ARTERIAL DISEASE

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Thrombosis may play an important role in the pathogenesis of the complications of peripheral arterial disease (PAD). Genetic polymorphisms of hemostatic factors may be involved in arterial thrombosis and may contribute to PAD complications. We evaluated the presence of inherited thrombophilic abnormalities (G20210A prothrombin and R506Q FV Leiden mutations, Antithrombin, Protein C and S deficiencies) in 282 patients with symptomatic PAD (Fontaine's stage: II n=176 and III/IV n=106) consecutively referred to our Unit. As control group we studied 209 apparently healthy subjects. Patients with PAD did not differ from control subjects with regard to age, sex distribution, prevalence of hypertension, and hyperlipidemia. Diabetes and smoking habit were more frequent in the PAD group. The prevalence of G20210A prothrombin mutation was similar in PAD patients and controls (6.0% *vs.* 4.3%, p=ns), but it was significantly increased in patients with Fontaine's stage III/IV in comparison with those with stage II and with controls (10.4% vs 3.4% vs 4.3%, p=0.02, respectively). In a logistic multivariate analysis, the relative risk (OR) of patients carrying the prothrombin mutation for critical ischemia (stage III-IV) was 5.77 (95%CI: 1.54-22.72, p=0.01), after adjustment for age, sex, diabetes, and smoking habit. The prevalence of the other thrombophilic alterations were not different in the PAD group and the control group, and no difference was observed with regard to the Fontaine's stages. G20210A prothrombin mutation is associated with more severe stages of PAD. Longitudinal studies will help to clarify if the prothrombin mutation is a genetic marker which predicts an individual's predisposition for developing complications of PAD.

P008

GENE EXPRESSION PROFILING IN RAT LEFT VENTRICLE AFTER 10-WEEK MILD EXERCISE TRAINING

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Whereas physical exercise is a known protective factor against cardiovascular morbidity and mortality, the effects of mild exercise (as recommended to most adult humans for cardiovascular fitness) are less clear and the underlying molecular mechanisms still remain to be explored. To identify the gene expression changes involved in the induction of this phenotype, a genomic approach was used in an animal model known to induce cardioprotection. Rats were trained at moderate intensity on a treadmill: 25m/min, 10%incline, 1h/day, 3days/week, 10 weeks; about 60% of the maximal aerobic power. By Affymetrix technology, we investigated gene expression profile induced by exercise training in left ventricle (LV) of trained (n=10) and control (n=10) rats. This exercise protocol did not induce cardiac hypertrophy and determined decreased infarct size (p=0.02) after ischemia/reperfusion experiments. Rats were sacrificed 48 hours after the last training session, in order to identify long-lasting changes in gene expression. We observed 10 genes differentially expressed in LV of exercised animals with respect to controls and 2 gene sets associated with training. We validated by real-time PCR the upregulation of three genes: caveolin 3, beta enolase, and hypoxia inducible factor 1 alpha. Moreover, caveolin 3 protein levels resulted higher in exercised rats than in controls by immunohistochemistry and Western Blot analysis. Our data indicate that Cav3, Eno3, Cyp27a1, Egln1, Cst3 and Tnfaip1 genes as well as GABA and ARENRF2 pathways show long-lasting expression changes in rat LV as a consequence of mild exercise training associated to cardioprotection without induction of hypertrophy.

P009

PLASMA LEVELS OF BETA2-MICROGLOBULIN, A BIOMARKER OF PERIPHERAL ARTERIAL DISEASE, ARE NOT AFFECTED BY MAXIMAL TREADMILL EXERCISE IN PATIENTS WITH INTERMITTENT CLAUDICATION

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Peripheral arterial disease (PAD) is a chronic atherosclerotic disorder involving the aortic, iliac and lower limb arteries and affecting a significant fraction of the adult population worldwide, with an associated strong increase in cardiovascular morbidity and mortality. Recently, plasma levels of beta2 microglobulin (β2M) were identified as a specific biomarker of PAD. β2M levels independently correlated with the severity of disease, as assessed by the ABI or by treadmill testing, and interestingly when the levels of this biomarker were assessed in a large cohort of patients with coronary artery disease, that were found to be higher only in those who also had PAD. The reason of this apparent disease selectivity is presently unknown and it has been hypothesized that B2M could be released in the systemic circulation as a result of the ischemia/reperfusion damage that typically occurs during exercise in patients with PAD. We have carried a study to confirm the elevation of β2M in PAD patients and to verify if the ischemia/reperfusion damage induced by a maximal treadmill testing in patients with intermittent claudication, generates an acute release of β2M in the circulation. We studied fortyfour patients with intermittent claudication undergoing blood sampling before and immediately after a maximal treadmill exercise (age 65±9 year; sex: 4 females and 40 males). Twenty two controls were selected among a population of age -and sex- matched, non diabetic, non dyslipidemic, normotensive, nonsmoking subjects with a normal creatinine clearance (mean age 64±9 year; sex: 6 females and 16 males). β2M levels, measured by ELISA, were significantly higher in PAD patients than in controls (2.28±0.17 *vs.* 1.52±0.17ng/mL; p<0.05) but were not affected by maximal treadmill exercise (before exercise 2.28±0.17ng/mL; after exercise 2.20±0.14 ng/mL; p=NS). Our data, in a relatively small but well characterized series of patients with intermittent claudication, confirm that B2M plasma levels are elevated in PAD patients, as compared with age- and sex-matched controls. On the other hand, the acute ischemia/reperfusion damage produced by a maximal treadmill exercise, and documented by effort-induced endothelial dysfunction (sVCAM-1: before exercise 803±36.5ng/mL; after exercise 985±45.5ng/mL; p<0.05), does not further enhance plasma B2M thus excluding this as the cause of the reported specificity of this biomarker for PAD patients as compared with other patients with clinical manifestations of atherothrombosis different from PAD.

P010

ENDOTHELIAL FUNCTION AND METABOLIC SYNDROME: THE ROLE OF INFLAMMATORY ADIPOCYTOKINE AND HYPERINSULINEMIA

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Endothelial function is altered in both macro- and microcirculation in type 2 diabetes (T2D). T2D is characterized by impaired endothelium-dependent vasodilation in response to insulin and vascular disease such as peripheral artery disease (PAD) and hypertension (HT). The mechanisms of alterations in the synthesis or enhanced inactivation of nitric oxide (NO) and an increase in endothelin-1 production are related to arterial dysfunction. NO is produced through L-arginine pathway by three different isoforms of nitric oxide synthase (NOS), an inducible form that can be activated by cytokines TNF-α. 20 PAD (stage IIb Fontaine)-T2D and 20 T2D (with microangiopathy) patients and 20 healthy subjects matched in sex, age, BMI and waist circumference were recruited to the aim of our experimental experience. NO plasmatic levels, endothelial damage markers [von Willebrand factor (vWF), platelet activation, TNF-alpha, PAI-1, I, glycosylated haemoglobin (HbA1c) and C-reactive-protein (CRP)] were detected. Insulin resistance was reported in both patient groups compared to healthy subjects and this condition was correlated with NO levels (insulin-stimulated NO synthesis is impaired, resulting in unopposed vasoconstriction). vWF plasmatic levels were increased in PAD-T2D compared to T2D patients and also found significant differences in platelet activation among two groups. In PAD-T2D, increased NO levels correlated with TNF-α, CRP, HbA1c and platelet activation showed greater endothelial damage than in T2D. These results described a prothrombotic state related to insulin resistance. Particularly, the cluster of an increased of vWF and TNF-α levels and, maybe, low NO bioavailability could be a key to lead to an higher risk of thrombotic events in PAD-T2D than in the T2D patients. Hence, endothelial damage in insulin resistance condition such as metabolic syndrome is the consequence of adipocytokine TNF-alpha that acting as *vasocrine* signalling with inhibition of insulin-mediated capillary recruitment. This action may explain relations between visceral fat, insulin resistance, and PAD.

P011

HEMORHEOLOGICAL PROFILE IN PERIPHERAL ARTERIAL DISEASE PATIENTS

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Introduction. Peripheral arterial disease (PAD), defined as a chronic obstruction of the arteries supplying the lower extremities, is a common manifestation of systemic atherosclerosis. Recently, many advances in the understanding of the development of such vascular disease have been reported, and a number of novel risk factors have been described. Hyperviscosity, due to alterations of blood cells and plasma components, may play a role in the pathogenesis of the disease. Aim of this study was to evaluate the possible association between hemorheological variables and PAD. *Material and Methods.* The hemorheologic variables [whole blood viscosity (WBV), erythrocyte deformability index (DI), plasma viscosity (PLV), fibrinogen] were analyzed in 90 patients (median age: 73, range 31-87 years; 70 M, 20 F) and in 180 healthy sub-

jects comparable for age and gender (median age: 70, range: 35-89 years; 140 M, 40 F). WBV and PLV were measured using a Rotational Viscosimeter (Contraves, Switzerland), whereas DI was measured by a microcomputer-assisted filtrometer (Myrenne, Germany). *Results*. EF and PLV, but not WBV at 0.512s-1 and 94.5s-1 shear rates were found to be significantly different in patients as compared to healthy subjects. In order to investigate the possible association between these parameters and the disease we divided the study population into tertiles of their distribution among the healthy control group. At the univariate analysis, we found a significant association between the highest tertiles of PLV (2nd tertile: OR 3.61, 95%CI 1.32-9.86, p=0.01; 3rd tertile: OR 12.1, 95%CI 4.88-29.88, p<0.0001), and DI (2nd tertile: OR 0.48, 95%CI 0.25-0.89, p=0.02; 3rd tertile: OR 0.49, 95%CI 0.26-0.93, p=0.03) and the disease. After adjustment for multiple potential confounders, at a multivariate analysis, the highest tertiles of PLV (OR 9.64, 95%CI 3.62-25.72; p<0.0001), and DI (OR 0.49, 95%CI 0.25-0.99; p=0.04) remained to be significantly associated with the disease, as compared to the lowest tertiles. *Conclusions.* Our data indicate that an alteration of hemorheologic parameters, namely PLV and DI, may modulate the susceptibility to PAD. Hemorheological profile in PAD patients could allow to identify patients who might benefit from hemodilution.

Vascular Biology

P012

GLYCOGEN SYNTHASE KINASE-3 NEGATIVELY REGULATES TISSUE FACTOR GENE EXPRESSION IN MONOCYTES INTERACTING WITH ACTIVATED PLATELETS A NOVEL MECHANISM LINKING THROMBOTIC RISK AND METABOLIC DISORDERS

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At the site of vascular injury platelets (Plts) interact with monocytes (MNs) and induce de novo synthesis of tissue factor (TF) that plays a key role in atherothrombosis. The signals required for the expression of TF in MN, in the specific context of a developing thrombus, remain unknown. The importance of identifying these signals relies on both, the understanding of the hyper-coagulation state in patients at high cardiovascular risk and the discovery of novel targets for anti-thrombotic interventions. Glycogen synthase kinase-3 (GSK-3), a serine-threonine kinase, down-stream insulin pathway, originally identified as the enzyme that phosphorylates and inhibits glycogen synthase, is now recognized to play a pivotal role in the regulation of many cellular functions including inflammatory gene expression. Using a well characterized *in vitro* model of human Plts-MN interaction that allows detailed analysis of TF activity, TF protein and gene expression we explored the regulatory role of GSK-3. Our results demonstrated that, in MN interacting with activated Plts: 1) GSK-3beta undergoes phosphorylation on serine 9, a process associated with reduction of enzyme activity, reaching a maximum at 6-8 hours and then declines toward basal levels. 2) According to the kinetic of GSK-3 phosphorylation, TF activity, antigen and mRNA were low until 5 hours and dramatically increased thereafter, up to 24 hours. 3) Blockade of GSK-3, by four structurally different inhibitors (SB216763, SB415286, azakenpaullone and LiCl) or blockade of PP1/PP2A phosphatase by calyculin-A increased TF activity, antigen and mRNA. In contrast, upregulation of GSK-3 activity by interferon (IFN)-gamma reduced TF expression. 4) GSK-3 blockade increased Plts-induced NF-kB, p65 subunit, accumulation in the nucleus. 5) According to the established role of GSK-3 down-stream insulin receptor, addition of insulin to mixed Plts/MN suspensions increased GSK-3beta-ser-9 phosphorylation and TF activity and gene expression. Therefore GSK-3 is a key molecular brake in the signaling pathways leading to TF expression in MN interacting with activated Plts. In contrast GSK-3 appears to mediate TF gene expression in MN challenged by endotoxin. Our study identify a novel mechanism linking increased thrombotic risk and metabolic or neurological disorders, in which GSK-3 activity in blood MN and macrophages may be altered as consequence of the primary disease or of pharmacological treatments.

P013

ANTICOAGULANT PROTEIN C EXPRESSION AND FUNCTION IN PORCINE ENDOTHELIAL CELLS UNDER XENOGENEIC STIMULI

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Background. Microvascular thrombosis following the activation of clotting cascade is a hallmark of porcine solid organ xenografts rejection. Xenografts rejection may predispose to vascular thrombosis because of putative cross-species functional incompatibilities between natural anticoagulants present on the donor endothelium and host activated coagulation factors. Changes in the balance between procoagulant vs anticoagulant factors at the endothelial surface following xenotransplantation may induce intravascular thrombosis. In human, protein C (PC) is the key factor for the protein C pathway that is known as an important regulation mechanism of blood coagulation. Activated protein C (APC), in complex with protein S (PS), inhibits coagulation by inactivating factor Va and factor VIIIa, which are critical components of tenase and prothrombinase complexes, respectively. On endothelial cells surface, the complex thrombomodulin (TM) with thrombin and endothelial PC receptor (EPCR), is required for the efficient activation of PC. *Aim of the study.* We studied the *in vitro* model of porcine aortic endothelial cells (PAEC) and human umbilical vein cells (HUVEC) after addiction of human PC and we evaluated the ability of these cells to activate protein

C. *Materials and methods.* HUVEC and PAEC cells were tested with immunofluoresce technique for the expression of two membrane proteins: EPCR and TM. Monolayers of cells were incubated with human thrombin and with human PC in different concentration. The reaction was terminated by adding the thrombin inhibitor, hirudin. The amount of activated PC generated was determined with a specific chromogenic substrate. *Results.* Immunofluorescence analysis demonstrated the coexpression on the endothelial membrane surface of EPCR and TM on PAEC and HUVEC cells. The generation of APC was higher on cultured HUVEC than on cultured PAEC. The reaction of activation of PC on PAEC cells was more slowly than HUVEC cells. *Conclusions.* PAEC cells simply requires the expression of human coagulation protein receptors such as TM and EPCR in order to activate clotting inhibitor systems and prevent clot formation. Engineering of the porcine genome for xenotransplantation studies in primate is a step towards clinical application.

P014

BIOSYNTHESIS OF PROTEIN S BY HUMAN MEGAKARYOCYTES AND CHARACTERIZATION OF PLATELETS PROTEINS

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Background. Protein S (PS) is a vitamin K-dependent plasma glycoprotein with molecular weight of approximately 70 kDa. About 40% of PS circulates as free protein, whereas the remaining 60% in a complex with complement C4b-binding protein (C4BP). Free PS acts as a cofactor for activated protein C (APC) in the proteolytic inactivation of the coagulation factors Va and VIIIa. PS plays a crucial role in regulating thrombin generation, and therefore controlling procoagulant activity. PS is syntesized by the hepatocytes, endothelial cells and osteoblasts. Platelets (PLTs) contain PS, but the origin of intra PLTs PS (synthesis or uptake of plasma PS by megakaryocyte) is still unknown. The correlation of PS levels in plasma and in PLTs remains to be determined. Inherited or acquired PS deficiency is generally associated with venous thrombotic disease *Aim of the study.* To clarify the origin of platelets PS we have developed a model of MK cultures obtained from healthy donors. Moreover, we characterized plasma and platelets PS. *Materials and methods.* Mononuclear cells from peripheral blood were isolated by the histopaque system and have been grown in a serum-free medium in the presence of thrombopoietin (TPO) and interleukin 3 (IL3). With immunohistochemistry and immunofluorescence techniques we studied the immunophenotype of these cells including the presence or absence of PS, coagulation protein C and FV. Plasma free PS and PLTs PS derived from healthy donors and PS deficient individuals were analyzed by immunoblotting technique. *Results.* The morphology of differentiated cells, similar to MKs, and their positive stain with anti-CD41 allowed us to conclude that these cells were indeed MKs. In addition, we detected FV in their cytoplasm whereas PC was not present as expected. PS was also present in the cytoplasm of MKs obtained from healthy donors. Plasma and PLTs PS Western blot pattern demonstrated different molecular weight of PS in some deficient PS individuals as compared to normal control. *Conclusions*. These preliminary results confirmed that MKs, in healthy individuals, can synthesize PS. Studies are ongoing on the analysis of MKs obtained from PS deficient patients and on the clarification of the molecular mechanism that regulates the levels of plasma and PLTs PS.

P015

TOBACCO SMOKE INTERACTION WITH INTERLEUKIN-1BETA PROMOTES DISSOCIATION OF VE-CADHERIN/BETA-CATENIN COMPLEXES, SUPPRESSION OF PTEN ACTIVITY AND INDUCTION OF COX-2 EXPRESSION THROUGH THE ROS PATHWAY

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Tobacco smoke (TS) interacts with inflammatory cytokines to produce endothelial dysfunction. We hypothesised that interleukin-1beta (IL-1beta) plus TS (TS/IL-1beta) induces COX-2 expression, suppresses PTEN activity and leads to disassembly of endothelial junctional complexes of VE-cadherin/beta-catenin by ROS-dependent pathways, and investigated molecular mechanisms that are ROS-modulated in this situation. Exposure of mouse cardiac endothelial cells to TS/IL-1beta increased ROS production and COX-2 expression, decreased PTEN activity through its phosphorylation of Ser residues, and disrupted adherens junctions through tyrosine phosphorylation (p-Tyrosine) of VE-cadherin and beta-catenin. Treatment of endothelial cells with antioxidant n-acetyl cysteine nearly abolished ROS production and activation of Src, EGFR and p38MAPK induced by TS/IL-1beta. Moreover, inhibition of Src/EGFR/p38 pathways activated by TS/IL-1beta decreased COX-2 expression, phosphorylation of PTEN, VE-cadherin and beta-catenin, and blocked opening of adherens junctions thus reducing endothelial permeability. Overexpression or silencing of PTEN modulated p-Tyrosine of both VE-cadherin and beta-catenin, and altered assembly of adherens junction complexes. Finally, exposure of ApoE-/- mice to cigarette smoke-induced phosphorylation of Src, EGFR, p-38MAPK, PTEN and beta-catenin, disrupted VE-cadherin/ beta-catenin complexes and increased COX-2 expression in cardiovascular tissue.In conclusion, TS interaction with IL-1beta modulates COX-2 expression and stability of VE-cadherin/ beta-catenin complexes through ROS/Src/EGFR-p38MAPK pathways. Moreover, PTEN deactivation is essential to increase VE-cadherin and beta-catenin p-Tyrosine and to disassemble VE-cadherin/ beta-catenin membrane complexes.

P016

GENETIC ANALYSIS OF POLYMORPHISMS IN GENES INVOLVED IN REMODELLING OF THE EXTRACELLULAR MATRIX AND SUSCEPTIBILITY TO ABDOMINAL AORTIC ANEURYSM

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Abdominal Aortic Aneurysm (AAA) has a multifactorial aetiology and the importance of genetic components is getting increasing interest. AAA is characterized by histological signs of chronic inflammation, depletion of vascular smooth muscle cells, and destructive remodelling of extracellular matrix. Contrasting data are available in literature on polymorphisms in matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) genes. We performed a genetic association study with polymorphisms in genes coding for MMPs, TIMPs, and the structural extracellular matrix elastin in AAA. Genomic DNA samples from 600 unrelated white subjects (AAA subjects, n=300; control subjects, n=300) were genotyped for 12 polymorphisms in 10 different candidate genes: MMP1 (-1607 G/GG), MMP2 (-735 C/T, -1306 C/T, -1575 G/A), MMP3 (5T/6T), MMP9 (-1562 C/T), MMP10 (A180G, Lys53Arg), MMP12 (-82 A/G), MMP13 (-77 A/G), TIMP1 (C434T), TIMP3(-1296 T/C), ELN (G1355A, Gly422Ser). Genotyping was performed by using a primer extension based microarray technology. All investigated polymorphisms resulted in Hardy-Weinberg equilibrium in patients and controls. Genotype distribution resulted significantly different between patients and controls for the following SNPs: 5T/6T MMP3 (6T6T 18.3% AAA vs 29.6% controls, p=0.001); -77 A/G MMP13(GG 21.4% AAA vs 14.2% controls, p=0.015); G1355A ELN (GA+AA 60.4% AAA vs 68.8% controls, p=0.015); C434T TIMP1 (CT+TT 0.0% AAA *vs.* 4.6% controls, p<0.0001). At the multiple logistic regression analysis adjusted for traditional cardiovascular risk factors (sex, age, hypertension, smoking habit, dyslipidemia, diabetes) and chronic obstructive pulmonary disease (COPD), 5T/6T MMP3 (OR=0.53, 95%CI 0.29-0.96, p=0.037) and -77 A/G MMP13 (OR=2.13, 95%CI 1.17-3.89, p=0.014) polymorphisms resulted independent susceptibility factors for AAA. These findings suggest that genetic variations in TIMP1, ELN and in an independent way MMP3 and MMP13 genes may contribute to the pathogenesis of AAA.

P017

GENERATION AND CHARACTERIZATION OF A STABLE RAT ADULT BRAIN ENDOTHELIAL CELL LINE

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Microvasculature brain endothelial cells, that constitute the blood-brain barrier, differ fundamentally from other vascular endothelial cells in their characteristics and functionality. Primary cultures of brain endothelial cells that are difficult to obtain pure, are phenotypically unstable, and rapidly undergo cellular senescence after limited number

of divisions. Establishment of a model of the blood-brain barrier has proven to be a difficult goal. To this end, we have transduced normal rat brain endothelial cells with lentiviral vectors containing human telomerase and SV40 T antigen, and many stable immortalized clones were obtained by sequential limiting dilution. All cell clones showed a normal endothelial morphology, cobblestone appearance, and positive staining with DilAcLDL. One of these cell lines, termed RBECs (rat brain endothelial cells), has been characterized. RBECs express telomerase and SV40T, and they grow indefinitely without phenotypic dedifferentiation (over the course of 40 passages). Moreover, RBECs express a number of endothelial markers (i.e. PECAM-1, VE-cadherin, ZO-1 and eNOS), but no astrocyte and oligodendrocyte markers (i.e.GFAP and MBP respectively). We have also evaluated whether this cell line maintains stable and physiologically normal endothelial phenotype. RBECs form networks of capillary-like vascular cords within three-dimensional extracellular matrix and they show gene up-regulation in response to inflammatory stimuli. LPS, PMA and inflammatory cytokines (IL-1beta and TNF-alfa) induce in RBECs the expression of cyclooxygenase-2 (COX-2) and plasminogen activator inhibitor-1 (PAI-1), and increase activity of tissue factor (TF) and production of reactive oxygen species (ROS). In conclusion, we provide an extensive phenotypic characterization of an immortalized RBECs, which stably maintains in culture most of the structural and biochemical properties of primary brain endothelium. RBECs might represent an important tool for the study of brain endothelial cells functions *in vitro*.

P018

FIBRILLIN1 POLYMORPHISMS IN PATIENTS WITH MARFAN SYNDROME AND RELATED DISORDERS

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Marfan syndrome (MFS) is an inherited connective tissue disorder with autosomal dominant transmission caused by mutations in the fibrillin-1 gene (FBN1), encoding the glycoprotein fibrillin1. Recently, mutations in the TGFBR1 and TGFBR2 genes, codifying for the receptors of TGF-beta, were reported in MFS patients. Major clinical manifestations of MFS affect cardiovascular and skeletal apparatuses and ocular and central nervous systems. FBN1 gene has also been shown to harbour mutations related to a spectrum of conditions phenotypically related to MFS, called *type-1 fibrillinopathies*. In addition to causative mutations, some polymorphisms in FBN1 gene have been found in MFS patients. Aim of the study is to determine if FBN1 polymorphisms may be associated to or may be responsible of the clinical MFS phenotype. We investigated 130 unrelated patients (76 men; 54 women) referred to the Centre for Marfan syndrome and related disorders (University of Florence, Florence, Italy). The clinical and differential diagnoses were made according to the Ghent criteria. DNA mutation screening was performed by DHPLC and sequence analysis on FBN1 gene. We identified 22 polymorphisms: 6 new and 16 already published. Among the 130 patients all affected by MFS or related disorders, we selected 37 patients carrying haplotypes made of at least 4 out of the 15 more frequent FBN1 polymorphisms. Experiments are in progress to analyse the presence and frequency of these polymorphisms/haplotypes among 100 healthy controls. In 17 patients (15 with classic Marfan syndrome and 2 with Thoracic Aortic Aneurysm (TAA) we identified also a pathogenetic FBN1 mutation. In the remaining 20 patients, we did not find any mutations in the FBN1 (including the 5'upstream region) and in the TGFBR1 and TGFBR2 genes. Among these 20 patients, 6 were affected by classic Marfan syndrome and 14 were affected by related mild disorders. In 3 out of 20 patients we performed immunohistochemical analysis showing a decrease in fibrillin 1 protein. In conclusion, the findings reported here suggest that FBN1 haplotypes are putative marker of an allele carrying a pathogenetic mutation or that the haplotypes represented by single nucleotide substitutions alter the regulation of the fibrillin-1 protein synthesis.

P019

LMWH BEMIPARIN AND ULTRA-LOW-MWH RO-14 INHIBIT ANGIOGENESIS INDUCED BY TUMOR CELLS

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Heparins may have a beneficial effect on survival in cancer patients, with a major role of LMWH over unfractionated heparin (UFH). Current investigation is aimed to understand the mechanisms by which LWMH might interfere with cancer biology. Since many effects of the heparins correlate with the length of their polysaccharidic chains, it is of interest to evaluate the anti-cancer action of heparins with different mean molecular weight (Mw). Aim of this study was to evaluate the impact of the LMWH Bemiparin (BMP, mean Mw 3.6 kDa) and of the novel UltralowMWH RO-14 (mean Mw 2.5 kDa), on tumor cell capacity to induce angiogenesis. Standard UFH was also used. Angiogenesis was evaluated by an *in vitro* capillary-like tube formation assay. Tumor cell conditioned media (TCM) were obtained from three human tumor cell lines, i.e. H69 (small cell lung cancer), MDA.MB.231 (breast cancer), and NB4 (acute promyelocytic leukemia). Human microvascular endothelial cells (HMEC-1) were incubated for 24h in Matrigel with TCM, or purified proangiogenic factors (VEGF, bFGF), in the absence or presence of increasing concentrations (from 0.01 to 10 IU/mL) of BMP, RO-14, UFH, or control medium. Tube formation was quantified by an image analysis software. The levels of the pro-angiogenic factors VEGF, bFGF and IL-8 in TCM were quantified by ELISA. All three TCM induced a significant ($p<0.05$) increase in total tube length (42-68% mean increase) compared to control medium. This increase was dose-dependently counteracted by BMP, RO-14 and UFH. Similarly, all heparins inhibited tube formation induced by standard VEGF and bFGF. Interestingly, the UltralowMWH RO-14 presents an anti-angiogenic activity similar to that of LMWH BMP. The evaluation of proangiogenic cytokine contents in TCM showed that VEGF was the main product in all of the three cancer cell lines. Bemiparin and RO-14 counteract the proangiogenic stimulus by tumor cells or standard proangiogenic factors on microvascular endothelium. These data support the hypothesis of a role of LMWH in the control of tumor progression. *Supported by Laboratorios Farmaceuticos Rovi S.A. (Madrid, Spain)*

P020

HAEMOSTASIS AND ANGIOGENESIS IN PLACENTAE FROM GESTATIONAL VASCULAR COMPLICATIONS

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Gestational vascular complications (GVC), such as preeclampsia (PE) and fetal growth restriction (FGR), are multifactorial diseases, whose pathogenesis is largely unknown. An abnormal endovascular invasion of syncytiotrophoblasts and an inadequate local neovascularization have been invoked. In a previous study, we showed in uncomplicated pregnancies a relationship between markers of haemostasis and of angiogenesis. To verify whether haemostatic and angiogenic markers are differently expressed in placentae from GVC in respect of controls and whether the relationships in uncomplicated pregnancy are maintained in placentae from GVC. RNA expression of haemostatic (TF, TFPI, TFPI-2, PAI-2, Anx V, TM) and angiogenic (Ang-1, Ang-2, PlGF, VEGF) markers in placentae from GVC (n=47) and from uneventful pregnancies (n=21) were investigated. TF, TFPI, PAI-2 and Anx V were significantly (ANOVA test, $p\leq0.05$) less expressed in PE±FGR (n=27) than in controls. Similarly, FGR group (n=20) showed a lower expression of all these markers (ANOVA test, $p\leq0.05$). In PE±FGR and in FGR , TM expression was higher than in controls (ANOVA test, $p\leq0.01$). In PE±FGR group Ang-1 and Ang-2 were higher expressed (ANOVA test, $p\leq0.01$). In the whole group of cases, VEGF, PlGF and Ang-1 were not correlated with anyone of the considered haemostatic markers. Factors involved in local haemostasis and VEGF appear to be reduced in GVC. The relationship between local factors involved in haemostasis and those involved in angiogenesis observed in at term placentae is impaired in GVC. *Abbreviations: TF: tissue factor; TFPI: Tissue Factor Pathway Inhibitor; PAI: Plasmino-*

gen Activator Inhibitor; AnxV: Annexin V; TM: Thrombomodulin; Ang-1 and 2: Angiopoietin 1 and 2; VEGF: Vascular Endothelial growth Factor; PlGF: Placental Growth Factor

P021

CENTELLA ASIATICA INHIBITS TNFALFA-INDUCED ADHESION MOLECULE EXPRESSION IN ENDOTHELIAL CELLS OF UMBILICAL CORDS FROM GESTATIONAL DIABETIC WOMEN

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Diabetes mellitus is associated with inflammatory endothelial activation and increased vascular leukocyte adhesion molecules expression, both playing a relevant role in the development of vascular complications. Centella Asiatica (CA) has shown anti-inflammatory properties in several experimental models: however, its actions on vascular adhesion molecule expression have not yet been tested. Thus, we evaluated the effect of CA on TNFalfa-stimulated adhesion molecule expression in endothelial cells obtained from umbilical cords of gestational diabetic (GD) and control women (C). Human Umbilical Vein Endothelial Cells (HUVEC) obtained at delivery form umbilical cords of 10 C- and 10 GD-women were stimulated with TNFalfa (1 ng/mL) after a 48 hours preincubation with CA (25 microg/mL). After 12 and 16 hours, vascular cell adhesion molecules (VCAM-1), intercellular cell adhesion molecules (ICAM-1) and E-Selectin protein levels (Western Blot) and their surface expression (flow cytometry analysis) were assessed. The functional consequences of C- and GD-HUVEC treatment with CA on VCAM-1 membrane exposure were also evaluated by human monocytoid cell (U937 line) adhesion assay. After a 12 hours TNFalfa stimulation, VCAM-1, ICAM-1 and E-Selectin protein levels were higher in GD- as compared in C-HUVEC ($p<0.05$, Western Blot analysis). Preincubation with CA significantly decreased the effects of 12 hours TNFalfa-stimulation on VCAM-1 protein levels in GD-HUVEC, while no effect was observed on C-HUVEC. Flow cytometry analysis demonstrated that, following CA preincubation, the percentage of cells positive for surface VCAM-1 and ICAM-1 expression was modestly but significantly lower both in C- and GD-HUVEC after 12 and 16 hours TNFalfa stimulation. In addition, as compared to cells not pre-exposed to CA, both VCAM-1 and ICAM-1 MFI ratio (Mean Fluorescence Intensity) was lower in both CA-preincubated C- and GD-HUVEC after 12 and 16 hours TNFalfa-stimulation. We also examined the functional consequences of C- and GD-HUVEC treatment with CA in terms of U937 cell adhesion to cells. In agreement with data on TNFalfa-increased VCAM-1 expression, treatment of C- and GD-HUVEC with CA for 16 hours produced a significant decrease in U937 cell adhesion ($p<0.001$). In conclusion, our *in vitro* study demonstrates a role for Centella Asiatica in mitigating the potentially dangerous effects on endothelium of chronic exposure to hyperglycemia *in vivo*. *Supported by Peter Italia, Rome. Patent Pending.*

P022

ENDOTHELIAL MICROPARTICLES AND VASCULITIS

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Background. Microparticles (MPs) are small (<1 micrometer diameter) phospholipids microvesicles released from stimulated or apoptotic cells after plasma membrane remodelling. Circulating blood contains MPs derived from platelets, blood cells, endothelial cells and several other cell types. These microvesicles express on their surface several different proteins according to their cell origin and with the process related to their origin. Recent studies demonstrated increased endothelial microparticles (EMPs) levels in a number of diseases characterised by endothelial injury, such as vasculitis.*Aim of the study*. To clarify the relation between MPs and systemic vasculitis, we measured EMPs plasma levels in patients with vasculitis compared with a control group. *Materials and methods*. After informed consent, 20 mL of blood were collected in 3.8% sodium citrate solution (1:9 vol/vol) from 30 healthy subjects and 30 patients with diagnosis of vasculitis. The samples for MPs determination were obtained from platelets-free plasma after centrifugation. Using flow cytometric techniques (EPICS XL-MCL, Beckman Coulter IL, Italia), we measured EMPs identified by the monoclonal antibody CD146 conjugated with R-phycoerythrin covalently linked to cyanine 5.1 (CD146-PC5). *Results.* In patients with vasculitis EMPs levels were (40,6±19,92 cell/microL) and the number of total MPs was (575,26± 560,38 cell/microL). In healthy individuals EMPs and total MPs levels were (30,41±16,71 cell/microL) and (1313,27±1549,28 cell/microL), respectively. Our results demonstrated higher EMPs plasma levels in patients with vasculitis than in controls and the difference was statistically significant ($pp<0.05$). In contrast, total number of MPs was higher in healthy subjects than in patients ($p<0.05$). *Conclusions.* Our study confirmed a correlation between EMPs plasma levels and endothelial diseases. EMPs provide important pathophysiological and diagnostic information regarding the endothelial injury associated with systemic vasculitis. Unexpectedly the total number of MPs was minor in patients with vasculitis than in healthy subjects; probably this result is due to the chronic assumption of immunosuppressive therapy in patients with vasculitis. Further studies are needed to clarify the role of EMPs in vasculitis and the effects of the immunosuppressive drugs on MPs release.

P023

DIFFERENT PATTERN OF MODIFICATIONS FOR HAEMATOPOIETIC AND ENDOTHELIAL PROGENITOR CELLS AFTER A STRENUOUS EXERCISE IN SEDENTARY HEALTHY MEN

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Introduction. Physical exercise has been reported to increase the number of circulating haematopoietic (HPCs) and endothelial progenitors cells (EPCs) in athletes and in moderately-trained subjects, but no data on the effect of exercise on the mobilisation of these cells in sedentary subjects are available. The aim of this study was to assess the effect of a maximal exercise test on HPCs and EPCs in a group of healthy sedentary men. *Methods*. Twenty men with a median age of 34 (range: 22-40) years underwent to a maximal incremented graded treadmill test. The number of HPCs and EPCs were determined pre-exercise (T0), immediately at the end of the exercise test (T1) and 30 minutes after (T2). Peripheral blood HPCs were defined as $CD34^+$, $CD133^+$ and $CD34^+/CD133^+$ while EPCs were defined as $CD34^+KDR^+$, $CD133^+KDR^+$ and $CD34^+CD133^+KDR^+$ by flow cytometry. *Results.* HPCs showed a pattern of modification that included a significant ($p<0.05$) increase ($CD34^+$: 4.31±3.1 *vs.* 3.14±1.7; $CD133^+$: 4.3±3.1 *vs.* 3.1±1.6; $CD34^+/CD133^+$: 4.3±3.2 *vs.* 3.1±1.8 cells/microL, for T1 and T0, respectively) for all the three types at T1, with a following significant decrease at T2 ($CD34^+$: 2.9±1.7; $CD133^+$: 2.9±1.7; $CD34^+/CD133^+$: 2.9±1.7 cells/microL; $p=0.002$). On the contrary, EPCs reported a specular pattern of modifications with a significant decrease immediately after the acute exercise ($CD34^+/KDR^+$: 0.06±0.04 *vs.* 0.08±0.06, $p=0.04$; $CD133^+/KDR^+$: 0.07±0.05 *vs.* 0.09±0.04, $p=0.02$; $CD34^+/CD133^+/KDR^+$: 0.06±0.05 *vs.* 0.08±0.04, $p=0.04$ for T1 and T0 respectively), and a subsequent increase at T2, 30 minutes after the exercise ($CD34^+/KDR^+$: 0.08±0.05; $CD133^+/KDR^+$: 0.09±0.06; $CD34^+/CD133^+/KDR^+$: 0.08±0.05). *Conclusions.* In conclusion, we documented that intensive physical exercise has different effects in modifying HPCs' and EPCs' circulating levels. In fact, while HPCs significantly augmented immediately after the acute exercise, probably due to the increase of the leukocyte turn-over, EPCs showed a significant decrease with respect to baseline, possibly determined by the release of inflammatory mediators that are highly produced during the acute phase of the exercise.

P024

RELATIONSHIP BETWEEN HAEMOSTASIS AND ANGIOGENESIS IN AT TERM PLACENTAS

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Few studies have been carried out to investigate whether distinct areas of at term placenta express different amounts of markers involved in the placental haemostasis and angiogenesis. A possible relationship between the expression of genes involved in the haemostasis and angiogenesis of human placenta has not been investigated. Twenty-eight fresh human placentas (35-41 weeks of gestation) from uneventful pregnancies were dissected with two different methods. Quantity mRNA expression of TF, TFPI, TFPI-2, PAI-2, Anx V, VEGF, TM genes was evaluated by quantitative real time PCR system. Histology of each sample was graded. Gene expression of all the considered markers was not significantly different in each area, using both the different methods of dissection. A significant correlation ($p<0.05$) was found between the expression of TF and TFPI-2. TF and TFPI-2 were significantly ($p<0.05$) associated with VEGF, whereas a stronger association ($p<0.01$) was found between TFPI and TFPI-2. TFPI and TFPI-2 were strongly associated with PAI-2 expression ($p<0.01$). In placentas with central cord insertion, gene expression is not dependent on the method of sampling site. A significant relationship between haemostasis and angiogenesis in at term placentas was shown.

P025

T-LYMPHOCYTES RENIN-ANGIOTENSIN SYSTEM IS UPREGULATED BY ANGIOTENSIN II

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The interplay between Angiotensin (Ang) II and adaptive immunity seems particularly intriguing because both experimental and human studies showed that Ang II can modulate the activity of immune cells and may be produced by these same cells. We investigated whether angiotensin II (Ang II) could affect mRNA expression for all RAS components and for interferon-gamma (INF-γ) by human isolated T-lymphocytes. T-cell angiotensin-converting enzyme (ACE) activity and Ang II content were also investigated. mRNA expression for all RAS components, obtained from peripheral blood of 30 healthy subjects was quantified with reverse transcriptase- polymerase chain reaction (RT-PCR). ACE activity was assayed in cell pellets and supernatants by measuring the hippuric acid formation by HPLC and Ang II cell content was measured by radioimmunoassay after HPLC separation. All determinations were performed under baseline conditions and in the presence of 10^{-13} Molar Ang II in lipopolysaccharide (LPS)-stimulated or unstimulated lymphocytes. 10^{-13} Molar Ang II significantly increased the T-cell gene expression of all RAS components and of INF-γ (Figure 1), T-cell ACE activity and Ang II content ($p<0.01$ *vs.* baseline for all).

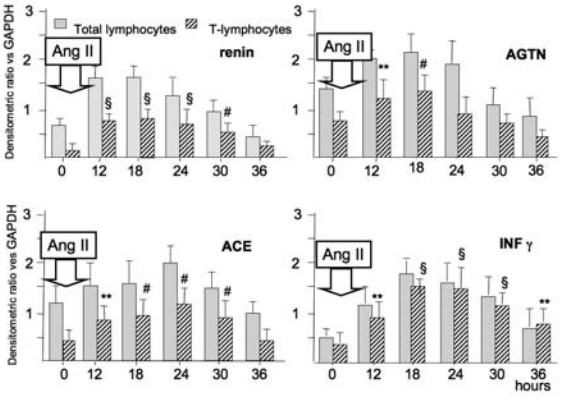

Figure 1. Angiotensin II upregulates RAS gene expression in T-lymphocytes.

These effects were further potentiated when T-lymphocytes had been prestimulated by LPS and completely inhibited by Irbesartan. Our findings strongly support the existence of a positive Ang II driven autocrine loop that upregulates lymphocytic RAS. The immuno-potentiating effect of Ang II can be deleterious when local RAS are unregulated as in cardiovascular atherosclerotic disease.

P026

BALANCE BETWEEN CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND MATURE CIRCULANTING ENDOTHELIAL CELLS IN RELATION TO THE SEVERITY OF PERIPHERAL ARTERIAL DISEASE

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Introduction. The maintenance of endothelial health depends, not only on the local milieu, but also on circulating endothelial progenitor cells (EPCs) derived from the bone marrow. Indeed, EPCs support the integrity of vascular endothelium and promote revascularization of ischemic areas. On the other hand, circulating mature endothelial cells (CECs) are considered a marker of endothelial injury. Previous studies demonstrated reduced number of EPCs in peripheral arterial disease (PAD) patients, but few data are available on CECs. Aim of our study was to contemporary assess EPCs and CECs in PAD patients in relation to the severity of the disease. *Methods.* In 30 PAD patients [22 M/8 F; median age: 69 (45-86) years] we measured circulating EPCs and CECs by using flow cytometry. EPCs were defined as $CD34^+KDR^+$, $CD133^+KDR^+$ and $CD34^+CD133^+KDR^+$, while CECs were defined as $CD146^+/CD31^+/CD45^-/CD61^-$. *Results.* A significant trend of decrease ($p<0.05$) in relation to the clinical severity of the disease, as seen by Fontaine's stages, was observed for $CD133^+/KDR^+$ EPCs [stage IIa: 0.093 (0.06-0.25); stage IIb: 0.049 (0.02-0.16); stage III: 0.03 (0.02-0.05); stage IV: 0.035 (0.02-0.08) cells/μL]. On the contrary, a significant ($p<0.05$) increase was showed by CECs [stage IIa: 0.077 (0.02-0.13); stage IIb: 0.084 (0.02-0.19); stage III: 0.15 (0.05-0.19); stage IV: 0.22 (0.08-0.33) cells/μL]. In order to evaluate the balance existing between EPCs and CECs in relation to the clinical progression of the disease, we calculated the CECs/EPCs ratio. By increasing Fontaine's stage, a progressive and significant ($p<0.05$) increase in ratio value was observed, indicating a prominent role of CECs with respect to EPCs number [stage IIa: 0.62 (0.2-2.30); stage IIb: 1.22 (0.23-7.67); stage III: 6.39 (1.43-7.71); stage IV: 6.14 (1-16)]. *Conclusions*. Our results demonstrate an unbalance between EPCs and CECs in PAD patients in relation to the progression of the disease, possibly indicating that the endothelial damage observed in these patients is not sufficiently repaired by a concomitant increase of the regenerative capacity of EPCs.

P027

THE PLASMA HYPERCOAGULABILITY AND VASCULAR ENDOTHELIUM ABNORMAL CYTOKINES' RELEASE MAY INFLUENCE THE PROGRESSION OF THE OSTEOPATHY IN HOMOZYGOUS β-THALASSEMIA PATIENTS

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Background. From years we reported that a chronic plasma hypercoagulation and vascular endothelium permanent dysfunctions are the major causative factors for thromboembolic complications in homozygous β-thalassemia (β-Th)(Musso R *et al.* Blood 1990; 75: 2467-2468; Musso R. *et al.* Haematologica 2005; 90: 87-88). *Aim.* In this scenario, an other emerging complication such as the osteopathy, which becomes certain in adult β-Th patients, has mainly been referred to the several endocrine deficiencies, iron overload and iron chelator drugs, while the contribution of the hypercoagulation and abnormal vascular endothelium cytokines' release has not been considered. *Methods.* 11 Sicilian homozygous β-Th patients (6 females and 5 males), aging 24-66 yrs, were studied. 11 Sicilian heterozygous β-Th subjects and 10 healthy individuals of comparable age served as controls. Bone density scans showed severely low bone mass in 7/11 and low bone mass in 4/11 respect to the control groups. The osteoblastic cytokines' net-work as the Platelet-derived growth factor (PDGF), Transforming growth factor-beta (TGF-β) and Interferon-gamma (IFN-γ) together with the osteoclastic cytokines such as the Interleukin-1 (IL-1), Interleukin-6 (IL-6) and Tumor necrosis factor-alpha (TNF-α) were determined by ELISA. *Results.* Our results showed a significant ($p<0.001$) increase of the osteoclast cytokines in homozygous β-Th respect to those observed in the controls group. The osteoblastic cytokines were in normal range in all groups (Tables 1 and 2). *Conclusions.* From these observations we suggest that the chronic red blod cels haemolysis, blood transfusions and iron chelation *quoad vitam* would determine continuously biochemical changes in β-Th leading also to an abnormal release of several cytokines from injured vascular endothelium/intima of the bone microenvironment. The osteoclastic cytokines' formation is dominant over the osteoblastic one and by reducing bone mineral density so potentiating the osteoporosis in adult homozygous β-Th patients. In this context, the chronic vascular endothelium suffering of the bone microenvironment together with the hypercoagulability, leukocytes and platelet hyper-activation could enhance further the osteoclast cells functions in β-Th.

Table 1. Osteoclast activation indexes: IL-1, IL-6, TNF-α.

	IL-1 (pg/mL)	*IL-6 (pg/mL)*	*TNF-α (pg/mL)*
β-Th pts (n=11)	404.6±181.7*	29.1±15.3*	1.315±329.6*
β-Th trait (n=11)	211.4±92.9	12.7±8.9	783±291
Healthy subjects (n=10)	181.3±126.4	9.9±7.7	744.4±201.9

**p<0.001 vs β-Th trait and healthy subjects*

Table 2. Osteoblast activation indexes: TGF-β, PDGF, IFN-γ.

	TGF-β (pg/mL)	*PDGF (pg/mL)*	*IFN-γ (pg/mL)*
β-Th pts (n=11)	1.350±637	23.7±19.2	325.7±163.6
β-Th trait (n=11)	1.151±796	20±21.7	287±201
Healthy subjects (n=10)	1.255±819	17.9±18.4	305.7±124.9

Inflammation and Thrombosis

P028

CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND RESIDUAL *IN VIVO* THROMBOXANE BIOSYNTHESIS IN LOW-DOSE ASPIRIN-TREATED POLYCYTHEMIA VERA PATIENTS

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Polycythemia vera (PV) is associated with high morbidity and mortality for thrombosis. We hypothesized that in PV altered sensitivity to aspirin might be related to dysfunction of the endothelial repair and/or of the nitric oxide (NO) system. Urinary thromboxane (TX)A2 metabolite (TXM), endothelial colony-forming cells (ECFCs), plasma asymmetric dimethylarginine (ADMA) and of von Willebrand Factor (vWF) were measured in 37 PV patients on low-dose aspirin and 12 healthy controls. Patients showed an approximately 2-fold increase in median TXM and plasma ADMA levels ($p<0.0001$), while ECFCs number was reduced by approximately 7 fold ($p<0.0001$) as compared to non-aspirinated control. These differences were more pronounced in patients with previous thrombosis. Eight-week aspirin did not affect ECFCs in controls. vWF and TXM correlated directly with ADMA, and inversely with ECFCs. By multiple regression analysis, lower ECFCs quartiles (β=-0.39; SE=0.17; $p=0.028$) and higher vWF levels (β=0.338, SE=0.002, $p=0.034$) were independent predictors of higher TXM quartiles ($R^2=0.39$). Serum TXB2, measured in 22 patients, was approximately 10-fold higher than aspirin-treated controls. PV patients appear to have an unbalanced ECFC/NO axis, and an apparent altered sensitivity of platelet TXA2 production, all potentially contributing to aspirin-insensitive TXM formation. Thus, additional antithrombotic strategies may be beneficial in PV.

P029

THE BALANCE BETWEEN PRO- AND ANTI-INFLAMMATORY CYTOKINES IS ASSOCIATED WITH PLATELET AGGREGABILITY IN ACUTE CORONARY SYNDROME PATIENTS

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Residual platelet reactivity (RPR) on antiplatelet therapy in ischemic heart disease patients is associated with adverse events. Clinical, cellular and pharmacogenetic factors may account for the variable response to antiplatelet treatment. We sought to explore the interplay of multiple pro-inflammatory and anti-inflammatory cytokines with platelet function in patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI) on dual antiplatelet therapy. *Methods.* In 208 ACS patients undergoing PCI on dual antiplatelet therapy we measured platelet function by platelet aggregation with two agonists [1mM arachidonic acid (AA) and 10 μM ADP]. Interleukin-1Beta(IL-1β), interleukin 1 receptor antagonist(IL-1ra), interleukin-4(IL-4), interleukin-6(IL-6), interleukin-8(IL-8), interleukin-10(IL-10), interleukin-12 (IL-12), interferon-inducible protein(IP-10), interferon-gamma(IFN-γ), monocyte chemoattractant protein-1(MCP-1), macrophage inflammatory protein 1-alpha(MIP-1α), macrophage inflammatory protein 1-Beta(MIP-1β), tumor necrosis factor-alpha(TNF-α), and vascular endothelial growth factor(VEGF) levels were determined by using the Bio-Plex cytokine assay (Bio-Rad Laboratories Inc, Hercules, CA, USA). We defined patients with RPR those with platelet aggregation by AA ≥20% and/or ADP (10 microM) ≥70%. We documented a significant association between IP-10, IFN-γ, IL-4 and RPR by both AA- and ADP-induced platelet aggregation after adjustment for age, sex, cardiovascular risk factors, ejection fraction, BMI, vWF and CRP. Patients with pro-inflammatory cytokines not compensated by anti-inflammatory cytokines had higher risk of RPR by both AA and ADP (AA: OR=3.85, 95%CI 1.52-9.74; ADP: OR=2.49, 95%CI 1.33-4.68) with respect to patients with balanced anti-/pro-inflammatory cytokines. Patients with anti-inflammatory response overwhelming pro-inflammatory response have lower risk of RPR (AA: OR=0.55, 95%CI

0.28-1.06; ADP: OR=0.47, 95%CI 0.26-0.87). Our study provides new insights into the interplay of anti/pro-inflammatory cytokines with platelet hyper-reactivity in high risk patients.

P030

ROLE OF MMP-2 IN THE PROTHROMBOTIC ACTIVITY OF ATHEROSCLEROTIC PLAQUES: EFFECTS ON PLATELETS

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Matrix metalloproteinases (MMPs) are a family of enzymes able to degrade and to remodel extracellular matrix in several physiologic and pathologic conditions and their involvement in atherosclerotic plaques formation and rupture has been reported. In particular, in atherosclerotic plaques, smooth muscle cells, T-lymphocytes and macrophages express some MMPs (MMP-1, MMP-2, MMP-7, MMP-9) and the degree of inflammation, expressed by cell infiltration, appears to be proportional to the plaque content of MMPs. This structural modification of the plaque can cause its rupture in particular sites, like the *plaque shoulder*, particularly rich of lymphocytes and macrophages. On the other hand, some MMPs, (like MMP-2) have been recently found to potentiate platelet activation in response to several stimuli. It can thus be hypothesized that the sudden release of some MMPs by a rupturing plaque may contribute to platelet thrombus formation. Aim of our study was to assess the role of MMP-2 present in atherosclerotic plaques in the regulation of platelet activation. Atherosclerotic plaque fragments were obtained from 52 patients undergoing carotid endoartectomy for high-grade carotid artery stenosis and the levels of MMP-2 in the plaques were measured by zymography and the effect of plaque extracts on platelet activation was estimated by platelet aggregometry. The MMP-2 content of plaques from patients with previous ischemic homolateral symptoms (n=21) was significantly higher compared to those from patients with asymptomatic atherosclerotic plaques (n=28) (34.9±16.2 *vs.* 25.6±14.4 ng/microg of plaque extract, $p<0.05$) and to those of patients with controlateral ischemic symptoms (n=3). Atherosclerotic plaque extracts (72 ng/mL) were incubated with gel-filtered platelets from healthy volunteers for 2 min before the addition of a subthreshold concentration of the thrombin-receptor activating peptide TRAP-6 and platelet aggregation was followed for 5 min; 46% (n=24) of the plaque extracts potentiated TRAP-6 -induced platelet aggregation, with an average 3.3±0.3 fold increase of aggregation ($p<0.05$). The preincubation with specific inhibitors of MMP-2 (MMP-2 inhibitor II, 10 microg/mL and TIMP-2, 3 ng/mL), significantly reduced the proaggregatory effect of plaque extracts on TRAP-6 induced platelet aggregation (-23.8±6.5%, $p<0.05$ and -46.7±7.4%, respectively, $p<0.05$). In conclusion, our data show that some plaque extracts can exert a prothrombotic platelet-aggregation-potentiating effect due to their high content of MMP-2.

P031

ASSOCIATION AMONG MYELOPEROXIDASE, OXIDANT STRESS AND SOLUBLE CD40 LIGAND LEVELS IN PATIENTS WITH ATRIAL FIBRILLATION

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High circulating levels of soluble CD40 ligand (sCD40L), a marker of platelet activation, were recently described as a risk factor for vascular events in patients with atrial fibrillation (AF). Oxidant stress was shown to play a key role in sCD40L expression but it has never been investigated if this occurs in AF. Aim of the study was to investigate if a relationship between mieloperoxidase(MPO), a reactive oxidant species-generating enzyme, and CD40L does exist. We studied 245 patients affected by AF (121 males, 124 females, age 72.6±9.9 years), 51 paroxismal and 164 permanent and 30 persistent, and 88 control ubjects matched for age and sex (males 41, females 47, age 72.6±8.8). In 87 AF patients (43 males, 44 females , age 70.8±8.9) serum levels of 8-OH-DG, a marker of oxidative stress, were also measured. MPO, 8-OH-DG and sCD40L were assayed by an ELISA test (R&D Systems, Minneapolis, MN, USA). MPO (575±323 *vs.* 164±96 pmol; $p<0.0001$), 8-OH-DG (4.79±2.31 vs 3.12±0.48 ng/mL; $p<0.002$) and sCD40L (5.04±2.09 *vs.* 2.45±1.2 ng/mL; $p<0.0001$) were significantly higher in AF patients compared to controls. Bivariate analysis (Pearson test) disclosed a significant correlation between MPO and 8-OH-DG (r=0.267; $p<0.02$) MPO and sCD40L (r=0.376; $p<0.0001$) and 8-OH-DG and sCD40L (r=0.573; $p<0.0001$) in AF patients. In order to establish the independent predictors of CD40L in AF, a multiple linear regression analysis (adjusted for age, gender, cholesterol, systolic and diastolic pressure and glycaemia) was performed. The independent predictive variable associated with CD40L was only 8-OH-DG (S.E.:0.088; standardized coefficient β: 0.603; $p<0.0001$) [R^2=36.5%]. This study shows an association among MPO, 8-OH-DG and sCD40L in AF patients, suggesting that MPO could enhance sCD40L via enhancing oxidative stress.

P032

ASSOCIATION OF LOW-GRADE INFLAMMATION AND PLATELET ACTIVATION IN HYPERTENSIVE PATIENTS WITH MICROALBUMINURIA

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Increased levels of soluble CD40 ligand (sCD40L) have been associated with enhanced *in vivo* platelet activation, and may represent a molecular link between inflammation and prothrombotic state. We aimed at analyzing the relationship among platelet activation, endothelial dysfunction low-grade inflammation and sCD40L in hypertensive patients with or without microalbuminuria (MA). A cross-sectional comparison of sCD40L levels was performed in 25 patients with essential hypertension and MA (MH) pair-matched for gender and age with 25 patients with essential hypertension (EH) and 25 healthy normotensive subjects (HS). Circulating C-reactive protein (CRP, marker of inflammation), sP-selectin (marker of *in vivo* platelet activation), asymmetric dimethylarginine (ADMA) and von Willebrand Factor (vWF) (markers of endothelial dysfunction) levels were analyzed in each subject. sCD40L levels were increased in MH patients compared to either EH ($p<0.001$) or HS ($p<0.0001$). A highly significant correlation between plasma sCD40L and sP-selectin ($p<0.0001$), vWF ($p<0.001$) or CRP levels ($p<0.05$) was observed in MH patients. Multivariate regression analysis showed that sP-selectin was the strongest independent predictor of sCD40L levels ($p<0.0001$) in MH patients. Hypertensive patients with both vWF and CRP levels above the median had the highest sCD40L levels ($p<0.0001$). Factorial ANOVA analysis of all hypertensive subjects confirmed that only MH patients with low-grade inflammation had elevated levels of sCD40L. sCD40L levels appear to discriminate a subset of patients characterized by microalbuminuria and low-grade inflammation, suggesting that inhibition of the CD40/CD40L system may represent a potential therapeutic target in hypertensive subjects at high risk for cardiovascular events.

P033

DETERMINANTS OF PLATELET ACTIVATION IN HEART FAILURE

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Introduction. Thromboembolism is a critical and relatively common complication of chronic heart failure (HF). *Methods.* We performed a cross-sectional study in 84 HF patients [33 M; 81±8 yr; 49 in I-II, 35 in III-IV New York Heart Association (NYHA) class] and 42 controls, using urinary (U) 8-iso-prostaglandin (PG) F2alpha and 11-dehydro-thromboxane (TX) B2 as non-invasive indexes of oxidative stress and platelet activation, respectively, B-type natriuretic peptide (BNP) as a biomarker of cardiac function, plasma asymmetric dimethylarginine (ADMA) as an index of endothelial dysfunction, C-reactive protein (CRP) and sCD40 ligand (sCD40L) as markers of inflammation. *Results.* Forty-two HF patients not on aspirin treatment had significantly higher U-11-dehydro-TXB2 excretion [Median (IQR): 1488(824-2130) *vs.* 440(313-611) pg/mg cr], 8-iso-PGF2α [528(430-702) *vs.* 304(228-364) pg/mg cr], BNP [363(196-659) *vs.* 78(56-98) pg/mL], ADMA (1.6±0.5 *vs.* 0.5±0.2 micromol/L), CRP [1.74(0.98-2.7) *vs.* 0.5(0.4-0.7) mg/L] and sCD40L levels [1342(653-2320) *vs.* 432(322-840) pg/mL] (all $p<0.0001$) than controls. Forty-two HF patients on low-dose aspirin showed significantly lower 11-dehydro-TXB2 [343(227-455) pg/mg cr, $p<0.007$] and sCD40L levels [820(535-1160) pg/mL, $p<0.02$] than HF patients not on aspirin. Patients in NYHA classes III-IV showed higher U-11-dehydro-TXB2 excretion than patients in I-II classes, independently of aspirin treatment ($p<0.05$). In the 42 HF patients not on aspirin, U-11-dehydro-TXB2 was correlated with BNP

(Rs=0.59), 8-iso-PGF2 alpha (Rs=0.58), and CD40L (Rs=0.61) (all p<0.0001). Multiple regression analysis revealed that higher BNP levels (Beta Coefficient=0.74), no aspirin therapy (-0.41), and higher sCD40L levels (0.32) (all p<0.0001), independently predicted the excretion rate of 11-dehydro-TXB2 in the 84 pts. *Conclusions.* Persistent platelet activation characterizes patients with heart failure. This phenomenon is related to disease severity and is largely suppressable by low-dose aspirin.

P034

ANTITHROMBOTIC AND ANTIINFLAMMATORY ACTIVITIES OF NEBIVOLOL, AND ITS ENANTIOMERS, ARE IN PART DEPENDENT ON NITRIC OXIDE SYNTHESIS

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Nebivolol (a racemic mixture of L- and D-nebivolol) is a selective beta1-adrenergic receptor antagonist that, besides its hypotensive effect, was reported to influence vasodilation and platelet aggregation by a mechanism involving the L-arginine/nitric oxide pathway. Our aim was to evaluate the *in vivo* antithrombotic and antiinflammatory properties of nebivolol and of its two enantiomers, D- and L-nebivolol, in different models in wild type (WT) and eNOS-/- mice. Bisoprolol was used as a reference compound. In WT mice nebivolol (Neb), L-nebivolol (L-Neb) and D-nebivolol (D-Neb) (2.5 mg/kg, po), but not bisoprolol (2.5 mg/kg, p.o.), significantly reduced mortality in a collagen+epinephrine-induced platelet pulmonary thromboembolism model (Neb: -44%, L-neb: -45%, D-neb: -29%; p<0.05; bisoprolol=0%). In a photochemically-induced acute femoral artery thrombosis model, time to occlusion was significantly prolonged by Neb, L-Neb and by D-Neb but not by bisoprolol (control: 7.6±0.5 min., Neb: 23±4.3 min. p<0.001 *vs.* control; L-Neb: 22.4±4.1 min. p<0.001 vs control; D-Neb: 18.4±4.9 min. p<0.01 vs control; Bisoprolol :6±1.26 min. p=NS vs control). When the femoral artery thrombosis experiments were carried out in eNOS-/- mice, unable to synthetize endogenous NO, Neb and D-Neb were still active while L-Neb lost activity (control: 7.7±0.33 min., Neb: 19.8±3.5 min., p< 0.01 *vs.* control ; D-Neb: 15.4 ±3.3 min., p<0.01 *vs.* control; L-Neb: 7.11±0.64 min. and Bisoprolol: 6.12±0.07 min., p=NS *vs.* control). Serum IL-6 was enhanced in WT mice 4 weeks after photochemically-induced arterial damage (IL-6: from 2.8±0.07 to 11.8±3.4 pg/mL) and was significantly reduced by Neb and L-Neb, but not by D-Neb and bisoprolol (Neb=-67%; L-Neb=-69%; p<0.01 vs control D-Neb=-7%;bisoprolol=-9%). Neb and D-Neb (2.5 mg/kg each, p.o., for 5 days), but not L-Neb significantly reduced systolic blood pressure in mild hypertensive eNOS-/- mice, but not in normotensive WT mice. In conclusion, our data show that Neb exerts antithrombotic and anti-inflammatory activities, and that these effects are partly mediated through a stimulatory activity on endogenous NO formation exerted by L-Neb.

P035

DECREASED PLASMA SOLUBLE RAGE IN PATIENTS WITH HYPERCHOLESTEROLEMIA: ASSOCIATION WITH OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION

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Background. The ligand - receptor for advanced glycation end products (RAGE) axis emerged as a novel pathway involved in atherosclerosis initiation and progression. A soluble RAGE isoform (sRAGE) neutralizes the ligand-mediated damage by acting as a decoy. In hypercholesterolemia, up-regulation of the ligand - RAGE axis may bridge oxidative stress with endothelial dysfunction. *Methods.* We measured in 60 hypercholesterolemic patients and 20 healthy controls plasma sRAGE levels, urinary 8-iso-prostaglandin (PG) F2α excretion, and plasma levels of asymmetric dimethylarginine (ADMA). *Results*. Plasma sRAGE was significantly lower, ADMA and urinary 8-iso-PGF2α were significantly higher in hypercholesterolemic versus control subjects. Interestingly, patients with a previous myocardial infarction on chronic statin treatment showed plasma sRAGE levels and urinary excretion of 8-iso-PGF2α respectively higher and lower than untreated patients without cardiovascular events. On multivariate regression analysis, 8-iso-PGF2α and ADMA independently predicted sRAGE levels. *Conclusions.* sRAGE might represent an endogenous protection factor against the occurrence of accelerated atherosclerosis mediated by oxidative stress and endothelial dysfunction in hypercholesterolemia. Moreover, the effects of statins on sRAGE and oxidative stress may contribute to the pleiotropic effects exhibited by these molecules.

P036

INTERLEUKIN-1 BETA GENE HAPLOTYPES, MYOCARDIAL INFARCTION AT YOUNG AGE AND INFLAMMATORY/PROCOAGULANT RESPONSE OF HUMAN MONONUCLEAR CELLS

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Background. We have recently shown that a polymorphism in IL-1 beta promoter was associated with the risk of ischemic vascular disease at young age. The same polymorphism affected the release of IL-1 beta from stimulated mononuclear cells and, in turn, the expression of TF on cell membranes. *Objectives.* To investigate whether IL-1B haplotypes are associated with the risk of myocardial Infarction (MI) at young age and with the release of IL-1B and expression of Tissue Factor pro-coagulant activity (TFA), after stimulation *in vitro* with lipopolysaccharide (LPS) of human peripheral blood mononuclear cells (PBMCs). *Methods.* 437 patients with MI at young age, frequency-matched for age, sex and recruitment center, with 412 healthy population-based controls were studied. For functional studies, PBMCs from 64 healthy volunteers were studied. For IL-1B gene, five single nucleotide polymorphisms (SNPs), unically identifying two haplotype-blocks, were genotyped by Real Time-PCR (TaqMan SNP Genotyping Assay): rs1143634 (corresponding to +3954C/T); rs1143633 (corresponding to +3877G/A) (for hap-block *A*); rs16944 (corresponding to -511C/T); rs1143623 (corresponding to -1464G/C); rs4848306 (corresponding to -3737C/T) (for hap-block *B*, in the promoter region). *Results.* Subjects carrying haplotype B2 (221) and B4 (211) showed a decreased risk of MI at young age in multivariate analyses [OR=0.69 (95%CI=0.52-0.92); p=0.01 and OR=0.62 (95%CI=0.40-0.95); p=0.03, respectively]. Subjects carrying haplotype B2 (221) also showed decreased levels of IL-1B (p=0.01 in multivariate analysis). No association was found between Il 1 beta haplotypes and TFA after stimulation of PBMCs with LPS. *Conclusions.* IL-1BETA haplotypes influence the inflammatory process of human PBMCs to LPS and affect the risk of MI at young age.

P037

DETERMINANTS OF PLATELET ACTIVATION IN PULMONARY ARTERIAL HYPERTENSION

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CD40 ligand (CD40L) is a transmembrane protein originally identified on $CD4^+$T cells. Subsequently, CD40L has been identified on the membrane of activated platelets from where a soluble form (sCD40L) can be shed. It is calculated that more than 95% of sCD40L is of platelet origin. Platelet activation by thrombin also causes *in vitro* and *ex vivo* release of vascular endothelial growth factor (VEGF). A potential role for VEGF in the process of structural vascular change in pulmonary arterial hypertension (PAH) has been suggested, and VEGF is expressed in high levels in the plexiform lesions of PAH, both idiopathic and secondary. We hypothesized that activation of platelets might represent a source of VEGF levels in PAH. To test this hypothesis, we measured plasma VEGF and sCD40L levels in patients with therapeutically controlled PAH. We studied 38 patients with severe PAH in NYHA functional classes III and IV. Patients were divided into 3 groups according to the type of PAH: (i) patients with primary PAH (n=9), (ii) patients with secondary PAH (n=24); and (iii) patients with chronic thromboembolic pulmonary hypertension (n=5). Thirty-one sex- and age-matched healthy subjects were used as controls. As expected, plasma sCD40L and VEGF levels were higher in PAH patients compared to controls [0.9 *vs.* 0.2 ng/ml and 51.7 *vs.* 6.3 ng/mL; p<0.05). VEGF significantly correlated with sCD40L (r=0.53, p<0.001). To further quantify the relationship among VEGF, sCD40L and clinical and pharmacological variables a multiple regression

analysis was performed in which VEGF was included as the dependent variable. Stepwise linear regression yielded a model in which only sCD40L plasma levels (regression coefficient=0.48, SEM=0.14, $p<0.01$) predicted VEGF levels, independently of all potential predictors (type of PAH, therapy with prostaglandins, phosphodiesterase type 5 inhibitors, anticoagulant and or endothelin receptor antagonists). The present findings add further evidence to the currently accepted hypothesis that platelet activation might represent an important contributing factor in pulmonary vascular remodeling and hypertension, and suggest a potential role for platelet released CD40L and VEGF in the pathogenesis of PAH.

P038

ENHANCED LIPID PEROXIDATION AND PLATELET ACTIVATION AS POTENTIAL CONTRIBUTORS TO INCREASED CARDIOVASCULAR RISK IN THE LOW-HDL PHENOTYPE

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Background. Low levels of high-density lipoprotein (HDL) cholesterol have been identified as a major independent inverse predictor of cardiovascular events, even in patients on statin treatment with very low low-density lipoprotein (LDL) levels. In addition to its cholesterol-transporting properties, HDL attenuates the expression of tissue factor and selectins, protects LDL from oxidation, directly and indirectly blunts platelet activation in animal models. Our study is aimed at examining whether HDL levels are related to *in vivo* oxidative stress and platelet activation, as potential contributors to increased cardiovascular risk. *Methods.* Urinary 8-iso-prostaglandin (PG)F2-alfa and 11-dehydro-thromboxane (TX)B2, *in vivo* markers of oxidative stress and platelet activation, respectively, were measured in 65 CHD normocholesterolemic patients with HDL <35 mg/dL (13 F, 52 M, aged 61±10 yrs), compared to 47 CHD patients with HDL > 35 mg/dL (17 F, 30 M, aged 63±11 yrs). *Results.* Patients with HDL ≤35 mg/dL showed significantly higher levels of 8-iso-PGF2alfa (310±142 pg/mg creatinine) and 11-dehydro-TXB2 (650±372 pg/mg creatinine) as compared to patients with high HDL (207±114 and 383±170 pg/mg creatinine, respectively). A significant direct correlation was found between urinary 8-iso-PGF2α and 11-dehydro-TXB2 in both groups of patients (Rho=0.78, $p<0.0001$ and Rho=0.81, $p<0.0001$, respectively). In contrast, HDL levels were inversely related to both 8-iso-PGF2α (Rho=-0.37, $p=0.002$) and 11-dehydro-TXB2 (Rho= -0.44, $p<0.0001$) only in the subjects with HDL ≤35 mg/dL. On multiple regression analysis, only urinary 8-iso-PGF2alfa (Beta 0.65, $p<0.0001$) and HDL levels (Beta -0.27, $p<0.0001$) predicted 11-dehydro-TXB2 excretion, independently of gender, age, smoking, hypertension, diabetes, previous myocardial infarction, total cholesterol, triglycerides. *Conclusions.* We conclude that a low HDL phenotype is associated with increased lipid peroxidation and platelet activation, thus providing novel insight into the mechanisms linking low HDL and occurrence of cardiovascular disease.

P039

PARACRINE UP-REGULATION OF MONOCYTE CYCLOOXYGENASE-2 BY PLATELETS: ROLE OF TGF-BETA1

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Introduction. Cellular interactions between platelets and leukocytes provide a crucial mechanism for intercellular communication in thrombosis and inflammation. We have examined the role of platelets and platelet-derived products on cyclooxygenase-2 (Cox-2) induction in adherent monocytes and addressed the signaling pathways involved. *Methods*. Human monocytes were co-cultured with autologous platelets or platelet releasates or exposed to mediators contained in platelet alpha-granules for 4-24 h. Cox-2 protein and mRNA were determined by Western and RT-PCR. Thromboxane B2 and prostaglandin E2 synthesis as index of Cox-2 activity, and levels of TGF-β1 in platelet releasates were measured by EIA. *Results*. Activated platelets induce rapid and transient Cox-2 *de novo* synthesis in adherent monocytes. The effect is dependent upon platelet number but not upon cell-cell contact. Platelet-induced Cox-2 was not affected by prevention of platelet TxA2 synthesis or microparticle formation but was blunted by inhibition of platelet alpha-granule secretion. TGF-β1 induced Cox-2 expression and activity at concentrations within the range of those detected in releasates from activated platelets. The time course of monocyte Cox-2 induction by TGF-β1 was identical to that observed with platelet releasates. Moreover, TGF-β1 receptor blockade completely abolished platelet-induced Cox-2 expression. p38 MAPK activation represents a common transduction pathway through which activated platelets and rTGF-β1 induce Cox-2 in monocytes. *Conclusions.* Data suggest that TGF-β1 released by activated platelets is pivotal in Cox-2 induction in monocytes and further supports the key role of platelets in inflammatory and reparative responses.

P040

SERUM HAPTOGLOBIN IS A MARKER OF VISCERAL OBESITY

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A recent theory looks at obesity as a low grade systemic inflammation condition. This view is supported by several lines of evidence including the association of obesity with elevated acute phase reactants. Haptoglobin (Hp) is a glycoprotein involved in the acute phase response to inflammation, and it has been demonstrated to constitute a marker of adiposity in humans. The aim of this study was to identify the relationship between Hp and various anthropometric measurements and serum parameters in a cohort of obese women. Eighty morbidly obese women (mean age 41+11, range 20-63 years; mean BMI 42,6+5,4, range 32,6-58,8) were enrolled. Each evaluation consisted of clinical and anthropometric measurements. Abdominal sagittal diameter (ASD) and visceral fat thickness (VF) were measured by ultrasound. Fasting blood samples were collected for assay of several metabolic and hormonal parameters. A negative correlation between Hp and age ($p<0,05$) was observed; a positive correlation between Hp and weight ($p<0,005$), hip circumference ($p<0,005$), ASD ($p<0,01$), VF ($p<0,0058$), BMI ($p<0,005$) was observed. No significant associations were found between Hp and the other anthropometric measurements. In a multivariate regression analysis only age ($p=0,001$) and VF ($p<0,005$) were independent determinants of serum Hp. Serum Hp was positively correlated with white-cells ($p<0,0005$), platelets ($p<0,005$), log VES ($p<0,0001$), log C-reactive protein ($p<0,0001$) and fibrinogen ($p<0,0001$). In conclusion, Hp is a marker of visceral obesity and it is associated with an inflamed obese phenotype. Whether Hp plays a role to increase the cardiometabolik risk remains a matter of investigation.

P041

GENE EXPRESSION PROFILING OF THE USE OF DONOR BONE MARROW MESENCHYMAL STEM CELLS FOR TREATMENT OF SKIN ALLOGRAFT REJECTION IN A PRECLINICAL RAT MODEL

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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. Aim of our study was to evaluate the effect of intravenous donor MSC infusion to obtain clinical tolerance induction in allogeneic skin graft transplantations in rats by microarray gene expression profiling. The experimental design included 4 arms: group A without treatment (control); group B with immunosuppressive therapy, cyclosporine A (CsA); group C with CsA and MSC; group D with MSC infusion. MSC were isolated from Wistar rats and administered in Sprague-Dawley rats receiving Wistar skin graft with or without CsA. Graft biopsies were performed at day 10 post transplantation in all experimental groups for gene expression studies. Total RNAs, prepared from skin biopsies of the four rat groups, were pooled, labeled with fluorochromes, and hybridized to 14,000 70 mer oligonucleotide microarrays. Quantitative PCR was used to validate the array results. Intravenous infusion with donor MSC in CsA-treated transplanted rats resulted in prolongation of skin allograft survival compared to control animals. Donor MSC infusion in immunocompetent rats resulted in a faster rejection as compared to control group. After data processing and application of the filtering criteria, the differentially expressed genes were 19 in the 3 treated groups, 127 in two 2 out of three treated groups, and 514 in at least one group of treatment. Microarray data underlined the pivotal role of inflammatory cytokine balance in determining the effect of MSC on the outcome of solid organ transplantation. In the CsA+MSC group an up-regulation of IL24, IL7 and a down-regulation of IL12a and IL3 was observed; whereas, in the MSC group IL1a, IL1b, and TGFa resulted up-regulated. The gene ontology analysis showed that alteration of several biological process is associated with skin graft tolerance observed in CsA+MSC: inflammatory and immune response (Hsp70-1, Hsp27, Cxcl2, Ccl2, Cxcl10), antigen presentation (Rt1-Bb, Trib1) and angiogenesis (iNos, Edn1, Rap1b, Pak1). Present data in a rat tissue transplantation model showed a possible immunogenic role for donor MSC and indicated that inflammatory environment could modulate the behaviour of MSC.

Thrombosis, Nutrition and Homocysteine

P042

GALLIC ACID, RESVERATROL AND QUERCETIN INTERACT WITH THE SUPPLEMENTARY BINDING SITE OF SALICYLATE ON PLATELET COX-1: IMPLICATIONS FOR POSSIBLE FOOD-FOOD AND FOOD-ASPIRIN INTERACTION

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Epidemiological studies suggest that polyphenol rich diets are associated with reduced risk of cardiovascular disease. Gallic acid, resveratrol and quercetin, belong to three different classes of polyphenols and are present in several beverages and foods of the Mediterranean diet, such as red wine, tea, fruits, vegetables. Based on our previous observation that gallic acid, a polyphenol structurally similar to salicylate, prevents the inhibitory effect of aspirin (a polyphenol-based drug) on platelet COX-1, we extended this study to the interaction of gallic acid with two natural polyphenols, i.e. resveratrol and quercetin. We first characterized the platelet antioxidant effect of the three molecules by measuring the intraplatelet Radical Oxygen Species (ROS) production induced by 2.5 microM arachidonic acid (AA). The compounds showed similar antioxidant effects, with IC50s of 10±4, 35±8 and 38±1 μM for resveratrol, gallic acid and quercetin, respectively. Resveratrol and quercetin also inhibited PRP aggregation induced by 0.6-1 mM AA (IC50: 44±18 and 13±18 μM) or 0.6-1.5 μM U46619 (IC50: 94±22 and 266±34 μM); resveratrol, but not quercetin, inhibited 1.5-2 μg/mL collagen-(IC50: 56±6 μM) and 10-20 μM TRAP-(IC50 270±55 μM) induced aggregation. Gallic acid, ineffective by itself, completely restored AA-induced platelet aggregation inhibited by the two other polyphenols. Similar effect was observed on AA-induced platelet TxB2 production. To unravel the structural basis of this interaction, in silico molecular modelling studies and molecular dynamics simulation were performed by docking the polyphenols into the crystal structure of COX-1. The results showed that gallic acid, resveratrol and quercetin all form a stable complex at Arg 120, the same binding site of salicylate, aspirin and other NSAIDs, with docking scores of -16.69, 20.70 and 22,71 kJ/mol and interaction energies of -172.10, 247.74 and 279.33 kJ/mol, respectively. These results provide a plausible molecular explanation to the pharmacological interaction between three different polyphenols at the level of platelet COX-1. A functional interaction has been also described for gallic acid with the platelet anti-COX-1 activity of aspirin. If these interactions will be confirmed *in vivo*, their possible relevance on the healthy value of the Mediterranean diet and on polyphenol components interference with aspirin antiplatelet effect should be investigated.

P043

EFFECT OF A DAIRY PRODUCT (PECORINO CHEESE) NATURALLY RICH IN CIS-9, TRANS-11 CONJUGATED LINOLEIC ACID ON LIPID, INFLAMMATORY AND HAEMORHEOLOGICAL VARIABLES: AN INTERVENTION STUDY

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Background. Some studies recently reported a beneficial role for conjugated linoleic acid (CLA)-enriched dairy products on plasma lipoprotein profile of healthy subjects. Aim of this study was to evaluate the influence of a short-term dietary intake of a sheep cheese naturally rich in CLA on several atherosclerotic biomarkers. *Methods.* Ten subjects (6 F; 4 M) with an average age of 45.6 (SD: 14.5 years) entered into a cross-over intervention study. After a run-in period, the subjects followed for 10 weeks a diet containing 200 g/week of cheese naturally rich in CLA (Test period) and for the same period a diet containing a commercially available cheese of the same quantity (Placebo period). These periods were alternated by a wash-out period of 10 weeks. We evaluated lipid parameters, inflammatory markers, platelet aggregation induced by collagen and arachidonic acid, and haemorheological profile. *Results.* The test period did not significantly affect body weight, blood pressure, and lipid profile. On the other hand, consumption of the dairy product nat-

urally rich in CLA determined a significant ($p<0.05$) improvement of inflammatory parameters such as interleukin-6 [pre: 10.9 (1.3-52.6) *vs.* post: 5.2 (0.4-43.8) pg/mL], and interleukin-8 [pre: 48.8 (21.2-135.9) *vs.* post: 28.4 (21.4-174.3) pg/mL,] whereas no significant differences in the placebo period were observed. With regard to haemorheological parameters, the test period significantly ameliorated erythrocytes' filtration rate (pre: 7.9±2.4% *vs.* post: 8.9±2.1%; $p=0.01$) with respect to the placebo period that showed no significant changes. Moreover, a favourable effect on platelet aggregation, induced by arachidonic acid [pre: 85.8± 6.8% *vs.* post: 79.6±11%] was observed during the test period in comparison with the placebo period [pre: 84±3.1% *vs.* post: 89.9±8.3%]. *Conclusions*. Dietary short-term intake of the tested dairy product naturally rich in CLA appeared to impose favourable biochemical changes of atherosclerotic markers, with regard to lower circulating levels of inflammatory cytokines, and platelet aggregation, as well as a better haemorheological profile.

P044

INFLUENCE OF SHORT-TERM FISH EATING ON LIPID, FIBRINOLYTIC, AND RHEOLOGICAL PARAMETERS IN HEALTHY SUBJECTS

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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). However, the mechanisms underlying this protection have not been fully elucidated. Aim of this study was to evaluate the influence of short-term dietary intake of fish on biomarkers related to the atherosclerotic process. *Materials and Methods*. For a period of 10 weeks, 10 healthy subjects (6 males; 4 females) with a mean age of 48 years consumed during their main meals contents of about 300 g per week of tuna meat, each subject consuming 150g dorsal and 150g ventral muscle slides from two bluefin tunas (Thunnus thynnus). The bluefin tunas which supply the muscle slides were captured as wild and transferred for five months in sea cage of a tuna farm until they reached around 44 kg live weight. During the fattening phase, fish were fed a mixed diet consisting of small raw pelagic seafood species (anchovies, mackerel, sardines, herrings, cephalopods). After tunas slaughtering and sectioning, dorsal and ventral muscles were analysed for proximate analyses, total lipid content and quantitative fatty acid composition (C23 as internal standard) using a 30 meters Stabilwax capillary column. Dorsal and ventral muscle slides contained respectively: 62.0% and 45.7% moisture; 22.5% and 16.2% protein; 12% and 35% lipid; 11.8% and 12.0% EPA; 14.3% and 15.2% DHA; 29.4% and 28.8% SFA; 32.3% and 32.5% MUFA; 33.4% and 33.8% n-3 PUFA. Mercury content was always below the safety threshold (CE 1881/2006, 9.12.2006). Each dorsal and ventral muscle slide was packed under vacuum, identified and stored at - 80°C until its use for the intervention trial. Guidelines were given to cook both dorsal and ventral samples: 2' in microwave oven, then add salt and/or olive oil. Lipid (total cholesterol, HDL-cholesterol, LDL-cholesterol and tryglicerides), fibrinolytic [clot lysing time (CLT), plasminogen inhibitor activator-1 (PAI-1), tissue factor pathway inhibitor (TAFI)], and haemorheological parameters [whole blood viscosity (WBV), plasma viscosity (PV), erythrocyte filtration rate (EF)], were determined in samples obtained at the beginning (T0) and at the end of the experimental period (T1). *Results.* Lipid profile showed a significant improvement at the end of the dietary intervention, as seen by lower levels of total cholesterol [T1: (200.2±49.5 mg/dL) *vs.* T0: (219.3±48.2 mg/dL); $p=0.01$], LDL-cholesterol [T1: (125.8±40.9 mg/dL) *vs.* T0: (140.2±46.6 mg/dL); $p=0.02$], and triglycerides [T1: (83.7±40.2 mg/dL) *vs.* T0: (112.1±57.8 mg/dL); $p=0.002$]. With regard to haemorheological parameters, a significant ($p<0.05$) improvement of WBV at both highest and lowest shear rates was reported (WBV 94.500 sec-1: 4.3±0.2 *vs.* 4.5±0.4; WBV 0.512 sec-1: 20.1±2.2 *vs.* 21.8±2.5, for T1 and T0, respectively). Moreover, interestingly, as regarding fibrinolytic parameters, dietary intervention with fish reported a significant increase of CLT, [T1: (57.7±9.5 min.) *vs.* T0: (47.1±14.7 min.); $p<0.05$], possibly determined by the concomitant increase of PAI-1 [T1: (20.8±15.9 mg/dL) *vs.* T0: (12.5±10.5 mg/dL); $p=0.01$] and TAFI [T1: (13.7±1.36 μg/mL) *vs.* T0: (11.6±1.36 μg/mL); $p=0.01$] levels. *Conclusions.* Dietary short-term intake of fish seems to impose favourable biochemical changes in healthy subjects, as showed by lipid and haemorheological parameters. An impaired fibrinolysis has been otherwise reported at the end of the dietary intervention.

P045

ORANGE JUICE INTAKE DECREASES THE PROCOAGULANT ACTIVITY OF WHOLE BLOOD IN HEALTHY VOLUNTEERS

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Background. Numerous epidemiological studies suggest that exposure to flavonoid-rich fruits have beneficial influences on risk factors for cardiovascular disease. Flavonoids contribute to this protection by counteracting oxidative stress, inflammation and reducing the expression of genes associated with the ischemic disorders. Since we have previously found that whole blood (WB) procoagulant activity (PCA) is positively associated with some cardiovascular risk factors we investigated whether intake of orange juice could affect WB PCA. Methods: The study was carried out in 17 healthy subjects (aged 31±SD), 10 males and 7 females. The subjects were randomized to receive according to a cross over design either *blood* or *blond* orange juice, i.e. containing or not anthocyanins, respectively. After a 2 week run-in period on a controlled diet, the subjects were randomized allocated to receive orange juice for 4 weeks. After 6 weeks of wash out, each subject was then crossed to the other treatment for further 4 weeks. Blood samples were collected after overnight fasting before and at the end of each treatment period. Blood was drawn and incubated with or without bacterial endotoxin (LPS), tumor necrosis factor-alpha (TNF-α) or both at 37°C for 2 h. At the end of incubation, PCA was evaluated by a one-stage clotting assay. Statistical analysis of data followed the two-stage procedure, with the test of Population-Attributable Risk calculations (PAR) performed at the nominal level of 0.5%. *Results.* WB expressed a strong PCA following stimulation with LPS, TNF-alpha or both. Both *blood* and *blond* orange juices prolonged the clotting time of unstimulated WB by 58.1±21.4 and 59.3±15.1 (sec±SE), respectively. By contrast, neither *blood* nor *blond* juice affected LPS- and TNF-alpha-stimulated WB PCA. When data were analyzed considering the two treatments as a whole, a statistically significant difference could be observed in LPS or TNF-alpha stimulated WB PCA of subjects before and after intake. *Conclusions.* Our results suggest that orange juice intake, by decreasing WB PCA, could exert a beneficial effect on risk factors associated with cardiovascular disease; moreover, mechanisms other than anthocyanin levels may play a role. *Supported by EC 6FP Food-CT-2005-007130.*

P046

ASSOCIATION BETWEEN DIETARY PATTERNS, CARDIOVASCULAR RISK FACTORS AND C-REACTIVE PROTEIN IN A LARGE HEALTHY ITALIAN POPULATION: RESULTS FROM THE MOLI-SANI STUDY

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Background. CVD risk factors and markers of inflammation are influenced by dietary habits. *Hypothesis-oriented* scores have generally been used, but they do not account for correlations between foods. *Aim.* To evaluate the association of non pre-defined, empirically derived dietary patterns with CVD risk factors and C-reactive protein (CRP). *Methods*. We analyzed 5.120 healthy subjects from the Moli-sani project, a cohort study of men and women older than 35, randomly recruited from a general population of Southern Italy. The EPIC food frequency questionnaire was used. Dietary patterns were generated using principal factor analysis (PFA), that provides a representation of how foods are consumed together, and reduced rank regression (RRR), that extracts the combinations of foods maximally associated with responses. We used 51 food groups as predictors and 5 CVD risk factors as responses: total cholesterol, triglycerides, systolic pressure, glucose and CRP. CVD risk was calculated applying the equations of the CUORE project. *Results.* Three dietary patterns were identified by PFA. The *Pasta & Meat* pattern, characterized by high intake of pasta, tomato sauce, red and processed meat, animal fats and alcohol, was positively associated with body mass index (BMI), glucose, total cholesterol, HDL, LDL, triglycerides, CRP, diastolic pressure and CVD risk. The *Olive Oil & Vegetables* pattern, characterized by high intake

of olive oil, vegetables, legumes, soups, fruits and fish, was associated with increasing values of BMI and with decreasing values of triglycerides and CVD risk in men. The *Eggs & Sweets* pattern, characterized by high intake of eggs, margarines, butter, sugar and sweets, was associated with increased values of CRP. The RRR pattern, characterized by high intake of pasta, animal fats and alcohol and by low intake of breakfast cereals and yogurt, was associated with high levels of total, LDL and HDL cholesterol, triglycerides, glucose, BMI, systolic pressure and CRP, and it was very similar to the *Pasta & Meat* pattern. *Conclusions*. In a large healthy Italian population, 2 empirically derived dietary patterns characterized by mostly unhealthy foods were associated with higher levels of cardiovascular risk factors and CRP, whereas a *prudent-healthy* pattern was not. This confirms that the relationship between diet and CVD risk can be reliably investigated without prior information on single food and health, using statistical tools such as PFA and RRR.

P047

MODERATE CONSUMPTION OF DARK CHOCOLATE IS ASSOCIATED WITH LOW PLASMA LEVELS OF C-REACTIVE PROTEIN IN A LARGE HEALTHY ITALIAN POPULATION: RESULTS FROM THE MOLI-SANI STUDY

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Background. Flavonoids are associated with reduced inflammatory reactions. Dark chocolate (DC) contains high concentrations of flavonoids, and may have anti-inflammatory properties. *Aim.* To evaluate the association of DC intake with C-Reactive Protein (CRP) levels. *Methods.* The Moli-sani Project is an on-going cohort study of men and women aged ≥35, randomly recruited from a Southern Italy general population. Until July 2007, 10,994 subjects had been enrolled. Among 4,849 subjects apparently free of any chronic disease, 1,317 subjects who declared they did not eat any chocolate during the past year (mean age 53±12; 51% males) and 853 subjects who did eat chocolate but only in the form of DC (50±10; 55% males) were selected. High sensitivity-CRP plasma levels were measured by an immunoturbidimetric method (IL, Milan, Italy). The EPIC Food Frequency questionnaire was used to determine nutritional intake. Differences in CRP distribution were estimated by binomial Poisson regression. *Results.* DC consumers were younger, had a higher social status and a lower physical activity than non-consumers ($p<0.0001$). They consumed less cereals, meat and alcoholic beverages ($p<0.0001$), more nuts and seeds, fish, sweet confectionary, sweet beverages, coffee and tea ($p<0.05$) and had a higher intake of total energy ($p<0.0001$), and of all micro and macro-nutrients with the exception of total carbohydrates and total dietary fiber. After adjustment for age, sex, social status, physical activity, systolic blood pressure, BMI, waist to hip ratio, food groups and total energy intake, DC consumption was inversely associated with CRP ($p=0.034$). When adjusted for nutrient intake, analyses showed similar results ($p=0.019$). CRP mean values were 1.28 (1.22-1.34) and 1.17 (1.10-1.24) mg/L, in non consumers and in DC consumers, respectively. The prevalence of non-consumer subjects with CRP>3 mg/L was 19% while it was 14% in DC consumers. A U-shaped relationship between DC consumption and CRP was observed: consumers with a low intake of DC (up to 6.7 grams/day) had CRP levels lower than either non consumers or high consumers. *Conclusions.* Moderate dark chocolate intake is associated with reduced C-reactive protein levels in a healthy adult Italian population. Our findings suggest that regular moderate dark chocolate consumption may reduce inflammatory response.

P048

EFFECT OF SHORT-TERM CONSUMPTION OF BREAD OBTAINED BY A SELECTED *HEALTHY* ITALIAN GRAIN VARIETY ON LIPID, INFLAMMATORY AND HAEMORHEOLOGICAL VARIABLES: AN INTERVENTION STUDY

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Background. Epidemiological studies have strongly suggested that dietary habits play a crucial role in the prevention of cardiovascular disease. In particular, consumption of fruits, vegetables, and grains has been associated with reduced risk of chronic diseases. To date, beneficial capacity of different grains varies substantially in relation to cultivar and location of the varieties. The aim of this study was to evaluate the influence of short-term dietary intake of bread obtained by a selected variety of grain grown in Tuscany, Italy, and found to be naturally rich in antioxidants and B-group vitamins on biomarkers related to the atherosclerotic process. *Methods.* After a run-in period, 20 healthy subjects (9 F; 11 M) with a median age of 39.5 years (range: 21-61) were studied. The subjects followed for 10 weeks a diet containing 150 g/die of bread obtained by the test grain (Test period) and for the same period a diet containing commercially available bread of the same quantity (Control period), after a wash-out period of 10 weeks. We evaluated lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides), and haemorheological profile [whole blood viscosity (WBV), plasma viscosity, erythrocytes' filtration rate (EF)] before and after dietary intervention. *Results.* A general linear model for repeated measurements after adjustment for age, and gender was conducted. The test period determined a significant improvement of total cholesterol (pre: 211.1±44.9 *vs.* post: 196.5±44.4 mg/dL; $p=0.01$), and LDL-cholesterol levels (pre: 133.7±33.1 *vs.* post: 120.9±36.6 mg/dL; $p=0.02$), whereas no significant changes during the control period have been observed. With regard to haemorheological parameters, the test period significantly decreased all the parameters investigated, namely WBV at high (pre: 26.1±2.2 *vs.* post: 24.8±3.3; $p=0.01$) and low shear rates (pre: 5.9±0.4 *vs.* post: 5.7 ± 0.4), as well as EF (pre: 8.4 ± 3.1% *vs.* post: 9.1±2.8%; $p=0.009$) with respect to the control period that showed no significant changes. *Conclusions*. Dietary short-term intake of bread obtained by a grain naturally rich in antioxidants and B-group vitamins seems to impose favourable biochemical changes, with regard to lower circulating levels of markers of atherosclerosis, such as lipid parameters, and haemorheological variables.

P049

FAVOURABLE INFLUENCE OF BERGAMOT JUICE DIETARY INTAKE ON LIPIDIC AND HAEMOSTATIC MARKERS

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Introduction. Bergamot is an italian citrus fruit, product in the province of Reggio Calabria. It recently has been found to be rich in flavonoids that appear to be associated with the prevention of cardiovascular disorders, and exhibit also anti-inflammatory and platelet anti-aggregant activities; it is hypotized but not demonstrated a lipid-lowering property. Aim of this study was to evaluate the influence of short-term dietary intake of Bergamot Juice on biomarkers related to the thrombotic and atherosclerotic risk and verify the hypolipidemic effect. *Material and Methods.* This 4 weeks study was conducted on a population of 24 healthy volunteers, 7 male, 17 female (mean age: 43.6 years, range 22-68). Exclusion criteria were: thromboembolic diseases, renal failure, diabetes, and treatment with any therapies, including vitamins. None of the subjects had significant dietary changes during the study. All the individuals drank 50 mL of bergamot juice daily for the first two weeks, then 100 mL for the second two weeks and were underwent laboratory evaluation at the first day (T0), after two weeks (T1) and at the end of the study (T2). We evaluated total, LDL and HDL cholesterol, triglycerides, plasma fasting homocysteine, tissue-type plasminogen activator (t-PA), Factor VIII (FVIII), plasminogen activator-inhibitor (PAI-1), D-Dimer (DD) and prothrombin fragment 1+2 (F1+2). Results were presented as mean ± SD; the statistical analysis was evaluated by using Student's T test for paired data (p-value significant if less than 0.05). *Results.* Total and LDL cholesterol were significantly lower in second part of the study (T0 *vs* T2 and T1 *vs.* T2). We had a significant reduction of FVIII levels considering all the 4 weeks of the study (T0 *vs.* T2); t-PA was lower considering the last two weeks (T1 *vs.* T2). Other parameters had no significant changes. *Conclusions.* Dietary short-term intake of Bergamot Juice seems to lead to favourable biochemical variations of lipid parameters; this changes seem to be dose-dependent related with the highest juice dosage; the effect on the haemostatic markers was limited only to t-PA and FVIII; the observed reduction of FVIII levels suggests an anti-inflam-

matory effect of bergamot juice. We think that the short period of the study could have limited the influence of the juice on all the atherosclerotic and haemostatic markers; further longer observational studies involving other inflammatory markers are necessary.

Table 1.

	T0 Mean (SD)	*T1* Mean (SD)	*T2* Mean (SD)	*T0 vs T1* p-value	*T0 vs T2* p-value	*T1 vs T2* p-value
Total cholesterol	228,6±45,8	223,8 40,7	210,3±37,9	0,3724	0,0102	0,0063
LDL cholesterol	151,6±37,5	147,5±39,6	139,4±37,7	0,3414	0,0039	0,0191
HDL cholesterol	48,7±11,1	48,5±10,6	47,6±10,9	0,8670	0,3433	0,3751
triglycerides	142,5±124,5	177,5±291,1	115,8±59,4	0,3936	0,2303	0,2978
HCY	14,1 ±11,5	12,2±5,1	13,1±7,9	0,1966	0,3451	0,2736
FVIII	114,0±37,7	106,8±30,5	98,9±28,4	0,1774	0,0109	0,1540
D-Dimer	191,5±43,3	210,0±95,5	193,7±35,3	0,3937	0,8084	0,4301
t-Pa	10,4±7,4	10,6±7,5	9,6±6,5	0,6217	0,0820	0,0226
PAI-1	33,3±22,8	34,8±23,5	33,1±25,1	0,6629	0,9593	0,7315
TAT	2,6±1,1	4,9±11,9	2,7±0,6	0,3563	0,5700	0,3774
F1+2	172,0±66,9	206,9 ±149,9	170,9±72,2	0,2371	0,9105	0,2432

p-value significant if less than 0.05.

P050

INFLUENCE OF ALCOHOL BEVERAGES AND DRINKING PATTERN ON HOMOCYSTEINE CONCENTRATIONS

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Introduction. Homocysteine (Hcy) levels have been shown to be strongly influenced by both genetic and nutritional factors. Among nutritional and lifestyle habits, some contradictory studies on the effects of alcohol consumption on Hcy have been reported. These conflicting results may derive in part from the different types of alcoholic beverages consumed. Aim of this study was to investigate, in a large group of healthy Italian subjects, the role of alcohol and different drinking pattern on Hcy and other emerging thrombophilic parameters such as lipoprotein (a) and B-group vitamins. *Material and Methods.* We tested the relationship between alcohol consumption and preference of alcoholic beverages on atherosclerotic and thrombophilic parameters in a cross-sectional study of 932 healthy Italian subjects [median age: 66 years (15-88); 122 M, 140 F]. Hcy (a) levels were measured by FPIA method (Abbot, Norway), and lipoprotein (a) by an ELISA method [Mercodia Lp (a), Uppsala, Sweden]. *Results.* Of 932 healthy subjects enrolled in the study, 701 (75.2%) were regular drinkers whereas 129 (13.8%) were abstainers and 102 (11%) teetotallers. Drinkers reported to have significantly higher Hcy levels [10.2 (4.4-80.6) *vs.* 9.5 (0.7-25.5) μmol/L; $p<0.05$) with respect to abstainers and teetotallers, whereas no significant differences for the other parameters investigated have been reported. In order to investigate the relationship between type and amount of alcohol consumption and thrombophilic risk factors we performed a general linear model after adjustment for possible confounders. By dividing the drinkers into categories according to the number of drinks consumed per day [<1 alcoholic unit (A.U.); 1-2 alcoholic unit; >2 alcoholic unit), we could observe that Hcy was significantly related to the amount of drinks consumed [<1 A.U.: 10 (9.5-10.5); 1-2 A.U.: 11.4 (10.6-12.3); >2 A.U.: 11.7 (10.7-12.7) μmol/L; p for trend <0.05] whereas no relationship between vitamin B6 and alcohol was observed. Moreover, when analyses according to the drinking pattern were performed, we could demonstrate that the influence of alcohol on Hcy levels remained to be significant only among wine drinkers (p for trend <0.05) but not among beer drinkers ($p=0.9$). *Conclusions.* This study indicate that alcohol consumption determines a significant increase of Hcy levels, whereas no significant difference for lipoprotein (a) and B-group vitamins has been reported. The present findings seem to confirm previous findings of a positive significant relationship between wine, but not beer, consumption and Hcy plasma levels.

P051

MILD HYPERHOMOCYSTEINEMIA IS ASSOCIATED WITH IMPAIRED PLASMA FIBRINOLYSIS: RELATIVE IMPORTANCE OF TAFI AND FIBRINOGEN

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Mild hyperhomocysteinemia (HHcy) has been shown to impair fibrinolysis by altering the fibrinogen structure and making clots resistant to pharmacological t-PA concentrations. Using an *in vitro* model of physiological relevance, we investigated whether and how HHcy influences plasma fibrinolytic potential. We studied 176 patients with previous venous thromboembolism, 58 with HHcy and 118 with normal total homocysteine (tHcy) levels (NHcy), at least 3 months after withdrawal of antithrombotic therapies. Plasma fibrinolytic potential was measured as the fibrinolysis time of tissue factor-induced clots exposed to 15 ng mL-1 t-PA. Fibrinolysis time was longer in HHcy than in NHcy patients (105±43 *vs.* 91±26 min, $p=0.008$). Moreover, HHcy patients displayed higher levels of TAFI (157±35 % *vs.* 139±34 %) and factor-VIII (162±66% *vs.* 135±44%) but similar PAI-1, fibrinogen and endogenous thrombin potential. By multivariate analysis, plasma tHcy was identified as an independent predictor of fibrinolysis time ($p<0.001$). The difference in fibrinolysis time between HHcy and NHcy was unchanged when native fibrinogen was replaced by purified fibrinogen but disappeared when the assay included the TAFIa inhibitor, PTCI. Opposite results were obtained when the assay was performed using 500 ng/mL t-PA. This suggests that hypofibrinolysis in HHcy plasma was mainly TAFI-mediated at physiological t-PA concentrations and principally fibrinogen-related at pharmacological t-PA concentrations. The acute increase of tHcy either *in vivo* (after an oral methionine load) or *in vitro* (after incubation of normal plasma with 0.5 mM DL-Hcy) had no effects on fibrinolysis or TAFI levels. In conclusion we describe a TAFI-related hypofibrinolytic state in mild HHcy which might have pathophysiological relevance and account for the reported heightened thrombosis risk; however, it is unknown whether HHcy is causally related to hypofibrinolysis or an associated bystander.

P052

IN VIVO OXIDATIVE STRESS AND PLATELET ACTIVATION IN SUBJECTS WITH MODERATE HYPERHOMOCYSTEINEMIA DUE TO MTHFR 677 C→T POLYMORPHISM

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Objective. The methylenetetrahydrofolate reductase (MTHFR) 677 C->T polymorphism may be associated with elevated homocysteine (Hcy) levels, an independent risk factor for cardiovascular disease. We evaluated *in vivo* lipid peroxidation and platelet activation in carriers of the MTHFR 677 C→T polymorphism and in non-carriers, and their correlation with Hcy and folate levels. *Methods.* A cross-sectional comparison of urinary 8-iso-prostaglandin (PG)F2α and 11-dehydro-thromboxane (TX)B2 (markers of *in vivo* lipid peroxidation and platelet activation, respectively) was performed in 100 carriers of the polymorphism (50 with and 50 without hyperhomocysteinemia), and 100 non-carriers (50 with and 50 without hyperhomocysteinemia). A methionine-loading test was performed in 12 carriers with Hcy levels <15 micromol/L. Furthermore, folic acid was administered to 23 polymorphism carriers with Hcy levels >15 micromol/L. *Results.* Urinary 8-iso-PGF2α and 11-dehydro-TXB2 were higher in carriers with than in those without hyperhomocysteinemia ($p<0.0001$). Non-carriers with hyperhomocysteinemia showed lower folate levels ($p<0.0001$), higher 8-iso-PGF2α ($p<0.01$) and 11-dehydro-TXB2 ($p<0.01$) than those with Hcy <15 micromol/L. Carriers with hyperhomocysteinemia had lower folate levels ($p=0.0006$), higher urinary 8-iso-PGF2α ($p<0.0001$) and 11-dehydro-TXB2 ($p<0.0001$) than non-carriers with hyperhomocysteinemia. On multiple regression analysis, high Hcy ($p<0.0001$), low folate ($p<0.04$) and MTHFR 677 C→T polymorphism ($p<0.001$) independently predicted high 8-iso-PGF2α excretion rates. A methionine-loading increased Hcy ($p=0.002$), and both urinary metabolites levels ($p=0.002$). Folic acid supplementation led to a reduction in plasma Hcy ($p=0.0001$), and urinary 8-iso-PGF2α and 11-dehydro-TXB2 ($p<0.0003$). *Conclusions.* Hyperhomocysteinemia due to the MTHFR 677 C→T polymorphism is associated with enhanced *in vivo* lipid peroxidation and platelet activation, reversible, at least in part, with folic acid supplementation.

P053

THE ROLE OF HYPERHOMOCYSTEINEMIA AND MTHFR C677T GENOTYPE IN PATIENTS WITH RETINAL VEIN OCCLUSION: THE EXPERIENCE OF TWO CENTRES

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Introduction. Elevated Homocysteine (Hcy) level (higher than 15 micromol/L) is considered a risk factor for vascular occlusive diseases. It also may be a result of the thermolabile C677 variant (C677T) of the Methylentetrahydrofolate reductase (MTHFR) gene. In the recent years several studies have suggested that hyperhomocysteinemia (HHcy) may be a potential risk factor in retinal vein thrombosis (RVT). The aim of this study was to investigate the relationship between homocysteine, C677T MTHFR genotype and RVT retinal vascular occlusion, in comparison with healthy controls. *Materials and methods.* We evaluated the Hcy plasma level of 3114 consecutive subjects visited in the Thrombosis Centres of Reggio Calabria and Catanzaro in a 3 years period. We found 235 patients with HHcy (7.5%). 99 of these had thrombotic events and 136 were healthy subjects studied for a familial history of thromboembolism or healthy women underwent screening for thrombophilia before taking oral contraceptives. 20 of these 99 patients had RVT: the high prevalence of HHcy in the RVT subgroup (20.2%) suggested to investigate a possible relationship between RVT and HHcy. To evaluate if HHcy may be an independent risk factor for RVT, a retrospective study was performed: 105 consecutive patients (46 M; 59 F) (age 22-86, mean 58.4) with recent diagnosis of RVT were compared with 226 age and sex matched healthy controls. Patient group exclusion criteria: any other prothrombotic risk factor, renal failure, cancer and other vascular diseases. The statistical analysis was performed using chi-square test; *p*-value was significant if less than 0.05. *Results.* HHcy was documented in 36/105 patients (34.3%) with RVT and in 32/226 of healthy control subjects (14.2%). The difference in prevalence of hyperhomocysteinemia was statistically significant ($p<0.001$) in comparison with controls. The MTHFR C677T genotype was found in 69/105 (65.7%) patients with RVOD (eterozygosity: 40/105, homozygosity: 29/105). The group of controls showed the presence of MTHFR C677T genotype in 169/226 subjects (74.8%) (eterozygosity: 100/226, homozygosity: 69/226): this distribution did not significantly differ between the two groups ($p=0.08$). *Discussion.* Our data suggest an association between RVT and HHcy: its assessment may be important in the investigation, management and follow up of patients with RVT but, as reported in literature, the C677T MTHFR genotype can't be considered an independent risk factor.

Coronary and Cerebral Thrombosis

P054

EFFICIENCY AND RISK OF BLEEDING OF THE ORAL ANTICOAGULATION TREATMENT IN ELDERLY ATRIAL FIBRILLATION PATIENTS

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Background. Randomised controlled trials have shown that oral anticoagulation therapy (OAT), at INR of 2.5, is highly effective in the reduction of ischemic stroke in patients with AF. Evidence-based clinical practice guidelines recommend the use of OAT in patients with AF at high risk of stroke, which included age >75 years. Nevertheless, OAT remains largely under-used in older patients, mainly related to physicians' fear of major bleeding complications. We evaluated the efficacy and safety of OAT in a elderly population with AF with respect to age and bleeding events. *Methods.* We retrospectively examined 430 patients > 65 years starting OAT for AF (target INR 2.5) and routinely followed at our Anticoagulation Clinic (mean follow-up 3.9 years). Comorbid conditions and potential interacting drugs have been also analysed. Anticoagulation control, the incidence of stroke, systemic embolism, major and minor hemorrhagic complications were assessed. *Results*. Of 430 included patients (236 M, median age 77.5 years), 28.4% experienced bleeding (20 major, 190 minor). There were 4 stroke (0.9%), 3 TIA (0.7%) and 6 (1.4%) systemic embolisms. The rate of bleeding complications was not statistically significantly different between AF patients and all the other patients in relation to gender ($p=0.58$), mean comorbidity ($p=0.50$), number of medications ($p=0.90$) and age ($p=0.80$), even stratified <75, ≥75<80 and ≥80 ($p=0.21$). The annual event rate of stroke/TIA, death, major and minor bleeding was 0.2%, 1.8%, 1.2% and 11.3% respectively and was similar to that recorded in randomised trials (differences [95% CI] -1.2% [-2.5±0.1%] for stroke, -0.5% [-1.2±0.2%] for TIA, -1.8% [-3.9±0.3%] for deaths, -0.4% [-1.8±1.0%] for major bleeding, 2.1% [-1.9±6.1%] for minor bleeding). On the other hand, our study patients were older than those in the randomized trials (>75 years, 68.4% *vs.* 20.0%; differece 48.4% [IC 95%, 43.3-54.1]). *Conclusions.* No significant association between age and bleeding emerged in this elderly cohort study, even in patients ≥80. The efficiency of OAT and hemorrhage rates in these practice settings was generally comparable with those reported in previous randomized trials, although our patients were older and sicker. A carefully monitored OAT can be used with reasonable safety in older patients. This preventive treatment is likely to confer additional benefit as it is more widely prescribed.

P055

DECREASE FACTOR VIIA/ANTITHROMBIN COMPLEXES IN A GROUP OF CHILDREN WITH ISCHEMIC STROKE

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Background. The procoagulant effect of FVIIa/TF complex that acts through FIX and FX activation, is inhibited by antithrombin (AT) throughout the formation of AT/FVIIa complexes and more rapidly, by TFPI throughout the irreversible formation of quaternary complex (TFPI,TF,FVII,FX). The clinical significance of plasma levels of AT/FVIIa complexes is still undefined. *Patients and methods*. We measured FVIIa/AT complex plasma levels in 40 children who experienced ischemic stroke and in 23 healthy controls comparable for age. FVIIa/AT complex levels were determined in plasma by a specific ELISA kit supplied by Diagnostica STAGO (Asniéres, France). *Results.* FVIIa/AT complex plasma levels (mean ± standard deviation, SD) were markedly reduced in subjects with ischemic stroke (175.25±87.7 pM) as compared to controls (225.65±88.2 pM). The difference between cases and controls was statistically significant ($p<0.03$). *Conclusions*. Low FVIIa/AT complex plasma levels in children with previous ischemic stroke could reflect the reduction in FVII plasmatic concentrations. Some authors reported an increased TFPI secretion associated with ischemic events. This could determine a rapid formation of quaternary complex with less disponi-

bility of FVII for FVIIa/AT complex formation. A reduction in FVIIa/AT plasma levels could be a marker of arterial ischemic damage.

P056

PITUITARY FUNCTION IN YOUNG ISCHEMIC STROKE: PRELIMINARY STUDY

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Both animal and human studies suggest that the GH-IGF axis is involved in the pathogenesis of ischemic stroke. The aim of this study was to assess the presence of endocrine alterations in young patients experiencing ischemic stroke. At this purpose, in 13 patients with a history of ischemic stroke, pituitary function was tested 6-12 months after the thrombotic event. In all patients (5 Males, 8 Females; aged 15-50 yrs; BMI 26.9±3.6 kg/m^2), basal endocrine parameters and the GH response to GHRH + arginine test (using BMI-dependent cut offs) were evaluated. Hypopituitarism was found in 38.5% of the patients. The most common pituitary deficits were, in decreasing order: GH deficit in 23.1%, LH/FSH deficit in 15.4%. In contrast, deficit of ACTH, deficit of TSH, and diabetes insipidus were not recorded in any patients. In conclusion, hypopituitarism was found in young patients 6-12 months after an episode of ischemic stroke. Thus, endocrine evaluation and neuroendocrine follow-up of patients experiencing ischemic stroke should be performed on a regular basis, in order to monitoring pituitary function and, eventually, providing appropriate replacement treatment. Whether this finding can influence the clinical outcome of the ischemic disease remains to be clarified.

P057

CD40 LIGAND AND MCP-1 LEVELS AS PREDICTORS OF CARDIOVASCULAR EVENTS IN LACUNAR AND NON-LACUNAR STROKES

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Background. Upregulation of soluble CD40 ligand (CD40L) and of the monocyte chemoattractant protein-1 (MCP-1) has been found in patients with acute cerebral ischemia. We asked whether (i) the two molecules are similarly upregulated among non-lacunar and lacunar strokes and (ii) CD40L and/or MCP-1 may predict the risk of cardiovascular events in the two subtypes of ischemic stroke. *Methods.* Ninety patients with type 2 diabetes mellitus presenting with an acute ischemic stroke (compared with 45 control subjects) were evaluated on admission and up to 36 months (median 24 months) after the event. *Results. P*atients with acute stroke had higher plasma CD40L and MCP-1 than controls (p<0.0001), with no significant differences among lacunar and non-lacunar ones. On multiple regression analysis only higher sCD40L quartiles and older age were associated with higher MCP-1 quartiles. Forty-eight % patients experienced vascular events. Cox regression analysis showed that only the presence of higher sCD40L values independently predicted the recurrence of vascular events. *Conclusions.* Platelet-monocyte interaction, through upregulation of inflammatory molecules such as CD40L and MCP-1, is involved in the advanced stage of atherosclerotic cerebrovascular disease.

P058

CEREBRAL VENOUS THROMBOSIS IN ADULTS: A TEN YEARS EXPERIENCE

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Background. Cerebral venous thrombosis (CVT) is an uncommon disorder most often affecting young adults, with a potentially disabling or fatal outcome. It may be a diagnostic challenge due to variability of clinical presentation, and some shadows still exist on its treatment. *Methods.* We describe 44 consecutive patients, aged 44.4±10.6 years, admitted for CVT between 1998 and 2007. The diagnosis was confirmed by cerebral MR with MR venography, and/or by angiography. Each patient was evaluated for congenital and acquired thrombophilia, and for other prothrombotic conditions. Anticoagulant therapy, namely i.v. UFH and/or s.c. LMWH, followed by warfarin was given to all patients. Eventual complications along the clinical course were recorded. Patients were followed for at least 6-12 months, and the outcome was assessed using the modified Rankin Scale (mRS). *Results.* Most of patients were females (68.2%), and 79.5% of cases were younger than 50 years. Prothrombotic conditions were detected in 37 (84.1%) patients. In particular, an inherited or acquired thrombophilia was identified in 14 patients, with a relevant prevalence of the prothrombin G20210A heterozygosis (6 cases). Other prothrombotic states were identified in 31 patients. Among females, the most frequent risk factor was oral contraceptives intake (66.6% of women). Multiple risk factors were seen in 15 cases. Clinical presentation was acute or subacute in 34 (77%) patients, thus requiring intensive care admission in 12 cases. Intracranial bleeding at CVT onset was seen in 18 (41%) patients; nevertheless, heparin was always given, and the progression of a pre-existing cerebral hemorrhage occurred only in 1 case. Despite anticoagulant therapy, one patient showed a CVT worsening, and she was successfully treated with local thrombolysis. Complications were seen in 10 (22.7%) patients, namely pulmonary embolism (6), psoas muscles haematoma (1), heparin-induced thrombocytopenia (1), deep venous thrombosis (1), and obstructive hydrocephalus (1). Only one patient died for CVT progression, whereas a favourable outcome was seen in most of patients, with a good mRS (0-1) in 84.2% of cases. *Conclusions.* In our experience, prothrombotic risk factors were often detected, either alone or combined, suggesting that patients with CVT need a careful investigation. Anticoagulant treatment was not only effective, leading to complete recovery in most of patients, but also safe even in the presence of intracranial bleeding.

P059

CRYPTOGENIC STROKE: TIME TO DETERMINE AETIOLOGY

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About 80% of strokes are ischemic. Strokes remaining without a definite cause after an extensive work-up are classified as cryptogenic(CS) and make up to about 30-40% of all strokes. Stroke etiology can remain undetermined because: a) the cause of stroke is transitory or reversible and the diagnostic work-out is not done at the appropriate time; b) known causes of stroke are not accurately investigated; and c) some causes of stroke are unknown. Recent studies have challenged the previous view that CS is a relative benign cerebrovascular event and showed that CS is associated with a high rate of recurrence and adverse outcome at long term follow-up. Determining stroke etiology is a valuable procedure against the risk of stroke recurrence, especially in young patients. We discuss new evidences on etiology of CS specifically focusing on patient with patent foramen ovale (PFO) and aortic arch atheroma (AAAs). The frequency of PFO is higher in patients with CS than in the general population. The combined evidence of PFO with atrial septal aneurysm seems to be associated with a stronger risk for recurrent neurological events. Paradoxical embolism of thrombus or small clots from the peripheral venous system during right-to-left shunt is suspected to be the main cause of stroke associated with PFO. There is no consensus on the optimal management strategy, but treatment options include antiplatelet agents, warfarin, percutaneous device closure and surgical closure. We reviewed the literature and designed a diagnostic and treatment algorithm for patients with PFO and AAAs. In clinical practice, stroke etiology can be identified in more than half of the patients by using routine diagnostic procedures. To reduce the proportion of stroke of undetermined etiology, the following examinations as part of the assessment of a CS should be performed: a) ECG Holter or event-loop recording (ELR 7-day ambulatory ECG monitoring) for detection of atrial fibrillation; b) transcranial Doppler is mandatory in patients with suspected PFO. If positive, transesophageal echocardiography(TEE) to confirm and characterize the atrial septal anatomy should be performed. Lower limb compression ultrasonography showing a deep vein thrombosis reinforces the relationship between PFO and stroke; c)TEE is able to detect AAAs and plaques greater than 4 mm that require an appropriate antithrombotic treatment.

P060

A PRASUGREL 60 MG LOADING DOSE ACHIEVES FASTER ONSET AND HIGHER LEVELS OF PLATELET INHIBITION COMPARED WITH 300 MG AND 600 MG CLOPIDOGREL LOADING DOSES

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Background. In recently reported studies, a prasugrel 60 mg (Pras 60) loading dose (LD) caused greater inhibition of platelet aggregation than a clopidogrel 300 milligrams (Clop 300) LD. A clopidogrel 600 milligrams (Clop 600) LD increased the speed of onset and magnitude of IPA compared to Clop 300. To date, the inhibition of platelet aggregation with Clop 600 has not been compared to Pras 60. This study compared the inhibition of platelet aggregation after LDs of Pras 60, Clop 600, and Clop 300 and maintenance doses (MD) of Pras 10 or Clop 75. Methods: LDs and MDs of Pras 60 and 10 were compared with Clop 600 and 75 or Clop 300/75 in 41 healthy subjects, without ASA, in a 3-period crossover study. The LD was followed by a 7-day daily MD and a 14-day washout period. IPA to 20 micromolar ADP was measured by turbidometric aggregometry. *Results.* As early as 30 minutes after LD, Pras 60 showed higher IPA (52.1%) than either Clop 600 (4.3 percent) or Clop 300 (1.3 percent) (p minor than 0.001). At 1 and 2 hr after Pras 60 LD, IPAs of 79.6 percent and 88.4 percent, respectively, were achieved. Both values were higher than the peak achieved at 6 hr by either Clop 600 (66.5 percent) or Clop 300 (48.6 percent) (both p minor than 0.001). During MD, Pras 10 achieved greater IPA than Clop 75 mg (p minor than 0.001) on all days (day 2-9; measured before daily dose). Conclusions: A Pras 60 LD achieved more rapid and greater IPA than LDs of Clop 600 or Clop 300. The Pras 10 MD achieved more consistent and higher levels of IPA than a Clop 75 MD. The results of the TRITON -TIMI 38 trial support the hypothesis that higher levels of IPA are associated with improved outcomes.

P061

RELATIONSHIP BETWEEN HIGH PLATELET TURN-OVER AND PLATELET FUNCTION IN HIGH RISK PATIENTS WITH CORONARY ARTERY DISEASE ON ANTIPLATELET THERAPY

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Introduction. Over the last years, an increasing interest on the phenomenon of the residual platelet reactivity (RPR) on coronary artery disease (CAD) patients under antiplatelet therapy has been evidenced. The unpredictable response to antiplatelet therapy can be attributed to clinical, pharmacogenetic and cellular factors. Reticulated platelets (RP) are immature platelets that reflect platelet production from megakariocytes so contributing to the rate of platelet turnover. A high platelet turnover rate could produce a population of platelets that could confer RPR through several different mechanisms. *Aim.* To asses the influence of RP on RPR in CAD patients on antiplatelet therapy. *Methods.* In 372 consecutive CAD patients undergoing percutaneous coronary intervention on antiplatelet therapy we measured RP by using the Sysmex XE-2100 haematology analyzer (Sysmex, Kobe, Japan). RP were expressed as the percentage of RP of the total optical platelet count (immature platelet fraction; IPF) and as the percentage of RP highly fluorescent (H-IPF). Platelet function was assessed by optical aggregometry (PA) on platelet-rich-plasma induced by 1 mmol arachidonic acid (AA-PA) and 10 micromol/L ADP(ADP-PA). RPR was defined as either AA-PA >20% or ADP-PA >70%. *Results.* A significant difference for IPF and H-IPF between patients with and without RPR was observed [AA-PA >20% IPF 4.7 (1.3-16.4) vs 3.8 (1.2-11.3) % p<0.005 H-IPF 1.3 (0.3-7.6) *vs.* 1.1 (0.3-8.0)% p<0.005; ADP-PA>70% IPF 4.6 (1.4-16.4) *vs.* 3.8 (1.2-13.7)% p<0.005 H-IPF 1.4 (0.3-7.6) *vs.* 1.0 (0.3-8.0) % p<0.001]. By dividing patients according to tertiles of IPF and H-IPF, a significant trend for an increase of platelet aggregation was observed (ADP-PA: p<0.0001; p<0.0001 AA-PA: p=0.002; p<0.001). Moreover, significant correlations between PA, IPF and H-IPF were found [ADP-PA and IPF r=0.24, p<0.0001; ADP-PA and H-IPF r=0.24, p<0.0001; AA-PA and IPF r=0.17, p<0.001; AA-PA and H-IPF r=0.20, p<0.001]. *Conclusions.* This study indicates that a high rate of platelet turnover, as suggested by the presence of RP, is a new mechanism which plays a role in determining RPR in high risk CAD patients.

P062

PLATELET ACTIVATION MARKERS IN PATIENTS WITH CARDIOVASCULAR DISORDERS TREATED WITH DUAL ANTIPLATELET THERAPY

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Background. Heightened platelet reactivity and/or resistance to antiplatelet, therapy may cause thrombosis in patients with coronary stents. We hypothesized that this event might involve adhesion mediated by glycoprotein Ib (GPIb)/von Willebrand factor, possibly resulting in the-generation of GPIb-positive platelet-derived microparticles (GPIb+P-MP). *Methods and Results.* We measured by flow cytometry GPIb+P-MP in the blood of patients with coronary stents as potential markers of a prothrombotic tendency, and membrane expression of P-selectin along with binding of the antibody PAC-1 as markers of the inhibitory effect of anti-platelet drugs. We studied 51 patients with stable angina (SA) who had received coronary stents without complications; 16 patients with a history of stent thrombosis (S-TH); and 29 normal individuals. All patients were treated with aspirin and a thienopyridine at the time of study. The number of GPIb+P-MP was similar in normal individuals and SA patients, indicating that anti-platelet therapy has no effect on the generation of these microparticles, but was unexpectedly lower in S-TH patients. Thus, GPIb+P-MP levels allowed discriminating SA from S-TH patients with significant sensitivity and specificity. P-selectin expression and PAC-1 binding were lower in patients than normal subjects but with no difference between SA and S-TH patients, evidence that platelet inhibition was similar in the two groups. *Conclusions.* Enhanced GPIb/von Willebrand factor interactions may alter generation and/or adhesion of GPIb+P-MP at sites of vascular lesion through mechanisms not inhibited by current anti-platelet therapy. A decrease of GPIb+P-MP in blood may indicate a prothrombotic tendency in patients with coronary stents.

P063

PHYSICAL ACTIVITY DURING LEISURE TIME AND PRIMARY PREVENTION OF CORONARY HEART DISEASE: AN UPDATED META–ANALYSIS OF COHORT STUDIES

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Objective. A vast body of evidence showed a clear preventive role for physical activity on the occurrence of cardiovascular disease. We systematically assessed the relationship between physical activity during leisure time (LTPA) and primary prevention of coronary heart disease (CHD) in an updated meta–analysis of prospective cohort studies. *Methods*. We searched MEDLINE, EMBASE, the Cochrane Library and bibliographies of retrieved articles. Studies were included if they reported relative risks and their corresponding 95% CIs for categories of LTPA in relation to CHD. *Results*. Eighteen cohort prospective studies, incorporating 363,553 subjects (16,103 CHD events) followed up for 4-23 years, met the inclusion criteria. We grouped all the categories of LTPA reported into 3 levels of intensity: high, moderate and low or sedentary. The high level of physical activity was determined in order to obtain a level of intensity attainable by the general population. Under a random–effect model, the overall analysis showed that individuals who reported to perform a high level LTPA had a significant protection versus CHD (relative risk 0.73 [95% CI 0.65-0.81], p<0.00001). A similar significant protection on the occurrence of CHD for subjects who practise a moderate level of LTPA has been also demonstrated (relative risk 0.86, [95% CI 0.81-0.92], p<0.0001). *Conclusions*. The present meta–analysis reports a significant protection of a moderate-to-high level of physical activity against the occurrence of CHD. These results strengthen the recommendations of guidelines indicating the protective effect against cardiovascular disease of physical activity profiles attainable by the ordinary people.

P064

THROMBOELASTOMETRY IN RECURRENT PREGNANCY LOSS A NEW POTENTIAL DIAGNOSTIC IMPACT IN CLINICAL PRACTICE

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The pathophisiology of placental thrombosis has been considered since the 1980 years as a cause for recurent fetal loss and acquired as genetic thrombophilia has been associated with such conditions. Women with previous unexepalined fetal loss could benefit from anticoagulant prophylaxis during next pregnancy. However, laboratory methods to study women at risk of fetal loss are essential. Risk factors may be identifide by testing for known thrombophilias but the relative risk associated with a single factor is small and the prognostic significance for an individual is unclear. Global haemostasis assays are the better methods to detect the hypercoagulable states. The ideal assay would measure endopoints for either thrombin and fibrin generation and lysis in presence of all blood components. Thromboelastometry (TEM) as an indirect measure of thrombin and fibrin generation, may provide extensive informations for thrombophilic states. TEM analysis, performed by the ROTEM, provides a velocity curve with new parameters: MaxVel, t-MaxVel, AUC. The Area Udere Curve (AUC)display a remarkable similarity with endogenous thrombin potential. The women with previous fetal loss, tested in our study, showed an hypercoaguble pattern. AUC values were significantly increased and correlated with F1+2 and TAFI levels. High AUC finding values do agree with F1+2 and TAFI levels. Enhanced thrombin generation and depressed fibrinolysis are just the mechanisms of an imbalance in overall hemosstasis. Therefore, the authors feel that TEM, discriminating an hypercoagulable state, such as in other thrombophilic conditions, results a valid tool to identify women at risk of fetal loss.

Venous Thromboembolism: Epidemiology and Risk Factors

P065

FACTOR V LEVELS IN A COHORT OF PATIENTS ELIGIBLE FOR ORAL ANTICOAGULANT THERAPY

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Factor V is characterized by a dual function in coagulation involved in both procoagulant and anticoagulant pathways. Activated FV (FVa) is cofactor in the prothrombinase complex whereas unactivated FV is cofactor in the APC-mediated degradation of FVIIIa. Generally, low coagulation factors are at risk of bleeding and high levels might favour thrombosis. Conversely, low FV levels might result in either thrombosis or haemorrhage depending on the severity and on the individual coagulation balance. For this reason, we tested by ProCGlobal test (Dade-Behring) FV dilutions (0-100%) and compared the results with those of identical FVIII, PC or PS plasma dilutions. Unlike FVIII behaviour, characterized by reduction in *APC-resistance* as FVIII levels decreased, FV showed a trend comparable to those of PC or PS molecule, ascribing it a prevailing anticoagulant role in this *in vitro* system. To explore a role of low FV levels in thrombosis, we assayed FV in a cohort of 1447 patients eligible for oral anticoagulant therapy [venous thrombosis (VT), n=497; arterial thrombosis (AT), n=498; cardiac valvular prosthesis (VP), n=452), and compared levels among groups. In addition, FVR506Q and FVH1299R (FVR2) have been screened to find interactions with levels and thrombotic risk. FV (activity %) was 116.5±22 in VT, 114.5±24 in AT and 113.5±20 in the VP group (non-thrombotic control group). VT patients had higher FV% than VP group (p<0.01). In addition, the percentage of patients with FV below 65% was 2.4%, 0.8% and 0.88% respectively among VT, AT and VP group, with an appreciable but non significant risk value of 2.7 (CI95% 0.90-8.65) comparing VT cases vs VP controls. As expected, FV506Q carriers were 19%, 5.3% and 3.9% of VT, AT and VP patients respectively. Similarly, FII20210A carriers spread as 13.2%, 4.7% and 5% respectively among the same subgroups. Conversely, FV1299R carriers were similarly distributed among the three subgroups of cases (12.4%, 13.3% and 12.6% respectively). Interestingly, stratifying FVR2 polymorphism by FV levels in the whole group of patients it was obtained an increase of R2-carriers as FV levels considered decreased (FV ≤ 85%, R2-carriers=21.4%; FV 115-125%, R2-carriers=13.2%; FV >145%, R2-carriers=5.5%; p=0.002). These results account for a strong role of FV in the onset and progression of thrombotic diseases and ascribe to FVR2 variant a significant FV modulatory role in both symptomatic and non-thrombotic patients.

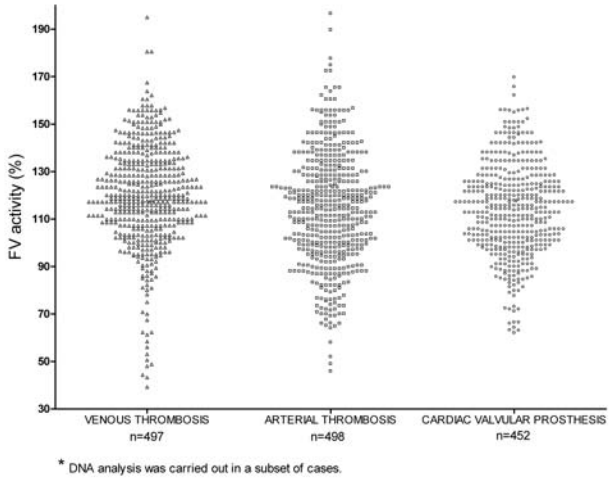

Figure 1.

P066

CARDIOVASCULAR RISK FACTORS AND THE RISK OF VENOUS THROMBOEMBOLISM

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The association between established cardiovascular (CV) risk factors and the risk of venous thromboembolism (VTE) is not yet entirely understood. However, CV risk factors such as hyperlipidaemia, hyperglycaemia, arterial hypertension, cigarette smoking, overweight, have recently been reported as potential risk factors for venous thrombosis. We have screened for hyperlipidaemia (hypercholesterolemia, hypertrigliceridemia and low HDL-cholesterol), impaired fasting glucose, diabetes mellitus, hypertension, cigarette smoking, obesity and overweight, 565 consecutive patients (256 M and 309 F; mean age 43.59±14.49 yrs) with a first episode of VTE: 358 DVT of lower extremities, 118 DVT associated with PE, 85 isolated PE, 4 PE associated with SVT. As many as 577 age- and sex-matched apparently healthy subjects (237 M and 340 F; mean age 42.29±12.31 yrs), from the same ethnic background, served as controls. Arterial hypertension was found in 146/563 (25.9%) of VTE patients and in 115/573 (20.1%) controls (p=0.020; OR:1.39; 95%CI 1.05-1.84; Chi-squared test). Hypercholesterolemia (cholesterol levels ≥190 mg/dL in repeated evaluations over a 3-yr period) was more common in VTE patients than in controls (306/490, 62.4% *vs.* 227/446, 50.9%; p<0.001; OR: 1.60, 95%CI 1.23-2.08, Chi-squared test), low HDL-C levels (≤35 mg/dL) were more frequent in patients (47/427, 11.1% *vs.* 18/279, 6.5% p<0.045 OR:1.8 95% CI 1.02-3.17). The association between obesity-overweight (BMI ≥25) and VTE was statistically significant (365/519, 70.3% *vs.* 305/556 54.9%; p≤0.001, OR: 1.95; 95% CI 1.51-2.5; Chi-squared test). The prevalence of cigarette smoking was 291/563 (51.7%) in patients *vs.* 240/572 (42%) in controls (p<0.001, OR:1.48; 95% CI 1.17-1.87; Chi-squared test). The prevalence of diabetes was not different between patients and controls (6.4% *vs.* 3.9%), impaired fasting glucose was not statistical significant for a while (78/456, 17.1% *vs.* 51/414, 12.3% p=0.056 OR 1.46 95% CI 1.00-2.15 Chi-squared test). The relevant association between some established CV risk factors and VTE implies that adequate treatment/prophylaxis of them should be seriously considered in primary and secondary prevention of VTE.

P067

INCIDENCE OF VENOUS THROMBOEMBOLISM AND PREVALENCE OF MEDICAL RISK FACTORS IN PATIENTS MANAGED AT HOME: A SIGHT INTO THE FAMILY MEDICINE PRACTICE

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Background. The amount of VTE risk in medical outpatients is still elusive; it also remains to be elucidated whether diseases like heart failure or COPD do confer the same risk if managed in hospital or at home. Some of the most widespread International guidelines differ substantially when recommending VTE prophylaxis only for medical inpatients (7th ACCP 2004) rather than extending it also to outpatients (International Consensus Statement 2006). Aim of the present study was to evaluate the incidence of VTE in the setting of Italian family medicine and its potential association with medical conditions known to be risk factors during hospital stay. *Materials and methods.* The study was a nationwide retrospective observation carried out according to a nested case-control methodology. 400 general physicians through Italy, recording all clinical information in an electronic database represented the national network data collecting. All cases recorded as VTE episodes had to be objectively documented. We present here the data on the incidence of DVT and PE and the prevalence of medical risk factors. RESULTS The eligible population was formed by 372.000 patients observed between 2001 and 2004. Overall, 1271 deep vein thrombosis (DVT) and 365 pulmonary embolism (PE) episodes were recorded. The incidence of DVT ranged from 8.1 (in 2004) to 9.7 (in 2003) per 10000 patients-years and that of PE from 2 (in 2001) and 3.1 (in 2003) per 10000 patients-years. Both PE and DVT incidences were higher in females. VTE incidence was lower than 5 per 10000 patiens-years for people in the third and fourth decades of life; it then increased with aging, being 44.5 per 10000 patients-years for people in their eighties (in 2003). Among known VTE risk factors, cancer was highly prevalent in family practice patients suffering from VTE: 16.1% in DVT patients and 15.3% in patients with PE. COPD was noticed in 14.2% DVT patients and in 18.6% of patients with PE. Heart failure was present in 3.5% DVT patients and in 6.8% of cases with PE. *Conclusions.* The incidence of VTE in the setting of Italian family medicine is consistent with that described by the epidemiology and is similar to that reported for the same setting in United Kingdom. Medical diseases known to be associated with VTE when patients are hospitalized appears to be highly prevalent also in cases of VTE occurring in family practice.

P068

RISK OF DVT IN WHEELCHAIR-BOUND OR BEDRIDDEN PATIENTS WITH MULTIPLE SCLEROSIS: A PROSPECTIVE STUDY

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Background. Multiple Sclerosis is frequently characterized by a progressive deterioration in mobility that may lead to paralysis of the limbs. The venous and lymphatic stasis and the resulting abolition of the vasoactive autonomous reflexes constitute potential risk conditions for venous thromboembolism (VTE). There is, however, no known data available about the frequency with which VTE complicates the evolution of multiple sclerosis in its later stages, characterized by severely reduced mobility. The aim of this study was to assess the frequency of deep vein thrombosis (DVT) in patients suffering from late-stage multiple sclerosis and therefore wheelchair-bound or bedridden, coming to a Neurology Centre for a period of rehabilitation and mobilization. *Patients and Methods*. From January 2006 to December 2007, 99 patients were enrolled, 61 women and 38 men, with a mean age of 58±11 years. The onset of the disease had occurred on average 18.7 years ago, the mean of bedridden hours were 9.5 a day and the wheelchair-bound hours were 14.3. Only 20 patients reported an ability to walk at home autonomously or with help. 55 patients presented lower limb edema, bilateral in 35 cases. During the first 24/48 hours of hospitalization, all patients admitted underwent an extended CUS examination with an assessment of the whole bilateral femoropopliteal and distal popliteal axis. Their plasmatic D-dimer was also determined by the immunoenzymatic technique. No patient was subjected to antithrombotic prophylaxis at home or during the period of rehabilitation. *Results*. The presence of DVT was found in 22 patients (21.78%), who presented complete or partial vein incompressibility in the femoropopliteal deep venous system. 13 of these had a history of previous thrombotic episodes. 18 of the 22 patients with DVT presented chronic lower limb edema. The edema was bilateral in 11 cases and monolateral in 7 cases. 13 out of the 22 DVT cases showed high D-dimer values (807.08±833.06 ng/mL. Of the remaining 77 subjects not affected by DVT, 48 had normal D-dimer values (193.37±67.28) and 29 abnormally high values (387.61±187.42). *Conclusions.* The data collected suggest that the frequency of DVT in late-stage multiple sclerosis may be over 20%. The long history of the disease does not allow the onset of each individual episode to be dated with any degree of certainty. A number of patients with DVT that we reported were found to be positive in the CUS examination but to have a negative D-dimer value and this could be evidence for a remote event and, in any case, the size of the risk of DVT in this category of patients, though assessed using a diagnostic technique that is less sensitive than phlebography, should lead one to consider taking long-term preventive measures systematically.

P069

D-DIMER AS A RISK FACTOR FOR OCCULT CANCER AND CARDIOVASCULAR EVENTS IN THE PROLONG STUDY

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Background. In the PROLONG randomized clinical trial (NEJM, 2006;355:1780-9) an abnormal D-dimer (D-d) at one month after VKA suspension for a first unprovoked episode of venous thromboembolism (VTE) was associated with a statistically significant higher risk for recur-

rence as compared with normal D-d. *Objectives.* To assess the predictive value of D-d for occult cancer and cardiovascular events in patients enrolled in the PROLONG study in 30 centers of the Italian Federation of Anticoagulation Clinics (FCSA). Patients were enrolled on the day of VKA suspension. D-d was measured at 30+10 days afterwards. Follow-up was 2.55 years and newly diagnosed cancers and cardiovascular events (AMI, stroke, coronary procedures, acute limb ischemia and sudden deaths) were registered. Results: In 222 subjects with abnormal D-d 9 cancers (1 pancreas, 1 larynx, 1 sarcoma, 2 brain, 2 prostate, 1 kidney, 1 lung; 4.9%) were observed, while in 386 subjects with negative D-d 9 new cancers (1 prostate, 3 lung, 1 uterus, 4 others ; 2.3%) were diagnosed, a non significant difference. In 222 subjects with abnormal D-d, 10 cardiovascular events were reported (4 AMI, 3 TIA, 2 strokes , 1 acute ischemia of the lower limbs; 4.5%), with a non significant relative risk of 1.38 (95%CI:0.88-2.2) when compared to 386 subjects with negative D-d, in whom 10 cardiovascular events (4 strokes, 2 AMI, 1 renal infarction; 1.04%) were reported. *Conclusions*. An abnormal D-d at one month after anticoagulation suspension does not seem to be associated either with an increased risk of cardiovascular events or with occult cancer.

P070

INCIDENCE OF SYMPTOMATIC AND ASYMPTOMATIC CHRONIC PULMONARY HYPERTENSION IN PATIENTS WITH A PREVIOUS EPISODE OF PULMONARY EMBOLISM: A PROSPECTIVE COHORT STUDY

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Introduction. The natural history of pulmonary embolism (PE) is not clearly established Although chronic thromboembolic pulmonary hypertension (CTPH) is believed to be extremely uncommon in patients with a previous episode of acute PE, in recent series, CTPH has been diagnosed in about 4% of patients with a previous PE. Furthermore, a study suggested that asymptomatic CTPH was even more common in these patients and that the development of CTPH was associated with a worst outcome at a long term follow up. However, evidences on the incidence of asymptomatic CTPH diagnosed with echocardiography Doppler remain limited. We therefore carried out a prospective cohort study to assess echocardiographic parameters in consecutive patients with a previous PE. *Methods.* Consecutive adult patients with a first episode of objectively confirmed PE were evaluated within 6-12 months after the index event. Patients were excluded if they suffered from other diseases that could cause chronic pulmonary hypertension (e.g. systemic sclerosis, severe emphysema or moderate to severe mitral valve regurgitation). Patients were evaluated with Doppler transthoracic echocardiography performed independently in duplicate by two operators blinded to results of other operator and unaware of the clinical characteristics of the patients. Pulmonary hypertension was defined as a systolic pulmonary artery pressure (PAPs) >40 mmHg at rest. *Results.* Of the 102 patients initially evaluated, 11 patients had one or more exclusion criteria and were thus not included in the study. Therefore, 91 patients (mean age 61.9+15.7 years; 39 men) were enrolled. Tricuspid regurgitation was detected in 72 patients. Eight patients (8.8%; 95% CI 4.5, 16.4) had chronic pulmonary hypertension: of these, 4 (4.4%; 95% CI 2.0, 9.3) were symptomatic (2 NYHA II, 2 NYHA III). The agreement between the two operators was perfect. Of note, 4 patients with normal echocardiographic findings at PE diagnosis developed chronic pulmonary hypertension Baseline characteristics of patients with and without CTPH were not statistically different. Pulmonary hypertension at the time of diagnosis of PE and incomplete recanalization at the control pulmonary perfusion scan were marginally significant predictors of CTPH at univariate analysis. *Conclusions.* Symptomatic and asymptomatic CTPH is not an uncommon finding after PE. Larger prospective trials with a longer follow up should assess the prognostic significance of asymptomatic CPTH.

P071

THROMBOPHILIA AS A RISK FACTOR FOR A FIRST EPISODE OF PULMONARY EMBOLISM

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Introduction. Pulmonary embolism (PE) and deep vein thrombosis (DVT) are grouped under the term venous thromboembolism (VTE). Although they share many risk factors, it is unclear whether thrombophilic abnormalities play a different role in the development of the two clinical manifestations. Thrombophilia has been extensively studied in DVT, but few data are available in PE. Aim of this study was to evaluate thrombophilic alterations in patients with a first episode of PE *Methods.* One-hundred-seventy consecutive patients [80 males and 90 females, median age 55 yr (range 14-89 yr)] with a first objectively confirmed episode of PE were compared with 177 matched healthy controls. Thrombophilic risk factors evaluated were antithrombin, protein C, free protein S, APC-resistance, factor V Leiden, prothrombin G20210A polymorphism, fasting homocysteine, lupus anticoagulant, anticardiolipin antibodies, factor VIII activity, lipoprotein(a). *Results.* Forty-nine patients (29%) had isolated PE and 121 (71%) had PE with DVT. PE was idiopathic in 97 patients (57%) and secondary to transient risk factors (recent surgery, trauma, immobilization, pregnancy and puerperium) in 73 (43%). After univariate analysis, thrombophilic risk factors associated with PE were APCR [OR 3.3 (95% CI, 1.7-6.4), p=0.001], prothrombin G20210A polymorphism [OR 6.8 (95% CI 2.3-20.0), p=0.001], hyperhomocysteinemia (>95th percentile) [OR 10.3 (95% CI 4.3-24.8), p=0.000], elevated lipoprotein(a) levels (>300 mg/L) [OR 2.9 (95% CI 1.7-5.1), p=0.000] and elevated factor VIII levels (>200%) [OR 2.8 (95% CI 1.4-5.8), p=0.005]. After multivariate analysis, adjusted for age, sex, acquired and thrombophilic risk factors, all these thrombophilic abnormalities, except factor VIII, were confirmed to be associated with PE. *Conclusions.* These results indicate the role of thrombophilia as a risk factor for a first episode of PE. Factor V Leiden, in agreement with previous reports, was not related to PE, whereas APCR was an independent risk factor.

P072

SEX RELATED TYPE OF VENOUS THROMBOEMBOLISM IN PATIENTS WITH AND WITHOUT THROMBOPHILIA

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Background. Whether patient's gender and thrombophilia are associated with the type of venous thromboembolism is unknown. *Methods.* The clinical manifestations of a first, objectively documented, episode of venous thromboembolism (isolated DVT, isolated PE , and DVT and PE) were investigated in 570 consecutive patients referred to our Thrombosis Center from May 2002 to December 2007. Medical histories were collected with particular regard to the presence of transient risk factors. Fifty-four patients were carriers of FV Leiden, 38 of the PT-G20210A polymorphism,140 of other thombophilic mutations (AT, PC, PS, LAC, MTHFR, elevated levels of factor VIII, IX and XI) and 338 with no thrombophilia. *Results.* Isolated symptomatic PE was more prevalent in patients without thrombophilia or with other thrombophilc abnormalities than in patients with FV Leiden [RR (95% CI) 3.36 (1.6 to 7.2) and 2.6 (1.2 to 5.7) respectively]. On the other hand the rate of isolated DVT was higher in patients with FV Leiden than in those without thrombophilia or with other thrombophilic abnormalities [RR (95% CI) 1.6 (1.3 to 2.0) and 1.7 (1.3 to 1.2) respectively]. Patients with prothrombin G20210A had a higher rate of DVT complicated by symptomatic PE than those with FV Leiden or without thrombophilia [RR (95% CI) 1.8 (1.0 to 3.5) and 1.8 (1.1 to 2.8) respectively]. Isolated symptomatic PE occurred in 133 of the 338 women , as compared with 50 of the 232 men [RR (95% CI) 1.8 (1.4 to 2.4)]. This risk remained unchanged also when patients without thrombophilia and those with other thrombophilic abnormalities were examined separately [RR (95% CI) 1.9 (1.4 to 2.6) and 1.8 (1.0 to 3.1) respectively]. The incidence of isolated DVT was higher in men than in women both in patients without thrombophilia and in those with other thrombophilic abnormalities [RR (95% CI) 1.6 (1.0 to 2.0) and 1.4 (1.0 to 2.0) respectively]. No difference in the incidence of isolated PE or DVT was observed in patients with FV Leiden. *Conclusions.* Carriers of FV Leiden have a lower risk of developing isolated PE whereas Prothrombin G20210A mutation is associated with DVT complicated by PE.

Women have a two fold increased risk of developing isolated PE, except those who are carriers of FV Leiden. Isolated DVT occurred more frequently in men.

P073

BIMODAL INCIDENCE OF DEEP VEIN THROMBOSIS IN INTENSIVE CARE UNIT PATIENTS

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Intensive care unit (ICU) patients are at high risk for deep vein thrombosis (DVT) and form a heterogeneous population characterized by the day-by-day change in risks of thrombosis and bleeding during the course of illness. ICU dedicated guidelines are lacking. This study was aimed to estimate a) the incidence of DVT in high risk ICU patients (SAPS SCORE>20) admitted for trauma, surgical or medical illness and b) if critical days with highest incidence of DVT could be identified. DVT prevalence (diagnosis within 48 hours of ICU admission) and incidence (diagnosis 72 hrs or more after ICU admission) was investigated in 386 out of 701 patients admitted to ICU from December 2004 to December 2006. Patients were enrolled when ≥18 years old and had been in ICU for ≥72 hours . Additional exclusion criteria: confimed DVT within the previous 6 months, congenital or acquired thrombophilic profile, high hemorrhagic risk, and a history of hypersensibility or thrombocytopenia to heparins of any type .All patients performed both pharmacological and mechanical DVT prophylaxis. The 1rst lower extremity compression ultrasound exam (CUS) was done within 48 hours from admission, the 2nd between the 4th and the 8th day and the 3rd between the 9th and 14th day. Logistic regression analysis was performed to evaluate the relationship among DVT and : age, diagnosis of admission (trauma, medical or surgical illness), malignancy, SAPS, length of stay and days of ventilation. One-hundred forty-two patients (56+4.8 yrs old, 104 M, 38 F), 70% admitted for trauma, 15% for medical disease and 10% for surgery, were considered eligible for the study. DVT was found in 12 /142 patients within 48 hours (prevalence 9%). During the ICU stay 13 patients developed DVT, for an incidence of 9,2%; 3 were diagnosed between the 3th and the10th day and the remaining 10 after 10 days (p<001). DVT was not associated to SAPS and malignancy but resulted to be significantly related to trauma as admitting diagnosis, days of ventilation and length of stay. The relationship between DVT and the length of stay remained significant after the adjustment for the other risk factors. In our ICU population lengh of stay resulted to be a strong and independent risk factor for DVT and the incidence of DVT showed a bimodal pattern with first early peak (within 48 hours) and a second late peak associated to an ICU stay >10days, never shown before in literature.

P074

IS OBESITY A RISK FACTOR FOR INCOMPLETE RESTORATION OF PULMONARY PERFUSION AFTER PULMONARY EMBOLISM?

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Introduction. An incomplete restoration of pulmonary perfusion after pulmonary embolism (PE) may contribute to the development of chronic thromboembolic pulmonary hypertension (CTEPH), a complication of PE associated with considerable morbidity and mortality, whose risk factors are poorly understood. This study was aimed at evaluating the restoration of pulmonary perfusion after PE and at identifying factors associated with the persistence of large perfusion defects. *Methods.* Eighty-three consecutive patients [34 males and 49 females, median age 58 yr (range 20-87 yr)] with a first objectively confirmed episode of PE underwent an integrated protocol including clinical evaluation, perfusion lung scanning, transthoracic echocardiography and thrombophilia screening. Perfusion defects were evaluated on lung scanning and expressed as percent of residual vascular obstruction (VO). Pulmonary hypertension was indicated by an estimated pulmonary artery systolic pressure of greater than 36 mmHg on echocardiography. *Results.* Sixty-seven patients (81%) showed a complete (VO=0) or nearly complete (VO≤15%) restoration of pulmonary perfusion; 11 (13%) had a VO of 16 to 30%, and 5 (6%) >30%. Only one of 83 patients had echocardiographic findings suggestive of CTEPH. The percentage of patients with at least one thrombophilic abnormality was not significantly different in patients with VO≤15% and in those with VO>15% (61% vs 58%). Obesity [BMI≥30 kg/m^2] was significantly associated with incomplete restoration of pulmonary perfusion (p=0.01). The proportion of obese patients was 33% among those with complete restoration of pulmonary perfusion, 40% with a VO up to 15%, 64% with a VO up to 30%, and 100% with a VO≥30%. *Conclusions.* Thrombophilia has no significant role in the persistence of perfusion abnormalities after PE. BMI seems to be inversely related to restoration of pulmonary perfusion. Further broad outcome studies are needed to confirm the association between obesity and development of CTEPH.

P075

HEREDITARY PROTEIN C DEFICIENCY AND RISK OF THROMBOSIS

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Protein C (PC) is a main component of a major anti-thrombotic regulatory system. Individuals with hereditary PC deficiency tend to have an increased risk of thrombo-embolism. In our Institute, from 1988 to 2007, hereditary PC deficiency has been diagnosed in 36 subjects (males 13, females 23, median age at diagnosis 32 years, range 7-80). In 22/36 cases -(61%), (males 6, females 16)- diagnosis has been made at the first deep vein thrombosis (DVT) episode, in 14/36 -(39%), males 7, females 7)- at the laboratory screening of asymptomatic relatives of index patients. In all subjects, PC, protein S (PS), antithrombin (AT) activity levels, homocysteinemia and factor V G1691A (FV Leiden), prothrombin G20210A and C677T MTHFR mutations were investigated. Data concerning living habits and use of oral contraceptives were collected. PC deficiency was defined as an activity level below 70% (normal range, mean + 2 SD, 70-115%). PC levels ≥5% and <30% were observed in 3 subjects, equal to 40% in 1 subject, >40% <50% in 11 subjects, ≥50% <60% in 14 and ≥60% <70% in 7 subjects. PS activity deficiency (normal range, mean +2SD, 64-123%) was observed in 2 subjects (60% and 62%, respectively). None showed AT deficiency and FV Leiden mutation. Two subjects were heterozygous for prothrombin G20210A mutation, 4 heterozygous and 3 homozygous for C677T MTHFR mutation. One subject was heterozygous for both prothrombin G20210A and C677T MTHFR mutations Out of 22 symptomatic subjects, 3 (13.6%) male patients showed PC activity level <30% as the only cause of DVT; the remaining 19 (86.4%) symptomatic subjects showed both reduced PC levels, ranging between 40% and 67%, and other risk factors for thrombosis as probable concomitant causes of DVT. In fact, 9 (47.4%) DVT were observed during pregnancy, 5 (26.3%) in women users of oral contraceptives (2 of these women were also heterozygous for prothrombin G20210A mutation), 3 (15.8%) in patients with auto-immune disorders, 1 (5.2%) in a patient affected by Hodgkin disease and 1(5.2%) in a patient older than 70 years. None of the studied asymptomatic relatives developed thrombo-embolic episodes during the follow-up. Whole median follow-up was 72 months (range 6-232). In conclusion, these data seem to suggest that PC deficiency alone could cause thrombo-embolic events if the activity levels are very low (e.g. <30%). On the contrary, if the levels are higher, thrombotic events could occur only if there is at least another concomitant risk factor.

P076

THE IMPACT OF DEEP VEIN THROMBOSISON ON OUTCOMES IN CRITICALLY ILL PATIENTS:A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background. The clinical consequences of Deep Vein Thrombosis (DVT) have the potential to be serious yet are frequently unrecognized in the Intensive Care Unit (ICU). We hypothesized that both undetected and clinically evident VTE would affect the prognosis of critically ill patients. *Purpose*. To systematically review whether a diagnosis of DVT in critically ill patients affects clinically important outcomes including length of stay, duration of mechanical ventilation and mortality. *Material and Methods*. Data sources used were the MEDLINE, EMBASE and

PUBMED databases. Studies selected evaluated one or more of the following outcomes: duration of patient stay in hospital and in ICU, hospital and ICU mortality, and duration of mechanical ventilation. Two investigators independently extracted and reviewed data from each study; including study and patient characteristics and outcomes. Statistical heterogeneity was evaluated using the X2 statistic; Cohen's Kappa for inter-rater agreement was used to assess inter-rater reliability. Data was pooled using the Mantel-Haenszel method and a random effects model using Review Manager. *Results.* Five studies were included in the systematic review. Patients diagnosed with DVT compared to those without DVT had increased ICU and hospital stay (7.3 days (95% confidence interval [CI] 1.4 to 13.2; p=0.02) and 16.5 days (95% CI 1.51 to 30.59; p=0.03), respectively. Duration of mechanical ventilation was increased by 3.41 days (95 % CI –1.12 to 7.94; p=0.14). Patients diagnosed with DVT also had increased relative risk (RR) for ICU mortality of 9.19 (95% CI 1.07 to 78.65, p=0.04) and a trend towards increased hospital mortality (RR 14.32 [95% CI 0.59 to 347.96, p=0.10]). *Conclusions.* A diagnosis of DVT upon ICU admission appears to affect clinically important outcomes including length of ICU and hospital stay and ICU mortality. Further research involving larger prospective study designs are warranted.

Table 1.

Outcomes

Study	*Duration of mechanical ventilation in days (DVT/NO DVT)*	*Hospitalization length In days (DVT/NO DVT)*	*ICU Stay In days (DVT/NO DVT)*	*Hospital rate mortality rate (DVT/NO DVT) n (%)*	*ICU mortality (DVT,n/NO DVT,n)*
Ibrahim 2002	18.9±19.7 / 14.6±12.9 p=0.310	31.4±21.7/ 27.5±18.2 p=0.375	18.6±14.6/ 15.9±1.04 p=0.388	8.9 (34.6%)/ 26.8(32.1) p=0.815	n/a
Velmahos 1998	Not given^	49±32/ 31±24 p≤0.05	34±31/ 19±18 p≤0.05	n/a	31%, 8.06/ 18%, 31.2
Major 2003	n/a	n/a	n/a	n/a	17%, 2/2%, 15 p=0.03
Patel 2005	n/a	26** (14,49)*/-	6** (3,15)*/-	70** (28.5%) [22.8-34.1])"/-	16.7%,41 [12.0-21.3]"/-
Cook 2005	9** (4,25)*/6 (3,13)* p=0.03	51** (24,73)*/23 ** (12,47)* p=<0.001	17.5** (8.5, 30.5)*/ 9** (5,17)*	17 (53.1%)/ 85 (37.4%) p=0.04	-,8 **/ -,62** p=0.78

PEPP, positive end-expiratory pressur. * IQR; ** median, " [95%CI]); ^ Necessity for ventilation measurated by PEEP. ≥10: DVT/no DVT: 11 (42%)/37 (21%)

P077

RISK FACTORS FOR ARTERIAL AND VENOUS THROMBOSIS: DATA FROM THE EPIDEMIOLOGICAL STUDY ON CONGENITAL AND ACQUIRED RISK FACTORS RELATED TO THROMBOEMBOLIC DISEASES IN LIGURIA REGION (ITALY)

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Background. Arterial and Venous Thromboembolism cover a wide spectrum of clinical conditions with great impact on people's health as well as on National Health Service costs. Many efforts have been made to acquire an accurate knowledge of the pathogenesis and risk factors of thromboembolic diseases. The aim of the epidemiological study on congenital and acquired risk factors related to thromboembolic diseases in Liguria region (SELT) was to analyse distribution and prevalence of risk factors related to thromboembolic diseases in a selected population in Liguria Region. *Methods.* Patients with thromboembolic diseases were enrolled throughout the Liguria area between 2004-2007, and included 115 patients with arterial disease including Stroke, Acute Coronary Syndromes (ACS) and Peripheral Arterial Disease (PAD), mean age of 57.9±15.2, 59 males and 56 females. Data were compared to those of 426 patients with venous thrombotic disease including Deep Vein Thrombosis (DVT), Pulmonary Embolism (PE), Superficial Vein Thrombosis (SVT), Cerebral Venous Thrombosis (CVT) and mesenteric venous thrombosis (MT), mean age 58.8±17.1, 193 males and 233 females. Each patient provided their full history and underwent physical examination and laboratory testing including a coagulation screening and genetic assays. *Results.* Analysis of preliminary data shows a significantly higher recurrence rate of arterial events in patients with arterial disease than in patients with venous disease (Stroke: 29.1% *vs.* 2.6% p<0.001; ACS: 12.7% *vs.* 4.5% p<0.01; PAD:11.4% *vs.* 1.4% p<0.001) as well as a higher rate of venous events in the subgroup of patients with venous disease (DVT: 38.0% *vs.* 8.9% p<0.001; PE: 13.8% vs 2.5% p=0.002; SVT: 10.1% vs 2.5% p=0.03). No significant difference was found in the prevalence of Factor II G20210A and Factor V R 506Q mutations or MTHFR C677T polymorphism in the two groups. Mean Factor VIII concentration resulted significantly higher in the venous group (155 % *vs.* 118 % p=0.007). Mean HDL cholesterol was lower (50 mg/dL *vs.* 56 mg/dL p<0.05) while plasma triglycerides were higher (150 mg/dL vs 126 mg/dL p<0.02) in the arterial disease group. *Conclusions.* Our preliminary data confirm the relevance of a previous thrombotic disease as a risk factor for developing a recurrent event and the role played by dyslipidemia in arterial diseases and suggest that Factor VIII elevation may be more than a mere bystander in the occurrence of venous disease.

P078

PLASMA COAGULATION PROFILE AND GENETIC THROMBOPHILIC MUTATION PATTERN CAN PREVENT THE IDIOPATHIC RECURRENT ABORTIONS, REPEATED FETAL LOSS AND INTRAUTERINE GROWTH RETARDATION IN HEALTHY FERTILE WOMEN

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Introduction. Cumulated evidences account for a hypercoagulation state frequently documented in apparently health women with adverse pregnancy outcomes. In this field, to many tests, too much conflicting data are until now described. So, testing for the identification of risk factors for pregnancy complications remains a complex and changing area of the laboratory. In this scenario, several factors have been considered as causative factors of hypercoagulable state: increased venous stasis, high circulating plasma levels of FI, II, VII, VIII, IX, X, misinterpreting temporary decreases in Protein C (PC), Protein S (PS) and Antithrombin III (ATIII) as inherited deficiencies, enhanced platelet activation, raised fibrin generation, lower fibrinolytic activity, latent or manifest vascular endothelium suffering/damage, immunological competence changes, increased complement activation, abnormal cytokines' release and up-expression or under-regulation of several cellular membrane receptors. Even if the scenario of the adverse pregnancy outcomes is intricate many attempts have been made to establish a test ordering pattern (Preston FE *et al.* Lancet, 1996; 348: 913-916. Brenner B *et al.* thromb Haemost 2000; 83: 693-697. Girardi G J Reprod Immunol 2005; 66: 45-51. Robertson L et al. BJH 2006; 132, 2: 171-196). Recently, the inheritable genetic thrombophilia mutations have been taken into account in women with recurrent enploid fetal losses (RFL) after 10 wweks, recurrent placenta's abruptions, several early onset preeclampsia or recurrent severe intra-uterine growth retardation (IUGR), oligohydramnios (so called maternal thrombophilias). Moreover, a strongest associations has been formed in the loss of 2nd/3rd trimester rather than 1st trimester miscarriage related to placental infarction which interferes with implantation/development of uteroplacental circulation. *Aim.* To define the prerequisites for the genetic thrombophilic mutations pattern as adjunctive factors in women with idiopathic recurrent abortion (ARI) and RFL. To establish a better prevention mediated by these risk factors with efficacious and safe strategies during the initial treatment phase and long-term treatment phase. *Methods.* 68 consecutive healthy fertile women, age ranging 18-52 yrs (median 39.9 yrs), with body mass index in normal average, with ARI from 2 to 9 in each subject and/or RFL at 2nd-3rd of pregnancy and without documented infections or endocrinopathies or anatomic uterine malformations were evaluated at the onset of their umpteenth gestation. We considered adverse pregnancy outcomes when a history of recurrent (≥2) fetal loss >10 weeks, recurrent IUGR, severe early-onset preeclampsia or massive abruptions with documented thrombophilia and characteristic placental pathology, as well as losses in 2nd/3rd trimester thought to be related to placental infarction, were present. 12 of them have had one birth in the list of their ARI and/or RFL. 11/68 had venous thromboem-

bolism episodes (n≤2) in juvenile age. Plasma routine coagulation tests, LAC, fibrinolytic status (PAI-1, D-dimer) were assayed by standard methods. Anticardiolipin antibodies (ACA IgG and IgM), anti-phospholipid antibodies (APA IgG and IgM) and anti-β2-glycoprotein-I antibodies (A- β2 –GP-I) were determined. Plasma homocysteine was also measured. Genetic thrombophilic mutations (13 tests) were performed by Reverse Dot Blot-Real Time PCR. *Results.* Our results showed in 49 females hypercoagulation proneness and/or several genetic thrombophilia mutations: ATIII deficit (n=1), PC reduction (n=3), PS deficiency (n=21), LAC positivity (n=14), APA (n=15), anti-β2-glycoprotein-I (n=11), increased PAI-1 activity (n=12), elevated D-dimer (n=31). Prothrombin mutation (n=19 etherozigousity and n=3 homozygous state), FV Leiden (Q506N=12 etherozigous and n=2 homozygous condition), FV R2 aplotype (n=7 etherozygous state and n=1 homozigousity), combined FII and FV Leiden (n=8), MTHFR (C677T n=30 etherozygous and 13 homozygous state A1298C n=11 eterozygousity and n=7 homozygousity, combined aplotypes n=13), hyperhomocysteinemia (21-115 nmol/L) was found in 15 women. *Summary and Conclusions.* The poliabortivity in apparently healthy fertile women is a complex disease. The pathogenetic mechanisms of recurrent abortions can recognize thrombotic predisposing/primary-secondary causes. In fact, a wide hypercoagulability state and heterogenous inherited thrombophilia are documented in apparently healthy women with adverse pregnancy outcomes. Some evidence exists that physicians have not kept on changes in this area because of the patients heterogeneity as well as laboratory variability. In addition, disparate perspectives of obstetrician, haematologist, rheumatologist, internist persist until now in this field. To identify risk factors for pregnancy complications thrombophilia testing: what else can go wrong? To what extent is ordering consistent with methodological approaches to define etiopathogenesis of ARI, RFL and IUGR? We suggest that an accurate clinical evaluation in conjunction with a well-reasoned screening for hypercoagulability and/or genetic thrombophilia profile must be considered in women with ARI or RFL. So, therapeutical approaches can be established to preventing better the idiopathic recurrent miscarriages in healthy women.

P079

HAEMOSTATIC EFFECTS OF STRONTIUM RANELATE IN THE TREATMENT OF POSTMENOPAUSAL OSTEOPOROSIS

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Introduction. Strontium ranelate (SrR) has been recently introduced with success in the treatment of postmenopausal osteoporosis. Its use has generally few and poor side effects specially interesting the gastrointestinal apparatus. Nevertheless in some phase III studies, patients undergoing treatment with SrR presented a relative risk for venous thromboembolism of 1.42 (CI 1.02; 1.98, p=0.036) as compared to placebo treated patients. *Objective.* Aim of this study was to evaluate, in a group of women with postmenopausal osteoporosis, the effects of therapy with SrR on some haemostatic parameters. *Material and methods.* 20 postmenopausal women (mean age 57±4 yr) with a T-score at the lumbar level < -2.5 SD were enrolled and treated for 12 months with SrR 2 gr/day, calcium 500 mg/day and vitamin D 400 IU/day. At the baseline, after 6 and 12 months were evaluated the following parameters: PT, APTT, fibrinogen, antitrombin III, protein C, protein S, platelets count and aggregation test. *Results.* During the study period none of the evaluated parameters changed significantly. *Conclusions.* SrR showed to be safe and well-tolerated in our patients, with no impact on haemostatic system.

Venous Thromboembolism: Diagnosis, Therapy and Prophylaxis

P080

PROGNOSTIC ROLE OF NATRIURETIC PEPTIDES IN ACUTE PULMONARY EMBOLISM: ANALYSIS OF LITERATURE STUDIES

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Background and aim. Whether thrombolysis improves prognosis of normotensive patients affected by acute pulmonary embolism (PE) with right heart dysfunction remains unclear. Measurement of brain natriuretic peptide (BNP) or its terminal portion (NT-proBNP) is now considered to improve risk stratification of acute pulmonary embolism (PE) beyond clinical and instrumental findings. The aim of this study was to analyse the risk predictive role of BNP and NT-proBNP in acute PE derived from literature studies. *Materials and Methods*. We searched the MEDLINE database for all English language studies published between January, 1980, and Febrauary, 2008, where the relationship between BNP and/or NT-proBNP concentrations and the risk of fatal PE and/or RHD and/or needing for vasopressive drugs and/or needing for Intensive Care Unit (ICU) and/or cardiopulmonary resuscitation 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) study population originating from a well-established cohort; (2) examination of the cross-sectional or longitudinal effects (or both) of BNP or NT-proBNP on end-points; and (3) appropriate consideration of and adjustment for potential confounders. *Results*. We identified 16 relevant studies, 11 studies for BNP, 7 for NT-proBNP (one study in common), enrolling a total of 1410 patients Mean age ranged from 53 to 79 years. 13 studies had fatal PE as primary endpoint, 8 for BNP, 5 for NT-proBNP. All studies found a positive correlation between high concentration of natriuretic peptides and acute mortality. 5 studies, 3 for BNP and 2 for NT-proBNP, evaluated and found a positive relation between concentration of BNP or NT-proBNP and RHD. 7 studies, 4 for BNP and 3 for NT-proBNP, analysed the relation between these biomarkers and serious adverse events other than acute mortality. All these studies revealed the positive association. 15 studies reported a threshold value. The relation between BNP/NT-proBNP and negative outcomes showed an high sensitivity (range 60-100%), whereas specificity was lower (range 33-94%). *Conclusions*. We conclude that high concentrations of BNP and/or NT-proBNP are highly sensitive to predict adverse outcomes in acute PE. Therefore measurement of these biomarkers should enter routinely in management of each patient with acute PE.

P081

COMPARISON BETWEEN TWO METHODS FOR THE DETERMINATION OF THE D-DIMER

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310 specimens from patients referring to our Chimical Chemistry and Haemathology Laboratory of Nuovo Ospedale San Giovanni di Dio, Azienda Sanitaria of Florence, have been analyzed with two different methods VIDAS D-Dimer Exclusion bio Merieux (immunoassay ELFA) and Innovance D-Dimer Dade Behring(immunoturbidimetric method). Our study has shown a good correlation among the two methods with a correlation coefficient of 0.934. We have considered as method of reference VIDAS D-Dimer Exclusion Bio Merieux, gold standard method by the International literature and used by our Laboratory from many years. 143 results are under the cut off of the method Bio Merieux, among these 25 (17.5%) are above the cut off of the method Dade Behring. 167 results are above the cut off of the method Bio Merieux, among these 4 (2.4%) are under the cut off of the method Dade Behring. For the use in the clinical practice of the method Innovance D-Dimer Dade Behring for the diagnosis of exclusion of the thrombus venous embolism further studies will be performed on specimens of patients with available clinical history for the definition of a new cut off. *Conclusions.*The two methods performed show a good correlation. The 29 results discording among 310 results from specimens analyzed show

the need of further studies taking in account also the clinical history of patients and other instrumental examinations.

P082

USEFULNESS OF VENOMETER TEST IN OUTPATIENT WITH CLINICAL SUSPICION OF DEEP VEIN THROMBOSIS

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Introduction. The suspicion of deep vein thrombosis (DVT) is a very common cause of medical referral to hospital. The clinical diagnosis is notoriously inaccurate (about 70% of patients with typical symptoms do not have venous thrombosis and about half of patients with confirmed venous thrombosis are asymptomatic); therefore the suspicion of DVT should always be confirmed by objective tests. Diagnostic procedures for DVT include invasive (contrast venography) and non invasive method (colour Doppler ultrasound or compression ultrasound). Strain gauge plethysmography has been marketed as a non invasive technique to rule out lower limb DVT and initially has also claimed to be a substitute for venography. Initial studies were promising, but more recent works show lower sensitivity than originally claimed, even for proximal DVT. *Aim of the study.* We have evaluated Venometer test, a technician operated machine that uses automated strain gauge plethysmography, as screening test for diagnosis of lower limb DVT. *Materials and Methods.* Over a three months period, 51 consecutive patients referred to our Centre with suspected DVT were assessed using Venometer test. The computer-assisted strain gauge plethysmography displays the results as positive or negative for DVT. All patients were also undergone to Colour Doppler ultrasound (CDUS). Furthermore, a patient's blood sample was also collected for D-dimer test. Data were analyzed using sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). *Results.* Overall, 6 of 51 patients have a confirmed diagnosis of DVT by CDUS; 3 proximal and 3 distal thrombosis, respectively. Venometer test was positive in 2/6 patients (sensitivity 33%; specificity 96%; PPV 50%; NPV 91%); however both the Venometer positive tests were in patients with proximal DVT with NPV= 98%. *Conclusions.* The automated Venometer test is a quick and non-invasive method and it is easy to use as initial screening test; however, it is not sufficiently accurate as definitive diagnostic test for DVT. Therefore, it should be used in combination with clinical risk assessment and D-dimer assay, and more definitive radiological investigations should be performed, if necessary.

P083

INFERIOR CAVA MALFORMATIONS AND RECURRENT THROMBOSIS

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Congenital IVC malformations are uncommon (prevalence in healthy individuals 0.5%) and may be associated with an increased risk of venous thrombosis, frequently underestimated.

Table 1.

	PATIENT 1	PATIENT 2	PATIENT 3	PATIENT 4	PATIENT 5
Age at diagnosis	34	33	14	33	46
Gender	M	M	M	F	M
Location of first thrombosis	Unilateral left iliac vein	Unilateral left iliac to femoral veins	Unilateral right iliac to femoral and popliteal veins	Unilateral right iliac to femoral and popliteal	Unilateral right iliac vein
Cava malformation type	Cava agenesia (renal segment)	Cava agenesia (renal segment)	Cava agenesia (post-renal segment)	Dysfunctional cours of VCI (pre-renal segment)	Cava ectasia enlargement (renal segment)
Associated malformation	No	No	No	Azygos ectasia and horseshoe kidney	Azygos ectasia
Method of diagnosis	CT	CT	CT	CT	CT
Thrombofilia testing	Negative	No	Negative	Negative	Heterozygous V Leiden
Other factors	No	No	No	Breast Cancer	No
Recurrent thrombotic occlusion	Yes	Yes	Yes	Yes	Yes
Continued anticoagulant treatment	Yes	Yes	Yes	Yes	Yes

These anomalies are caused by aberrant development in the sixth to eighth weeks of gestation and an inadequate collateral circulation could result in venous stasis and an increased risk of deep venous thrombosis (DVT). We describe five patients with iliac vein thrombosis and anomalies of the IVC (Table 1). Age of presentation of first thrombosis is around 35 years and the most frequent malformation are in the renal segment. All patients found recurrent thrombotic occlusion when anticoagulation treatment was suspended. Therefore IVC malformations should be investigated in patients less than 35 years with recurrent DVT of the lower extremities involving the iliac veins and in these patients the oral anticoagulation should be considered lifelong.

P084

WHEN PRE-TEST PROBABILITY CAN BE DECEPTIVE

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Diagnosis of venous thromboembolism (VTE) is a key medical issue in reducing the large number of undiagnosed cases and the high fatality rate from pulmonary embolism. Over the last decades clinical evaluation tools that have enabled us to accurately stratify the risk to patients and guide further strategies have been developed. Pre-test clinical probability assessment (PTP) has been established as a first essential diagnostic step and it has even been suggested that – in the context of Accident & Emergency (A&E) and a primary care setting – the Wells clinical score alone with a single protective dose of heparin are sufficient to safely postpone imaging tests with estimated as having a moderate-to-high risk of VTE. We present 2 outpatients whose PTP was highly indicative of deep vein thrombosis (DVT) while further evaluation showed a considerably conflicting outcome. The first was an 80-y-o diabetic and hypertensive male who had undergone surgery 3 weeks previously for right pertrochanteric femoral fracture, on regular thromboprophylaxis with enoxaparin 0.4mg/d; following progressive right lower limb swelling attributed to DVT his medication had been increased to 0.4 mg bid by rehabilitation clinic physicians in the previous 2 days before his admission to the A&E for aggravated pain and swelling. His Wells' revised score was 4 and D-dimer 885 ng/mL. An urgent US scan being unavailable on a Sunday, the patient was admitted to the medical ward, where low haemoglobin levels and a compressive ultrasonography showing a partially non-compressible right common femoral vein led staff to suspect inguinal haematoma. As creatinine >2 mg/dL contraindicated a CT scan, surgical exploration was performed during which laceration of medial branch of deep femoral artery was found and sutured, and haematoma was drained. The second was a healthy 76 y-o male complaining of painful swelling of the right thigh. While resting due to a right lower limb trauma he observed a progressive swelling of the injured limb: his primary care physician (PCP) prescribed LMWH on a suspicion of DVT (Wells score 3) and referred him to the A&E the following day. An urgent US scan showed haematoma of right quadriceps muscle, leading to hospital admission. *Conclusions.* whilst a valuable tool for VTE diagnosis, PTP alone can be misleading and an integrated approach is recommended. PCP must apply caution in administrating also a single therapeutic dose of LMWH in surgical or traumatic patients.

P085

CLOTS DISTRIBUTION, TROPONIN I AND RIGHT HEART DYSFUNCTION IN PATIENTS WITH ACUTE PULMONARY EMBOLISM

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Background. Both in hypotensive and in normotensive patients, the presence of right heart dysfunction (RHD) detected by echocardiogram is related to adverse outcomes in acute pulmonary embolism (PE). Thus to detect RHD and to stratify prognosis are crucial in acute management. The aim of present study was to evaluate the relation between RHD, troponin I and clots distribution in pulmonary vascular tree and short term prognosis of patients affected by PE. *Materials and methods.* We retrospectively analysed the echocardiographic data of 70 patients, 34 females and 36 males, with mean age ± SD 72.54±14.80 years (range 30-98), discharged from our Hospital from 2004 to 2007 with diagnosis of PE confirmed by pulmonary helical computer tomography hCT (61 patients by Internal Medicine ward, 9 patients by Cardiology ward). We considered the end-diastolic right/left ventricles ratio in four chambers

> 1 and systolic pulmonary arterial pressure (PAPs) > 30 mmHg as indexes of RHD. Echocardiographic data were compared with troponin I values ((Access 2, Beckman, USA, sensitivity 97%, specificity 95%, cut-off 0.06 ng/dL) and with clots distribution in pulmonary vascular tree such as hCT findings. Moreover for each patient we calculated the shock index (heart rate in beats for minute/systolic blood pressure in mmHg, normal <1, shock ≥1). *Results*. Hospital mortality was 8.5%. Mean age of dead patients was significantly higher compared to alive (85.67±10.80 *vs.* 71.57±13.82 years, $p<0.05$). 41% of patients revealed an unilateral PE, whereas 59% had bilateral. In 10% of patients main pulmonary artery was interested by clot, 48% of patients had involved one of the main branches, 90% had involved at least one of the lobar branches, 59% at least one of segmental branches of pulmonary arteries. 74% of patients had contemporary involvement of two or more arterial pulmonary branches. Echocardiogram was performed in 57 patients (81.5%, 52% of them revealing RHD. Mortality in RHD patients was significantly higher compared to no RHD patients (14.8% *vs.* 8%, $p<0.05$). Mean values of troponin I were significantly higher in RHD patients compared to no RHD patients ((0.37±0.07 *vs.* 0.12±0.05, $p<0.05$). 75% of RHD patients had increased values of troponin I compared to 33% of no RHD patients. Shock index was ≥1 in 37.5% of RHD patients, instead 20% of no RHD patients. RHD patients revealed non significantly higher values of mean heart rate and lower values of systolic blood pressure compared to no RHD patients. Patients with RHD had a significantly major involvement of main pulmonary artery and its main branches and bilateral involvement compared to patients without RHD (Figure 1). *Conclusions.* Our study shows that the presence of RHD is related to troponin I values and proximal and bilateral clots distribution in pulmonary vascular tree. Patients with RHD at admission have more severe prognosis. The presence of RHD could represent a criteria to start thrombolysis also in normotensive patients with PE.

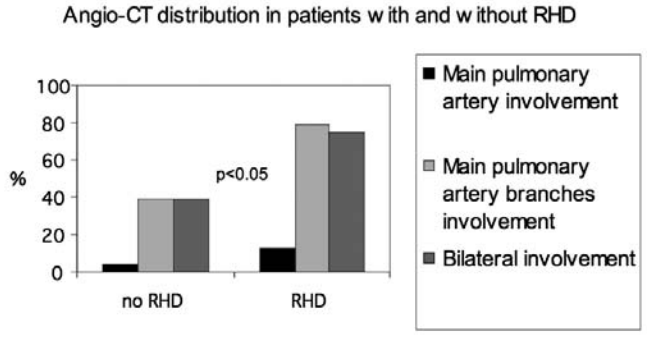

Figure 1.

P086

CLINICAL USEFULNESS OF HELICAL TC IN THE FOLLOW-UP OF PULMONARY THROMBOEMBOLISM

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Background. It's not clear which is the role of the thromboembolism events outcomes in pulmonary arteries that can determined recurrences or in of the secondary pulmonary hypertension. Timely diagnosis of pulmonary embolism (PE) is crucial because prompt appropriate management can decrease mortality and morbidity. Often all the perfusion CT scan deficits in the pulmonary arteries have considered as recurrences. *Methods.* We have done a retrospective study to evaluate the prevalence of perfusion deficit in pulmonary arteries performing spiral CT scan after one year from acute event. Of 69 patients (pts) hospitalized in our hospital have been excluded 21 neoplastic pts (30%), 1 pts thrombolyse (1%), 3 pts for recurrence under oral thrombolytic therapy (TAO). We have evaluated 43 pts for a median follow-up of 11.5± 2.2 months. During the follow-up 7 pts (16%) are dead (3 for myocardial infarction ,1 for brain hemorrhages, 2 for cardiac failure, 1 for PE recurrence). 3 pts (7%) resulted neoplastic and 3 pts (7%) were lost to follow-up. Only 28 pts have performed spiral CT scan after 1 year from PE. For all pts we collected at the diagnosis anamnesis, blood pressure, arterial blood gas analysis, ECG, D-Dimer value. R*esults.* On 28 pts enrolled, 10 (36%-Group B) presented at spiral CT scan, performed after 1 year from diagnosis and TAO thrombotic, residues in the pulmonary arteries. The other pts were collected in group A. The difference between two groups are shown in Table 1. *Conclusions.* The prevalence of thrombotic residues in the pulmonary arteries is 36% of pts. Spiral CT scan performed at the end of TAO should be useful for to evaluate the presence of perfusion deficit. In fact, the PE recurrence are causes of significant mortality and morbidity

Table 1.

Symptoms/Signs	*Group A 18 pts (100%)*	*Group B 10 pts (100%)*	*p*
Dyspnea	14 (78%)	9 (90%)	0.35
Chest pain	15 (67%)	0 (0)	>0.01
Syncope	1 (11%)	7 (70%)	<0.01
Right ventricular strain	4 (33%)	9 (90%)	<0.01
D-dimer	4.22±1.88	7.76±3.9	

P087

PHARMACODYNAMIC OF LOW MOLECULAR WEIGHT HEPARIN IN PATIENTS UNDERGOING BARIATRIC SURGERY: A PROSPECTIVE, RANDOMISED STUDY COMPARING TWO DIFFERENT DOSES OF PARNAPARIN (BAFLUX STUDY)

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Background. The optimal dose of low-molecular-weigh-heparin (LMWH) to prevent venous thromboembolism (VTE) after surgery in morbid obese patients remains controversial. *Aims.* Aim of this study was to evaluate the pharmacodynamic parameters of two doses of the LMWH parnaparin administered to patients with Body Mass Index (BMI) >36 undergoing bariatric surgery. *Material and methods.* Patients were enrolled in a multicentre, open label, pilot study and were randomised to receive 4250 IU/day [n=36; 30 females; median age: 38 years (23-56); median BMI: 46.7 Kg/m^2 (36.5-58.8)] or 6400 IU/day [n=30; 24 females; median age: 42 years (22-63); median BMI: 43.7 Kg/m^2 (36.1-64.1)] of parnaparin s.c. for 9±2 days. The pharmacodynamic effects of parnaparin were analysed by measuring the anti Factor Xa activity on day 0 (12 hours after the first parnaparin injection), day 4 and day 6 after surgery (before and 4 hours after parnaparin injection). *Results.* In 98.3% of patients receiving 4250 IU/day the peak anti Xa levels were in the range of 0.1-0.4 IU/mL. Higher anti Xa levels were observed in patients receiving 6400 IU/day: in 62.3% of these patients the peak anti Xa levels were greater than 0.4 IU/mL. The anti Xa levels measured 4 hours after injection on days 4 and 6 were not statistically correlated with BMI for either parnaparin dosage [Spearman correlation coefficients: -0.232 (p=0.077) and -0.118 (p=0.401) for 4250 or 6400 IU/day dose, respectively]. *Conclusions.* The dose of 4250 IU/day seems adequate to achieve prophylactic anti Xa levels in morbid obese patients undergoing bariatric surgery. On the other hand, most of the patients receiving 6.400 IU/day show anti Xa levels higher than the recommended prophylactic values.

P088

INCIDENCE OF CARDIOVASCULAR EVENTS AFTER VENOUS THROMBOEMBOLISM. A SYSTEMATIC REVIEW AND META-ANALYSIS

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An association between venous thromboembolism (VTE), cardiovascular risk factors and preclinical atherosclerosis has been claimed. Recent studies suggest a higher incidence of arterial events in patients with idiopathic VTE as compared to patients with VTE associated with risk factors.We performed a systematic review and a meta-analysis aimed at assessing the incidence of cardiovascular events (CV) after VTE. Unrestricted searches of MEDLINE and EMBASE were performed from 1982 to December 2007 using the terms *pulmonary embolism* or *deep vein thrombosis* or *venous thrombosis* or *venous thromboembolism*. Review articles and bibliographies were manually searched. Studies were included if they included patients with VTE and: 1) reported on the incidence of CV

events (ischemic stroke and acute myocardial infarction [AMI]); 2) had a follow-up longer than 3 months. Overall, 63 studies were included in the analysis. Thirty-eight studies reported data on fatal CV events (15344 patients, median follow up 17.65 months, range 6 to 144 months). The pooled incidence rate of fatal ischemic stroke, fatal AMI and fatal CV events was 0.25, 0.33 and 0.59% patient-year, respectively. Fourteen studies (49473 patients; median follow up 32.4 months, range 6 to 240 months) reported data on fatal and non fatal CV events, 5 randomized controlled trials (RCT) (3821 patients; median follow up 16.8 months, range 6 to 32.4 months) and 9 observational studies (45652 patients; median follow up 45 months, range 6 to 240 months). Overall, the incidence of fatal and non fatal CV events was 0.33% patient-year (0.59% in RCT and 0.33% in observational studies, p=NS). Incidence of ischemic stroke and AMI was 0.11 and 0.22 patient-year, respectively. Three observational studies reported separate data on CV events in patients with idiopathic and secondary VTE. An incidence rate meta-analysis of these studies (44403 patients; median follow up 145.5 months, range 38 to 240 months) was performed. The risk of CV events in patients with idiopathic VTE was higher than in patients with secondary VTE (incidence rate ratio [IRR] 2.00; CI 1.63-2.47). The separate analyses for AMI and ischemic stroke confirmed the higher risk in patients with idiopathic VTE with respect to secondary VTE (IRR 2.02, CI 1.62-2.53 and IRR 1.99, CI 1.69-2.35 respectively). Patients with idiopathic VTE have a higher risk of acute myocardial infarction, ischemic stroke and CV events in the long term follow-up, in comparison with patients with secondary VTE.

P089

RESIDUAL VENOUS OBSTRUCTION AND D-DIMER AS RISK FACTORS FOR RECURRENCE AFTER ANTICOAGULATION WITHDRAWAL FOR A FIRST IDIOPATHIC DEEP VEIN THROMBOSIS IN THE PROLONG STUDY

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In a single centre study we have shown that D-dimer (D-d) but not residual venous obstruction (RVO) is a risk factor for recurrent venous thromboembolism (VTE) after anticoagulation suspension. We assessed the predictive value of D-d and RVO in combination for recurrent VTE in the multi-centre (30 centres) randomized open label PROLONG study (NEJM, 2006;355:1780-9). *Methods.* Patients with a first episode of idiopathic proximal deep vein thrombosis of the lower limbs were enrolled on the day of anticoagulation suspension when RVO was determined by compression ultrasonography according to the method of Prandoni et al. (Ann Intern Med 2002; 137:955-960). D-d (cut-off value: 500 ng/mL) was measured at 30+10 days afterwards. Follow-up was 1.5-year. *Reults.* Recurrences occurred in 8.9% of subjects (36/404) overall, in 6.8% (21/309) of subjects and in 13.7% (13/95) of subjects with normal and abnormal D-d, respectively. Recurrences occurred in 9.6% of subjects (25/259) with absent RVO and in 7.6% of subjects (11/145) with RVO. The multivariate hazard ratio for recurrence was 2.49 (p<0.003) for abnormal D-d and 1.2 (p>0.05) for RVO. The recurrence rate was 4.6% (17/202) for normal D-d without RVO and 8.4% (5/107) for normal D-d with RVO, a non significant difference. The rates of recurrence were not significantly different between abnormal D-d without or with RVO, 12.2% (7/57) and 15.8% (6/38), respectively. *Conclusions.* The results of the multi-centre PROLONG study confirm that while D-d at 30+10 days after OAT withdrawal is a risk factor for recurrent VTE, RVO at the time of OAT withdrawal does not increase the risk of late recurrence.

P090

CHRONIC DISEASES AS RISK FACTOR OF VTE RECURRENCE AFTER OAT WITHDRAWAL

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Oral anticoagulant treatment (OAT) is able to prevent most episodes of venous thromboembolism (VTE) recurrence. Several factors are associated with an increased risk of recurrence, such as male sex and elevated D-dimer levels 1 month after OAT withdrawal. However, the positive predictive value of this parameter is only 15%, not sufficient to select patients requiring prolonged therapy. Aim of our study was to assess if recurrent VTE is associated with the presence of a chronic disease. We prospectively investigated 384 patients (205 males; 179 females), median age 62 years (12-92). Twenty fivepatients had associated chronic disease (10 patients underwent kidney transplantation, 9 patients had connective tissue diseases, 6 patients had inflammatory bowel disease). Follow-up [median 24 months (1-132)] started after OAT withdrawal. Median OAT duration was 10 months (3-72). VTE recurrence was recorded in 53 patients (13.8%) after a median time of 11.5 months (1-114). Recurrences were more frequent in patients with chronic disease than in those without [9/25 (36%) *vs.* 44/359 (12.2%), respectively (p=0.03); OR 4.0 (95%CI 1.7-9.7), p=0.001]. We confirmed that recurrence was more frequent in patients with elevated D-dimer levels than in patients without [27/51 (53%) vs 86/319 (27%) respectively, (p=0.000); OR 3.0 (95%CI 1.7-5.6), p=0.000]. A multivariate analysis adjusted for age, sex, presence of chronic disease and elevated D-dimer levels, showed that elevated D-dimer levels and chronic diseases are independently associated with recurrence [OR 2.9 (95%CI 1.5-5.3), p=0.001 and OR 3.3 (95% CI 1.3-8.3), p=0.01 respectively]. In addition the contemporary presence of chronic disease and elevated D-dimer levels seems to further increase the risk of recurrence [OR 5.7 (95%CI 1.7-19.3), p=0.006]. In conclusion our findings suggest that the presence of chronic disease help to identify patients at high risk who request a careful evaluation for tailoring the better duration of OAT.

P091

ELEVATED D-DIMER AND F1+2 LEVELS ONE MONTH AFTER OAT WITHDRAWAL AND RISK OF VTE RECURRENCE

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Oral anticoagulant treatment (OAT) is able to prevent most episodes of venous thromboembolism (VTE) recurrence. To define predictors of recurrence is the key question at OAT withdrawal. D-dimer, prothrombin fragment 1+2 (F1+2) and thrombophilia were studied. However, few studies have evaluated the role of the combination of these parameters as predictors of recurrence. The purpose of our study was to assess if VTE recurrence could be better predicted by the association of these parameters evaluated 1 months after OAT withdrawal. We prospectively investigated 370 patients (202 males; 168 females), median age 62 years (12-92) and median OAT duration 10 months (3-72). Follow-up [median 24 months (1-132)] started after OAT withdrawal. VTE recurrence was recorded in 51 patients (13.8%) after a median time of 11.5 months (1-114). Recurrences were more frequent among males (19.3%) compared with females (7.1%) (p=0.001). One or more thrombophilic alterations were detected in 22.6% of patients and no relationship was found between common thrombophilia and VTE recurrence (p=0.3). After OAT withdrawal, elevated D-dimer levels were found in 27/51 (53%) patients with recurrence and in 86/319 (27%) patients without recurrence [OR 3.0 (95% CI 1.7-5.6); p=0.000]. Elevated F1+2 levels were found in 23/49 (47%) patients with recurrence and in 86/295 (26.4%) patients without recurrence [OR 2.5 (95% CI 1.3-4.6); p=0.004]. When high D-dimer and F1+2 values were considered contemporary present OR was 4.1 (95% CI 2.0-8.7; p=0.000). The addition of F1+2 to D-dimer measurement only slightly increase the ability of identifying patients at higher risk of recurrence after a first episod of VTE.

P092

BOSENTAN FOR CHRONIC THROMBOEMBOLIC PULMONARY HYPERTESION: A SYSTEMATIC REVIEW

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Background. After acute pulmonary embolism, chronic thromboembolic pulmonary hypertension (CTEPH) is diagnosed in about 1% of patients. The endothelin receptor antagonist bosentan could be a therapeutic alternative for CTEPH patients who are not candidates to pulmonary endarterectomy (PEA) and for persistent CTEPH after PEA. *Methods.* We searched in MEDLINE and Embase using the terms *pulmonary hypertension* and *bosentan*. Papers were included in this review if they reported on patients with objectively confirmed CTEPH treated with bosentan. The efficacy measures were the improvement in NYHA class and 6 minute walking distance (6mwd) and in hemodynamic parameters (cardiac index, pulmonary artery pressure, pulmonary vascular resistance). Mortality and safety were also assessed. *Results*. Overall, 367 papers were found. Thirty-four papers focused on CTEPH were retrieved for full text examination. Seven single-arm cohort studies (125 patients) were found in which patients with CTEPH were treated with bosentan. Follow-up was at three months (3 studies), six months (3 studies) and 4 months (1 study) after the initiation of bosentan therapy. The weighted mean increase in 6mwd after 3-6 months of treatment was 62.6 meters (95% CI 60.8 to 64.3; $p<0.001$). The additional increase of 6mwd at one year was 8.5 meters (95% CI 7.1 to 9.9) (3 studies, 63 patients). About 25% of patients had an improvement on functional NYHA class at 3-6 months. Data on hemodynamic parameters were available in six studies, 109 patients. The mean weighted increase in cardiac index at 3-6 months was 0.16 l/min/m^2 (95% CI 0.13 to 0.19); the mean weighted decrease in pulmonary artery pressure at 3-6 months was 2.75 mmHg (95% CI 2.38 to 3.12). One patient died within 3-6 months (0.8%) and 2 additional patients died within one year (3%). *Conclusions.* Bosentan therapy is associated with an initial improvement of functional status in patients with CTEPH. Further treatment is able to maintain the functional improvement but it is associated with a modest improvement in hemodynamic parameters. These findings should be confirmed in placebo controlled mortality studies on the top of optimal treatment.

P093

THE ATTITUDE TO PRESCRIBE COMPRESSION STOCKINGS IN PATIENTS WITH ACUTE DVT: FINDINGS FROM MASTER REGISTRY

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Background. The adherence of clinicians managing the acute phase of venous thromboembolic diseases to the current guidelines is reported to be high for the pharmacological approach but not for the prescription of compressive elastic stockings. Results of surveys and registries show limited use of compression stockings in patients with DVT. The aim of this report is to provide information on the extent to which elastic stockings are used by patients with acute DVT managed by Italian doctors and to identify the predictors of failure to prescribe. *Materials and methods.* MASTER is a multicenter prospective registry collecting information about the clinical characteristics and management of patients with DVT and PE at the time of the relevant event by an electronic data network. *Results.* A total of 2119 patients were enrolled, of whom 1913 (90.2%) had DVT. Deep vein thrombosis affecting lower limbs was observed in 1772 subjects. Of these, 1277 (72.1%) were given elastic stockings on discharge from hospital. The following conditions were associated with a more frequent prescription: age under 60 years (OR 1.58; 95% CI 1.06-2.43), presence of oedema (OR 2.68; 95% CI 2.09-3.45) and in-hospital management (OR 2.28; 95% CI 1.84-2.83). The presence of thrombophilic conditions was associated with a trend towards an increased use of elastic stockings while the opposite was true for cases of DVT affecting both legs. In a multivariate analysis young age, presence of oedema and in-hospital management remained significantly associated with more frequent use of elastic stockings. *Conclusions.* The rate of prescription of elastic stockings by Italian clinicians is higher than previously reported in European registries, however it still appears to be below the optimum level. Special attention should be paid to patients treated at home for whom reasons other than clinical factors (i.e. logistical problems) may interfere with the prescription of elastic stockings.

P094

FONDAPARINUX IN HEPARIN-INDUCED THROMBOCYTOPENIA: A CASE REPORT

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An 82-years old woman was admitted for the resection of colon cancer causing stenosis of the left colon. She had no cardiac or thoracic history. Blood counts were normal. Twelve hours after surgery, antithrombotic prophylaxis was started with enoxaparin. The platelet count increased progressively in the following days until 374×10^9/L on day 9. On day 10 after surgery, the patient developed dyspnea. A spiral CT scan showed segmental embolism of the lower and apical right lung. The platelet count at that time was normal (224×10^9/L). Anticoagulant therapy with full dose enoxaparin was then started. The following day, a drop in the platelet count was recorded (141×10^9/L). A subsequent control (4 days later) showed severe piastrinopenia (33×10^9/L), suggestive of heparin-induced thrombocytopenia. Enoxaparin therapy was therefore suspended, and fondaparinux was started at a dose of 5 mg/die. Antibodies anti-platelet factor 4/heparin (HIPA) were strongly positive. In the following days platelet counts progressively increased, reaching 100×10^9/L another 4 days later. Oral anticoagulant therapy was then started, and fondaparinux was suspended upon an INR of 2. Eight days after the interruption of heparin, platelet count was normal (255×10^9/L). The patient was discharged. This case of heparin-induced thrombocytopenia reinforces the evidence that fondaparinux can be considered a sound alternative to anticoagulant drugs, such as lepirudin, difficult to manage, particularly in a surgical environment, for its potential bleeding complications.

P095

SCOPE: STUDY ON THE CLINICAL COURSE OF PULMONARY EMBOLISM

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Background. The incidence of chronic thromboembolic pulmonary hypertension (CTPH) after pulmonary embolism (PE) is higher than expected, averaging 4% in the first 2 years. The risk factors of such an adverse outcome are not well known; persistent right ventricular dysfunction and residual pulmonary thromboemboli are likely factors. Interestingly enough data from PE studies clearly show a very high prevalence (>50%) of residual pulmonary thromboemboli at 6 months.

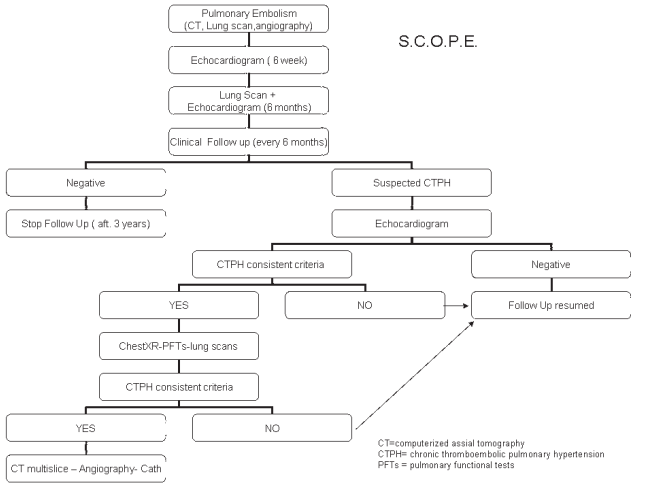

Figure 1.

The evidence of relationships among residual thromboemboli, PE relapses and CTPH is inadequate. The cohort multicenter, nationwide, prospective SCOPE is designed to assess the incidence of CTPH after an acute episode of symptomatic or asymptomatic PE, and the correlations among the development of CTPH and PE relapses, residual pulmonary thromboemboli and right ventricular dysfunction. *Methods.* Eligibility criteria are a first objectively documented episode of acute symptomatic or asymptomatic PE, with or without deep vein thrombosis. Exclusion criteria are age lower than 18 years, pregnancy, expected survival less than 2 years, previous documented cardiac or lung diseases, logistic difficulties, poor compliance, consent refusal. A standardised severity score of instrumental PE diagnosis (TC, lung scan, angiography) is recorded as well as type and duration of anticoagulant therapy. All patients undergo echocardiographic examination after 6 weeks and 6 months, lung scan and possibly compressive echography after 6 months. A centralised reading and scoring of scintigraphic images is then made by and independent committee. Subsequently a clinical follow-up of 3 years is scheduled. When clinical suspicion of PE or CTPH develops, a confirmatory standardised diagnostic strategy is performed; the cause of death is recorded or adjudicated by independent physicians, if unknown. All data are then recorded and collected centrally by electronically means. The study is ongoing and to date there are 73 active centers and 87 patients enrolled.

Inherited and Acquired Thrombophilia

P096

RS10757274 SINGLE NUCLEOTIDE POLYMORPHISM IN CHROMOSOME 9 AND RISK OF ISCHEMIC STROKE AT YOUNG AGE IN TWO EUROPEAN WHITE POPULATIONS

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Introduction. Genome wide association studies consistently found an association between loci on chromosome 9 (Chr9) in the region 9p21 and coronary artery disease. Arterial stroke at young age accounts for 2% to 19% of all ischemic strokes. Thus, efforts to identify novel risk factors for cerebral ischemia at this age have relevant public health implications. *Aims*. Italian white patients with first-ever ischemic stroke (IS) (n=253) before the age of 45 years and control subjects (n=191) recruited from the general population in the surrounding area, with no known history of vascular disease were included in the study. Consecutive German white patients (n=151) aged less than 50 years and control subjects (n=198) without a history of vascular disease randomly selected from the population of the same region were included. A meta-analysis of all available studies on this topic was performed. Data from different studies were combined by using the general-variance-based method. *Results.* Allele frequencies of rs10757274 were 0.52 (95% CI, 0.47-0.57) and 0.48 (0.43-0.53), respectively, for allele G and A in Italian controls and 0.58 (0.53-0.63) and 0.42 (0.37-0.47) in cases (p=0.099 for alleles difference). In Germans, G/A allele frequencies were 0.50 (0.45-0.55) and 0.50 (0.45-0.55) in controls, and 0.47 (0.42-0.52) and 0.53 (0.48-0.58) in cases (p=0.47). No association between rs10757274 polymorphism and the risk of IS at young age was found in Univariate and multivariate analyses either in the Italian or in the German population or according to any type of genetic inheritance model or etiological stroke subgroup. Two additional articles on the association between 9p21 loci and IS were found. Altogether, a total of 1,151 cases and 15,636 controls were considered, with 80% power to detect odds ratio >1.30 for the association with stroke. None of the individual studies showed any significant association. The overall genotypic-specific odds ratios were 1.09 (0.96-1.23) and 1.16 (0.94-1.43) when all studies were pooled. *Conclusions*. Our data support the suggestion that the variant rs10757274-G on 9p21, while strongly associated with increased cardiovascular disease risk, is not apparently involved in the risk for ischemic stroke.

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TISSUE FACTOR GENE POLYMORPHISMS AND HAPLOTYPES AND THE RISK OF ISCHEMIC VASCULAR EVENTS: THREE STUDIES AND A META-ANALYSIS

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Background. The exposure of flowing blood to tissue factor (TF) is the initial step in the coagulation process and plays an important role in thrombogenesis following atherosclerotic plaque rupture. There is a strong inter-individual variability in TF exposure on cell membranes upon stimulation and in its circulating levels, that could be at least partially explained by variation in TF gene. *Aims.* The aim of the present study was to address the role of genetic polymorphisms and haplotypes of tissue factor (TF) gene in the risk of ischemic vascular disease and in

the expression of procoagulant activity (PCA) in mononuclear cells upon stimulation. Finally a meta-analysis of our findings and studies retrieved from literature was performed. *Methods.* The following groups were compared: 431 Italian patients with juvenile myocardial infarction (MI) and 438 controls, 817 US cases with MI and 1,021 controls and 267 Italian cases with juvenile ischemic stroke and 212 controls. rs1361600 rs3917629 (rs3354 in US population), rs1324214 and rs3917639 Tag single nucleotide polymorphisms were selected from Seattle SNP database. Additionally, a meta-analysis of all previous studies on TF loci and the risk of ischemic vascular disease was performed. Genetic regulation of TF procoagulant activity (PCA) was studied before and after *in vitro* stimulation of mononuclear cells with lipopolysaccharide. *Results.* There was no statistically significant association between genotypes or common inferred haplotypes and MI either when haplotype effect was fit as additive, dominant or recessive, both in univariate and in multivariate analyses. In multivariate analyses, homozygotes for T allele of the rs1324214 TagSNP was associated with a reduced risk of stroke (OR (95%CI) =0.36 (0.14-0.90), p=0.03) as compared to CC homozygotes. However, no association was shown between common inferred haplotypes and stroke. The results of the meta-analysis confirmed the lack of association between rs1361600 and the risk of coronary artery disease. The functional test showed no association between TagSNPs or haplotypes and PCA expression either at basal condition or after stimulation. *Conclusions.* TF genetic variations investigated in this study neither affect the risk of MI nor the *in vitro* stimulation of PCA by mononuclear cells. Additional studies are necessary to define the role of TF polymorphisms in the risk of ischemic stroke.

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GENETIC VARIATION OF ALCOHOL DEHYDROGENASE 3 (ADH3) AND MODIFICATION OF VASCULAR RISK FACTORS BY MODERATE ALCOHOL CONSUMPTION: RESULTS FROM THE IMMIDIET STUDY

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Introduction. Moderate alcohol consumption is protective against cardiovascular disease and total mortality. Alcohol dehydrogenases (ADHs) are major enzymes of alcohol metabolism. The ADH3 polymorphism has two alleles, gamma 1 and gamma 2. *Aims.* The aim of our study was to investigate the effect of alcohol consumption on vascular risk factor in relation to ADH3 variants. *Methods.* IMMIDIET is a cross-sectional study of healthy male-female pairs living together, randomly recruited in three European general medical practices. Recruitment took place in Belgium, in Italy and in London. Mixed pairs of Belgian and Italian origin, leaving in Belgium, were also recruited. ADH3 genotypes were available for 535 Italians, 507 Belgians, 502 English, and 403 subjects from Belgian/Italian mixed couples. The ADH3 polymorphism (rs698) was genotyped by Real Time-PCR (TaqMan SNP Genotyping Assay). *Results.* The Italian population (living either in Italy or in Belgium) showed a higher prevalence of gamma 1 homozygous as compared to Belgians or English (0.74 as compared to 0.61-0.62). The intake of alcohol did not vary in relation to ADH3 genotypes (multivariate ANOVA adjusted for country, age, sex, tobacco, social status and total energy intake, both in men and in women). BMI, waist, blood pressure, HDL and cholesterol levels positively correlated with alcohol intake in men (multivariate ANOVA), while no association was found between alcohol and risk factors in women. The associations of alcohol with HDL, blood pressure and cholesterol levels were independent of ADH3 genotypes. However, significant interactions between ADH3 genotypes and alcohol consumption on BMI (p=0.006) and waist circumference (p=0.031) were found in men. The regression coefficient for alcohol and BMI was -0.01 for gamma 1 homozygous, 0.02 for heterozygous and 0.05 for gamma 2 homozygous. The regression coefficient for alcohol and waist circumference levels was -0.0045 for gamma 1 homozygous, 0.08 for heterozygous and 0.12 for gamma 2 homozygous. *Conclusions.* A marked, significant interaction between the ADH3 genotype and alcohol consumption in relation to BMI and waist circumference was found in men from different European origin. Men who drank daily and were homozygous for the gamma 2 allele had a substantial increase in both BMI and waist values. In contrast, ADH3 variants did not interact with the effects of alcohol intake on HDL, blood pressure or cholesterol levels.

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ANNEXIN V HAPLOTYPE M2 INFLUENCE EXPRESSION IN HUMAN PLACENTAE

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Annexin V (Anx V) is a placental anticoagulant protein expressed on normal villi. Recently, in a group of women with recurrent pregnancy loss without known thrombophilias, it has been shown that four consecutive nucleotide substitutions transmitted as a haplotype (M2), confers a two-fold higher risk of fetal loss than non-carriers. Reduced Anx V expression has also been showed immunohistochemically in placentas from women with preeclampsia (PE), may lead to a hypercoagulable state in the intervillous space and may be associated with the development of fetal growth restriction (FGR). Therefore, we hypothesized that placental expression of Anx V could be influenced by the carriership of this haplotype. Twenty-six women, without inherited or acquired thrombophilia, with a complicated pregnancy (12 with PE, 14 with FGR) and 7 with uneventful one (controls) were investigated. Amplified DNA fragments between the promoter and intron 1 region of the Anx V gene were subjected to direct sequencing analysis. Placental quantity mRNA expression of Anx V gene was evaluated by ABI 7700™ quantitative real time PCR system. Gene expression levels were calculated using the deltaCt-method to quantify comparable mRNA levels. Eleven cases (33.33%, 3 PE and 8 FGR) and 1 control (3.03%) showed M2 haplotype. Anx V in cases was 3-fold less expressed in respect of controls (ANOVA test p≤0.01). Women (n=12) carrying M2 haplotype (36.36%) showed Anx V gene expression 2- fold lower than that observed in women (n=21) not carrying it (63.64%; ANOVA test p≤0.01). It has been demonstrated that *in vitro* M2 haplotype reduced Anx V promoter activity. A direct link between reduced Anx V expression levels and a prothrombotic placental environment, leading to FGR, has been established immunohistochemically in PE patients. In a group of placentas from patients with PE or FGR we showed a reduced Anx V gene expression in those carrying the M2 haplotype. This is the first *ex vivo* demonstration that Anx V gene expression in placentas is dependent on the M2 haplotype. We conclude that M2 haplotype could influence Anx V gene expression, not only *in vitro*, but also *ex vivo*. The anticoagulant function of Anx V and the localization on syncytiotrophoblast may reflect a critical role in the occurrence of obstetric complications. Thus, the M2 haplotype, that is associated with a reduced Anx V gene expression, could be responsible, at least in part, for pregnancy complications.

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ENOS GENE INFLUENCES PLATELET PHENOTYPE IN HIGH RISK VASCULAR PATIENTS ON DUAL ANTIPLATELET TREATMENT

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Background. Nitric oxide (NO) regulates the platelet function. Aim of our study was to evaluate the role of eNOS –786T>C, 894G>T and 4a/4b polymorphisms in modulating the platelet phenotype in high risk vascular patients on dual antiplatelet therapy. *Methods and Results.* In 1442 consecutive ACS patients on dual antiplatelet therapy, platelet aggregation on platelet-rich plasma after collagen (2 µg/mL), ADP (10 µM) and arachidonic acid (AA) (1 mM) stimuli and the genetic analysis of eNOS polymorphisms were assessed. In patients carrying the eNOS 4a allele significantly higher maximal platelet aggregation values after AA and collagen stimuli in comparison to non-carriers were observed (p=0.04 and p=0.03, respectively). A significantly higher percentage of patients with AA-induced high residual platelet reactivity was found in subjects carrying the eNOS 4a allele (p=0.03). At multivariate linear regression analysis, we observed that eNOS 4a allele was independently associated with AA-induced platelet aggregation (p=0.01) and a trend in association for collagen stimulus (p=0.05). At logistic multivariate

analysis, the eNOS 4a allele significantly influenced the AA-induced high residual platelet reactivity (p=0.02). *Conclusions.* This study evidences a role for eNOS gene in moderately, but significantly, modulating the platelet phenotype in a high-risk population on dual antiplatelet treatment.

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POLYMORPHISMS OF THE Z PROTEIN PROTEASE INHIBITOR AND RISK OF VENOUS THROMBOEMBOLISM: A META-ANALYSIS

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Background. Two non-sense polymorphisms (W303x and R67X) of Z-dependent protease inhibitor (ZPI), a serpin involved in the regulation of coagulation has been identified. These mutations may be associated to an increased risk of venous thromboembolism (VTE). However, clinical studies gave contradictory results. Therefore, to assess the risk of VTE associated with these mutations, we performed a systematic review and a meta-analysis of all studies in which the prevalence of ZPI mutations in patients with VTE and in a control group of healthy subjects without a history of thromboembolic disease or genetic relationship with the patients was compared.*Methods.* Studies were identified using the MEDLINE and EMBASE (to February Week 4 2008) electronic databases. Study identification, study selection and data extraction were performed independently in duplicate by two reviewers. Odds Ratios (Ors) and 95% confidence intervals (CIs) were calculated for each trial and pooled using a random-effects model. Statistical heterogeneity was evaluated using the I^2 statistic. *Results.* Five studies for a total of more than 5000 patients were included in our systematic review. The inter-observer agreement for the study selection was excellent. Prevalence of R67X was evaluated in 2310 VTE patients and in 2815 controls. Prevalence of W303X was evaluated in 2311 VTE patients and in 2821 controls. Both R67X and W303X mutations were uncommon in the populations of patients with VTE (2.38% and 0.61%, respectively). R67X mutation of ZPI was not associated with an increased risk of VTE (OR 1.63; 95% CI 0.84, 3.16). Heterogeneity among studies was low (I^2=50%). Also W303 X mutation was not associated with an increased risk of VTE (OR 1.21; 95% CI 0.29, 4.98). Again, the heterogeneity among studies was not significant (I^2=48%). *Discussion*. Our systematic review failed to demonstrate an increased risk of VTE in patients with one of these two ZPI mutations. Although our conclusions are based only on the results of five studies, the inclusion in these studies of more than two thousand patients and almost three thousand controls strengthens the validity of our results. Thus, currently available evidence does not support routine screening for ZPI mutations patients with VTE.

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MODULATION OF COAGULATION PHENOTYPES BY LRP AND ABO BLOOD GROUP GENOTYPES IN THROMBOTIC WOMEN

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Background and objectives. Genes involved in biosynthetic processes and clearance from plasma of coagulation factors might influence their circulating levels. Polymorphisms of the multifunctional LRP receptor and of the ABO locus, and their combinations, could be useful tools to investigate the contribution of modifier genes on plasma factor levels and particularly on high FVIII and FXI levels, known risk factors for venous thrombosis. *Results.* Two hundred Italian women (mean age±SD: 34±9 years), randomly selected from those referred for investigation of thrombophilic states after a single episode of deep venous thrombosis, were studied. None of the enrolled females was pregnant, on oral contraceptive or hormonal replacement therapy. Carriers of known thrombophilic defects or mutations were excluded. Blood sampling was performed at least three months after the thrombotic episode and three weeks after withdrawal of any antithrombotic treatment. The LRP -25 CC genotype, which predicts lower LRP expression, was associated with: a) 15% higher FVIII activity (1.93 95% CI 1.83-2.02 IU/mL) as compared with G carriers (1.65 95% CI 1.47-1.83 IU/mL); b) 8% higher FXI activity (1.15 95% CI 1.10-1.21 IU/mL) as compared with G carriers (1.06 95% CI 0.98-1.14 IU/mL) and c) decreased APC sensitivity ratio (0.96 95% CI 0.93-0.99 *vs*. 1.04 95% CI 0.97-1.11). ABO genotypes influenced FVIII, APC resistance and APTT levels, in accordance with previous findings, and in addition slightly influenced FXI levels, that were 4% higher in non-O (1.15 95% CI 1.10-1.20) as compared to O subjects (1.10 95% CI 1.00-1.19). Combination of the ABO/LRP-25C/G genotypes produced a gradient of FVIII levels. Levels in the non-0/CC women (2.02 95% CI 1.91-2.13 IU/mL) were 53% higher than those in the 0/G carriers (1.32 95% CI 1.06-1.59 IU/mL). The LRP genotypes were found to influence FXI levels only in subjects with non-O blood group genotypes. Particularly the non-0/CC genotypes were associated with the highest FXI levels (1.16 95% CI 1.11-1.22 IU/mL). *Conclusions.* The LRP genotypes are biologically plausible genetic determinants of coagulation factor levels, particularly of FVIII. Our study highlights a frequent genetic combination (non-0/LRP-25CC) characterized by high FVIII levels and APC resistance. The influence of combined genotypes on thrombosis liability needs to be investigated.

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NEW MISSENSE MUTATION WITHIN PZ EX 8 IN ABORTERS

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Protein Z (PZ) is a vitamin K-dependent glycoprotein regulating coagulation cascade because of protein Z-dependent protease inhibitor. Reduced circulating levels of PZ have been suggested to play a role in the occurrence of bleeding, deep vein thrombosis and in early, as well as late fetal losses, although data about this issue are conflicting. Among a group of women with otherwise unexplained fetal losses, 7/124 (5.6%) showed PZ levels under the 5th percentile (i.e. 0.52 micrograms/mL), calculated in a control group formed by 104 parous women with at least one uneventful pregnancy and no fetal loss. Five (4.8%) out the 104 controls showed PZ levels under the 5th percentile. We decided to investigate, by direct sequencing, whether PZ sporadic mutations could be present in these 7 cases (mean ag ± SD: 33.1±4.3 yrs) compared to 104 controls (mean age ± SD : 37±5.8 yrs). Two (28.6%) cases and 18 (17.3%) controls were heterozygous for the intron A G -103A mutation; AA genotype was not observed in cases, whereas it was present in 3 (2.9%, p>0.05) controls. Genotype AG for the Intron F G79A gene variant was observed in 3 (42.9%) cases and 32 (30.8%, p>0.05) controls; AA genotype was not observed in cases and was present in 5 (4.8%) controls. 2 patients carried an unreported missense mutation within the exon 8 (T14928C, accession number AF 440358) causing the substitution of a Leu with a Pro in the trypsin-like serine protease domain of the protein. PZ levels in these patients were 0.25 and 0.40 micrograms/mL, respectively, that means below the 2.5 percentile (0.49 micrograms/ml) of the controls. It is known that there is a relationship between the structure and function of proteins. The alpha-helix segments are solidified by having a hydrophobic amino acid in every third to fourth position of the primary structure. When an alpha-helix structure is formed, all these amino acids line up on one side of the helix. Thus, the presence of Pro disrupts the alpha-helix. It is very suggestive to believe that non-conservative substitution of Leu with Pro would affect protein Z structure. This residue is highly conserved among different species. Among the controls, no woman carried this mutation. More interestingly, this mutation was not found in 197 patients with deep vein thrombosis or in the 197 age-matched controls previously investigated. This unreported sporadic mutation within the PZ gene could explain the very low PZ levels in our patients.

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DETECTION OF THE THROMBOPHILIC MUTATIONS: A COMPARISON BETWEEN SMART CYCLER DX SYSTEM AND GENEXPERT SYSTEM

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Genetic risk factors for venous thrombosis include a sequence variant in the prothrombin gene (20210G > A) and factor V Leiden (1691G > A). These single nucleotide polymorphisms can be diagnosed with restriction fragment length polymorphism analysis, after manual DNA extraction, which is technically demanding and labor-intensive. GeneXpert System has been recently introduced in clinical care. The GeneXpert System performs, in approximately 35 min, the three processes required

for real-time PCR molecular testing: automated nucleic acid isolation, amplification and fluorescence-based quantitative PCR. The aim of this study was to compare this fully automated method with the real time PCR in use in our laboratory (Smart Cycler DX system) for determining mutations in the coagulation FV and FII genes. We studied 30 subjects who underwent a screening for thrombophilia (median age 40y, range 2-82; 12 men and 18 women). For factor V Leiden mutation there was complete agreement between both methods in detection of wild-type (n=18), heterozygous (n=11) and homozygous (n=1) subjects. Similarly, the prothrombin G20210A mutation showed complete agreement for wild-type (n=22), heterozygous (n=4) and homozygous (n=4) subjects. The assay was also able to separate heterozygous from homozygous subjects. This study demonstrates that the GeneXpert is a rapid and reliable system for simultaneous detection of FV Leiden and FII mutations and could be particularly useful for laboratories with a large volume of thrombophilia test requests.

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AN AUTOMATION EXPERIENCE IN MOLECULAR BIOLOGY: THE GENEXPERT DX SYSTEM FOR FV LEIDEN AND FII 20210A MUTATIONS DETECTION

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Background. The association of Factor II (20210 G→A) and Factor V (Leiden, 1691 G→A) mutations with an increased risk for venous thrombosis have been well documented. Factor II 20210A mutation refers to the G to A transition at nucleotide position 20210 in 3' untranslated regions of the gene and it is associated with increased plasma levels of prothrombin. Factor FV Leiden refers to the G to A transition at nucleotide position 1691 of the FV gene, resulting in the substitution of the Arginine aminoacid by Glutamine in the FV protein, causing Resistance to cleavage by Activated Protein C(APC. FII 20210A and FV Leiden are present in 2% and 5% of the general population respectively. *Aim.* evaluate the performances of the new GeneXpert (Cepheid) instrument for the detection of the two above mentioned mutations with the Xpert HemosIL FII & and FV assays. *Methods.* samples collected in sodium citrate, 109 consecutive patients with suspect of thrombo- philia, were examined initially with Light Cycler (Roche) and after with GeneXpert for FII 20210A and FV Leiden mutations. Differently from Roche methodology, the GeneXpert automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in single or complex samples using real-time PCR. The system consist of an instrument and a computer, with an easy proprietary software for running tests on blood samples and viewing the results and interpreting genotype information. Single-use disposable GeneXpert cartridges contains the PCR reagents and handle the PCR process. Cartridges are self-contained so that cross contamination between samples is eliminated. *Results.* using the Light Cycler, which has been considered as reference method, we have found 71 normals, 33 heterozygous and 5 homozygous for FII 20210A mutation; 70 normals, 38 heterozygous and 1 homozygous for FV Leiden mutation with an agreement of 100% in terms of sensitivity and specificity. *Conclusions.* the new GeneXpert instrument shows very impressive performances mainly in terms of user friendly , TAT reduction (less than 35' are necessary for both mutations), less samples manipulation, better standardization and a very good correlation with conventional methods.

Table 1. Factor V and factor II agreement.

	ROCHE FII	GeneXpert FII	AGREEMENT %	ROCHE FV	GeneXpert FV	AGREEMENT %
NORMAL	71	71	100	70	70	100
HETEROZYGOUS	33	33	100	38	38	100
HOMOZYGOUS	5	5	100	1	1	100
TOTAL	109	109		109	109	

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GENE VARIANTS ASSOCIATED WITH VENOUS THROMBOEMBOLISM: OUR EXPERIENCE

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Thrombophilias are inherited or acquired conditions that predispose individuals to thromboembolism. The Factor V Leiden (FV G1691A) and the prothrombin mutation (PT G20210A) are a significant risk factor for venous thromboembolism (VTE). The aim of our study was to determine the prevalence of FV G1691A and PT G20210A in 212 patients (119 women and 93 men) referred to our laboratories, with at least one objectively confirmed episode of VTE (upper and lower extremities, mesenteric, portal and retinal veins and Budd-Chiari syndrome) with or without pulmonary embolism. From all patients we obtained a personal and family history of VTE as well as presence or absence of circumstantial vascular risk factors (recent surgery, trauma or immobilization, oral contraceptive use, pregnancy, post partum period and malignancy). The DNA mutations were analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Of all patients with thrombosis, 45 (21,2%) were heterozygous and 1 (0,5%) homozygous for the FV G1691A. The PT G20210A was found in 30 patients (27 heterozygous and 3 homozygous) which represents the prevalence of 12,7% and 1,4% respectively. The VTE in 33% of the our patients was considered to be secondary to recent surgery, trauma or immobilization, pregnancy and post partum period, oral contraceptive use and malignancy. These data suggest that in our population (Campania – South Italy) FV G1691A and PT G20210A are the most common genetic risk factors for VTE in presence of circumstantial risk factors and that this laboratory screening has an important role to identify venous thrombosis risk.

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THROMBOPHILIA IS NOT A RISK FACTOR FOR STENT THROMBOSIS

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Stent thrombosis (ST) after percutaneous coronary intervention is an uncommon and potentially catastrophic event with an incidence of 0.5-2%, that might manifesta s myocardial infarction and sudden death, with a morbilità of 60-70% and a mortalità of 20-25%. Although multiple factors play a major role, in determining the occurence of stent thrombosis (procedural factors, stent lenght, bifurcation lesions , antiplatelet agents, thrombophilc conditions), the incidence of these single risk factors remains under debate (or unknown). No data in litterature have shown a pathogenetic role of thrombophilia in stent thronbosis. The aim of our study is to analyze retrospectively the incidence of a genetically determinated thrombophilic condition, in a group of patients with angyographic diagnosis of stent thrombosis compared to a control population. *Materials and Methods.* we studied 33 patients (M: 28; F 5), (age average 66 years) who were seen consecutively at our Hospital. All patients were under antiplatelet treatment. A venous blood sample was obtained from patients and controls for the screening of the mutation G1691A of FV, G20210A of FII and C677T of the enzyme MTHFR and the mutation TAFI 140C/T. *Results.* None of the mutations studied showed a significant difference between patients and controls:FV Leiden (χ^2 0.916; p 0.327), FII G20210A (χ^2 0.0049; p 0.944), C677T MTHFR enzyme eterozygous (χ^2 0.5112;p:0.774) and homozygous χ^2:0.4821; p=0.487). This included also homozygous polymorphism of TAFI 140 C/C (χ^2:1.0760; p=0.584). *Conclusions.* Thus it appears that the most common genetically determined hypercoagulable states are not implicated in the pathogenesis of stent thrombosis, therefore our data confirm that antiplatelet therapy is the first choise for the treatment of these patients.

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PRIMARY ANTIPHOSPHOLIPID SYNDROME PATIENTS CONSUME MORE WEEKLY WARFARIN THAN INHERITED THROMBOPHILIA PATIENTS AT SIMILAR TARGET INR

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Weekly warfarin consumption in primary antiphospholipid syndrome (PAPS) patients on oral anticoagulation (OA) was compared to that of

inherited thrombophilia (IT) patients and on OA at similar target INR 2-3 along a follow-up period of 8 years. A further reference group of patients with mitral valve replacement (MVR) (target INR 3-4) was not included in the statistics (Table 1). PAPS used more weekly warfarin than IT (and MVR) independently from the vitamin K epoxidase polymorphim. PAPS homozygous for the MTHFR C677T transition (n=7) had greater warfarin use than heterozyogous or un-mutated PAPS patients (60±13 *vs.* 36±17 mg, $p \leq 0.05$) and than homozygous MTHFR C677T in the IT group (n=11) (60±13 *vs.* 25±10 mg $p \leq 0.01$). PIVKA II correlated with INR in PAPS (r=0.56, $p=0.02$) and in IT (r= 0.62, $p=0.01$). Log IgG aCL titre correlated to weekly warfarin consumption (r=0.42, $p=0.006$). PAPS patients on OA require greater weekly warfarin doses than IT on OA at similar INR Homozygous MTHFR C677T and IgG aCL titre confer a degree of warfarin resistance in PAPS possibly through hepatocyte oxidation/epoxidation cycles.

Table 1.

	PAPS	*IT*	*MVR*	*p-value*
No	48	74	33	
Female (%)	56	43	84	
Age (mean±SD)	44±16	43±15	59±14	
INR (mean)	2.33	2.39	3.11	
Warfarin (mg weekly)	48±18	39±18	35±14	$p=0.03$*

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VENOUS THROMBOEMBOLISM AND ANTIPHOSPHOLIPIDS ANTIBODIES TOWARD PROTEIN C/PROTEIN S SYSTEM: A CASE-CONTROL STUDY.

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Background. Although an association between venous thromboembolism (VTE) and anti-Protein C (PC) and anti-Protein S (PS) antibodies have been hypothesized, the role of these autoantibodies in the pathogenesis of VTE is not clarified yet. *Aim of the study*. To determine the risk of VTE in carriers of anti-PC and/or anti-PS antibodies as compared to non-carriers. *Materials and methods*. We performed a retrospective case-control study. For each subject a complete screening for inherited thrombophilia was performed. Detection of anti-PC, anti-PS and anticardiolipin antibodies IgG and IgM was performed with specific ELISA assays. For the detection of LAC, the guidelines recommended by the subcommittee for standardization of the International Society on Thrombosis and Haemostasis were followed. The 90° percentile of autoantibodies concentrations measured in controls were considered as cut off. *Results.* 135 cases and 164 controls were enrolled. Among cases there was a higher prevalence of anti-PC antibodies both IgG (O.R: 2.10, CI 1.07-4.13) and IgM (O.R: 2.76, CI 1.43-5.30) than controls. Anti-PS were higher in cases than in controls eventhough the difference was not statistically significant. After adjustment for age, sex and the presence of other auto-antibodies in a logistic regression analysis only anti-PC IgM >90° percentile were confirmed to be a significant risk factor for VTE (OR 3.615; 1.647-7.935). *Discussion.* Among auto-antibodies against the PC/PS system, anti-PC IgM auto-antibodies revealed to be an indipendent risk factor for VTE. Their presence conferred a 3,6-fold increased risk. Anti-PS auto-antibodies were not associated with VTE. This retrospective study does not allow to exclude that anti PC/PS antibodies is an epiphenomenon and not the cause of thromboembolic events. Other larger prospective studies are needed to confirm these findings.

P110

DRVVT TEST FOR LUPUS ANTICOAGULANT RESEARCH HIGHLY CORRELATES WITH THROMBOSIS IN THE ANTIPHOSPHOLIPID SYNDROME

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The antiphospholipid syndrome is characterized by the presence of antiphospholipid antibodies in plasma of patients with thromboembolic complications. A major problem in defining the syndrome is that serologic assays to detect antiphospholipid antibodies have a low specificity. Here, we studied the clinical relevance of detecting Lupus Anticoagulant (LA) with different tests, in order to define which test (or pattern of tests) highly correlates with thrombosis. We valuated sera from 200 patients, all positive for LA research two times (6-12 weeks), as indicated by current criteria. LA detection was performed by SCT, KCT, STA-CLOT-LA and dRVVT tests. 72 patients received the diagnosis of Antiphosholipid Syndrome (APS). In the remaining 128 patients with or without a history of autoimmune disease, LA was not associated with clinical events suggestive for APS. An increased frequency of thrombosis was found in the group of patients with LA detection positive with dRVVT (dRVVT+/thrombosis+69,70% *vs.* dRVVT+/thrombosis-36,73%, $p<0,001$). Among the different tests proposed for LA searching, dRVVT highly correlates with thrombosis in the antiphospholipid syndrome. This message should be kept in mind in order to attribute different prognostic values to LA positive tests on the basis on the method resulted positive, also concerning prophylaxis strategies to prevent thromboembolic events.

P111

LUPUS ANTICOAGULANT TESTING IN PIEMONTE AND VALLE D'AOSTA: TWO YEARS OF MULTILABORATORY EXTERNAL QUALITY ASSURANCE PROGRAMS

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Aim of this study is to investigate differences in LA, testing and reporting practices among diagnostic laboratories of Piemonte and Valle d'Aosta. A survey of 5 plasmas to screen for LA was sent in 2007 and 2008 to all 17 laboratories enrolled. The following information were requested to each participating centre: manufacturer/type of assay; values used to define negative/positive and semi/quantitative cut off and how they were determined; whether interpretative comments were provided and their content. *Results and Conclusions.* Survey 2007: a) Only half (8 out of 17) of the laboratories followed SSC-ISTH LA guidelines for LA diagnosis; b) all the laboratories were able to confirm LA presence in the medium/high LA positive sample (sensibility = 100%); c) most of the laboratories were able to confirm LA negativity in OAT, factor deficiency and negative samples (Specificity respectively: 76.5%, 88,.2%, 88,2%); d) more difficulties were found by laboratories in LA confirm test in the weak LA sample (sensibility = 58.8%); e) major problems were found in the execution of LA confirm test for all the screening test assayed;

P112

THE FACTOR V HR2 HAPLOTYPE (FV A4070G) AMONG WOMEN WITH VENOUS THROMBOEMBOLISM

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Background. The HR2 haplotype in the factor V (FV) gene produces a mild increase of the plasma activated protein C -resistance. It is considered a mild risk factor for thrombosis and it is uncertain wheter the coinheritance of FV Leiden increases the thrombotic risk conferred by FV Leiden alone. Whether the presence of FV HR2 could be relevant in some situations leading to acquired APC-resistance such as pregnancy or use of oral contraceptives, is unknown. *Aims.* The present study is aimed to investigate the prevalence of the FV HR2 among women with venous thromboembolism (VTE) due to different provoking factors. *Patients and Methods.* We investigated 393 women with VTE. The first clinical presentation was deep venous thrombosis (DVT) of the legs in 348 cases (in 87 of them with pulmonary embolism, PE) and isolated PE in 45 cases. The median age at the first thrombosis was 33 years (range 14-82). The first VTE event was provoked in 303 patients (pregnancy or puerperium n= 101; oral contraceptives n=84; surgery and other transient risk factors n=118). A group of 204 healthy women was also investigated (median age 37, range 19-61). Laboratory investigation for inherited thrombophilia (deficiency of antithrombin, proteins C and S, factor V Leiden, prothrombin [PT] G20210A) was carried out in all individuals. The presence of the FV HR2 was checked by a PCR assay for the A4070G polymorphism in the FV gene. *Results*. Inherited thrombophilia was found in 141 patients (35.8%) (deficiency of natural anticoagulants n=22, heterozygous FV Leiden n= 65, homozygous FV Leiden n=6, PT G20210A n=33, multiple abnormalities n=15) and 17 controls (8.3%) (FV Leiden n=8, PT G20210A n=9). The FV A4070G was found in 59 patients (1 homozygous) (16.7%) and in 18 controls (2 homozygous) (8.8%). The odds ratio (OR) for VTE associated with FV A4070G was 1.9 (95% CI 1.1-3.4) and 2.0 (95% 1.1-3.6) after adjustment for inherited thrombophilia. The prevalence of FV A4070G was similar among the overall patients with different circumstances of the first VTE ($p=0.3$), and either in the sub-

groups of patients with thrombophilia (p=0.07), with heterozygous FV Leiden (p=0.11), or no other inherited traits (p=0.47). *Conclusions*. The HR2 haplotype in the FV gene (FV A4070G) is a mild risk factor for VTE; however, the prevalence is uniform among the patients, independently either of the circumstances of the first thrombotic event or of the presence of other inherited thrombophilic traits.

P113

THE JAK2 V617F MUTATION IS NOT FREQUENT AMONG PATIENTS WITH UNPROVOKED DEEP VENOUS THROMBOSIS OF THE LEGS AND / OR PULMONARY EMBOLISM

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Background. Thrombosis is a common cause of morbidity in patients with polycythemia vera (PV) or essential thrombocythemia (ET); major venous vessels are involved up to one third of the events, especially in PV. The JAK2 V617F somatic mutation is a molecular hallmark of chronic myeloproliferative disorders (CMD) and can be recognized in about one third of the patients with unexplained splanchnic venous thrombosis not meeting all the criteria for diagnosis of PV or ET. *Aims.* The present study is aimed to investigate the prevalence of the JAK2 V617F mutation among patients with unprovoked venous thromboembolism (VTE) at usual sites and without overt CMD. *Patients and Methods*. We investigated 194 patients (M/F 128/66) with unprovoked VTE and without overt CMD. The first clinical presentation was deep venous thrombosis (DVT) of the legs in 175 cases (in 66 of them with pulmonary embolism, PE) and isolated PE in 19 cases. The median age was 51 years (range 18-89) at the thrombotic event and 56 years (range 18-89) at the time of investigation. Laboratory investigation for inherited thrombophilia was carried out in all patients. A group of 322 healthy controls was also investigated for the presence of the JAK2 V617F mutation (M/F 208/114, median age 38, range 18-60). The JAK2 V617F mutation was checked by a PCR assay according to Baxter *et al.* (Lancet 2005; 365:1054). *Results.* Inherited thrombophilia was found in 75 patients (38.6%) (deficiency of natural anticoagulants n= 14, factor V Leiden n=39, protrombin G20210A n=18, multiple abnormalities n=4). The JAK2 V617F mutation was found in none of the control individuals and in 2 patients (1.0%, 95% CI 0.2-3.6). The first case was a 71 y.o. woman who had had DVT + PE two years before. The second case was a 76 y.o. woman heterozygous for prothrombin G20210A who had had DVT + PE ten years before. Accordingly, the prevalence of the JAK2 V617F mutation among the patients without inherited thrombophilia was 1 of 119 (0.8%, 95% CI 0.1-4.6). *Conclusions.* The JAK2 V617F mutation is infrequently associated with unprovoked VTE at usual sites in the absence of overt CMD. The reason of the close association between the presence of the mutation and the location of venous thrombosis in the splanchnic vessels deserves further investigation.

P114

INCREASED ENDOGENOUS THROMBIN POTENTIAL IN CARRIERS OF INHERITED CLOTTING INHIBITORS DEFECTS

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Introduction. There are limited informations on ETP in subjects with inherited clotting inhibitors defects. Aim of our study was to evaluate ETP in carriers of Antithrombin (AT), protein C (PC) and protein S (PS) defects and compare it with that found in their relatives without defects *Methods.* We collected 283 subjects belonging to 51 families of consecutive probands referred to our department with documented VTE and inherited defects of PC, PS or AT. Among these subjects 80 belonged to 17 families with AT defect, 103 to 17 families with PC defect and 100 to 17 families with PS defect. They were assigned to 4 groups as follow: 1) presence of defects and previous VTE, 2) defects without previous VTE, 3) no defects but previous VTE, 4) no defects and no previous VTE. The ETP was determined in plasma by the commercially available method ETP (Dade Behring, Marburg, Germany) that evaluates the total amount of thrombin generated in the sample using a chromogenic method. ETP represents the area under the kinetic curve (AUC). *Results.* Among subjects belonging to families with AT defect, ETP values (mean ± SD) were significantly higher in group 1 (464.9±106.25 mA) than group 3 (376±37.13 mA) and 4 (389.8±54.64 mA) (p .013 and .023, respectively). The ETP value in group 2 (480.4±86.41 mA) were significantly higher than in group 3 (376±37.13 mA)(p .0002). No significant differences were observed either between groups 1 and 2 or between groups 3 and 4. As for families with PC or PS defect no significant differences between the four groups were detected. *Conclusions.* Family members with AT defects presented with significantly higher ETP as compared to non carriers. No difference was present in previously symptomatic vs asymptomatic carriers or non-carriers of AT defects. Thus, the defect in itself is responsible for increased ETP. On the contrary the presence of PC or PS defect did not determine an increase in ETP. The new ETP test may be a promising tool for monitoring thrombin generation in patients with AT defects during risk situations and prophylaxis for VTE. possible modifications of the test with the introduction of thrombomodulin or activated PC might make the test more sensitive also to the presence of PC and/or PS defects.

P115

ANTITHROMBIN TYPE I DEFICIENCY IN A YOUNG NIGERIAN MAN WITH RECURRENT VENOUS THROMBOSIS: MOLECULAR CHARACTERIZATION OF A NEW FRAMESHIFT MUTATION LEADING TO A STOP CODON

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The role of inherited thrombophilia as a risk factor for venous thromboembolism (VTE) in the black African population is still unknown. In addition, no valid estimates of the prevalence of inherited clotting inhibitor deficiencies in Africans suffering from VTE are available. We identified a new and never described gene lesion responsible for type I AT deficiency in a 34-year-old Nigerian man who presented with recurrent VTE. Antithombin activity and antigen were 57,6% and 51% respectively in patient's plasma, consistent with a diagnosis of heterozygous type I deficiency (defect of synthesis). Genomic sequencing of the AT gene demonstrated a single base (cytosine) deletion at nucleotide 2762, in exon 2 of the Antithrombin gene, resulting in a frameshift which leads to the creation of a premature stop codon at position 102. Restriction fragment length polymorphism (RFLP) using a DdeI-based restriction protocol confirmed the presence of the single base deletion. Immunoblotting analysis excluded the presence of abnormal Antithrombin molecules. Antithrombin deficiency is a genetic defect strongly associated with venous thromboembolism (VTE), and the prevalence of heterozygotes in the general population is around 1 in 5.000. Considering the extremely low prevalence of genetic mutations leading to Antithrombin deficiency in the general population, and the fact that this particular deletion was never previously described, we hypothesize that the ATIII 2762delC mutation could reasonably be native of Africa.

P116

PLASMINOGEN ACTIVATOR INHIBITOR (PAI-1) ACTIVITY AND RECURRENT PREGNANCY LOSS

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Introduction. A recurrent pregnancy loss (RPL) was defined as two or more spontaneous losses of the fetus before the 20th week of gestation. RPL has a well-established association with congenital and acquired thrombophilia. Plasminogen activator inhibitor-1 (PAI-1) is the major plasmatic physiologic inhibitor of tissue-type plasminogen activator: PAI-1 low fibrinolitic activity is related to increased plasma levels, that are associated to high risk of ischemic events. The aim of this study was to investigate the relation between the PAI-1 and RPL. Materials and *Methods.* A total of 60 Caucasian women were studied: 26 patients (range 32-54; mean age: 38.9) and 34 healthy controls (range 22-68; mean age: 43.7). Exclusion criteria were: presence of genetic prothrombotic polymorphisms (Factor V Leiden, Prothrombin G20210A), antiphospholipid syndrome, hyperhomocysteinemia, deficiency of one or more physiological coagulation inhibitors (Antithrombin, Protein C or S) and treatment with any therapies; we also excluded women with other risk factors for pregnancy complications (uterine malformations, abnormal kariotypes, etc.). PAI-1 levels in plasma were determined by a chromogenic method. The Coaliza PAI-1 Kit (Chromogenix, Instrumentation Laboratory SPA, Milan, Italy) was used. The significance of the different prevalence of PAI-1 levels observed between the groups was tested by the chi-square analysis; statistical significance was considered a P value less than 0.05. *Results.* PAI-1 levels were highest in patient group (12/26,

41.1 %) than in controls (6/34, 17.6%). The difference in prevalence was statistically significant (p=0.017). *Discussion*. Our study suggests that increased PAI-1 levels could be a possible reason of RPL. PAI-1 level could be investigated in women with RPL in absence of other prothrombotic risk factors and without apparent mechanical, anatomical, endocrinological or immunological causes. The investigation of prothrombotic risk factors is today recommended in women with unexplained RPL, but further larger studies are needed to confirm the utility of the PAI-1 screening in women with recurrent abortions and other pregnancy complications.

P117

OBSERVATIONAL STUDY OF 150 WOMEN WITH HEREDITARY THROMBOPHILIA AND HISTORY OF RECURRENT MISCARRIAGE

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Introduction. Recurrent pregnancy loss (RPL) represents a heterogeneous condition. Whilst acquired thrombophilia is a well-known cause of RPL, the contribution of inherited thrombophilia to RPL is still debated. This study was aimed at evaluating the prevalence of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C, prothrombin A20210G and Factor V Leiden polymorphisms in a cohort of women with RPL or failure to achieve pregnancy after assisted reproductive procedures (at least three), but without any history of acquired thrombophilic conditions. *Methods.* We prospectively recruited 150 women (age range 27-45; 63% older than 35 years) who were referred to our unit by two distinct Women's Health Care and Fertility Centers for counselling about their thrombophilic risk. 54 (36%) had a history of RPL, 11 of whom had been submitted to *in vitro* fertilization (IVF) procedures. The remaining 96 were scheduled for IVF because of unexplained primary (n=65) or secondary infertility (n=31). All genetic tests were performed by RT-PCR-based assay (LightCycler 2.0 Roche). *Results.* Heterozygous Factor V Leiden and prothrombin A20210G mutations were detected in 6% and 4% of women, respectively. Heterozygous and homozygous MTHFR A1298C gene mutations were found in 6% and 20% of women, respectively. MTHFR C677T homozygous mutation was detected in 24%, whereas the heterozygous mutation was present in 47% of women. Women with MTHFR gene mutations always had homocysteine levels below the currently accepted cut-off value of 15 micromol/L. No differences were observed in the distribution of MTHFR genotypes between patients with RPL or with a history of infertility (TT 26% *vs.* 22%, CT 46% *vs.*, 47%, and CC 28% *vs.*, 31%, respectively). Nonetheless, the prevalence of TT genotype was significantly higher in comparison with the frequency found in the general population in Europe, which is about 12%. *Conclusions.* These results are suggestive of a possible association between MTHFR C677T polymorphism and RPL or primary infertility.

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THROMBOPHILIA AND PREGNANCY: OUR EXPERIENCE

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During pregnancy the increase in procoagulants, the decrease in anticoagulants, the acquired activated protein C resistance and increases in fibrinolytic activity may result in an acquired hypercoagulable state. Further, association between inherited thrombophilias such as factor V Leiden mutation and prothrombin gene G20210A mutation, and adverse pregnancy outcome has been cleared up by several studies.[1] However not all thrombophilias carry the same risk during pregnancy. Only low grade recommendations are available on treatment of thrombophilic pregnant women and these are based on observational studies including small series of patients mostly treated with low molecular weight heparin.[2] Here we describe our experience in managing this kind of patients. Thirty seven healthy women with spontaneous pregnancies were investigated for combinations of the commonest thrombophilic alterations (Factor II G20210, Factor V leiden, Protein S/C and Antithrombin deficiency). Two patients had a story of recurrent fetal loss, thirteen patients showed a family history of DVT (Deep Vein Thrombosis), one patient had DVT, one patient had thromboembolism, two patients had a story of gestational hypertension, one a story of gestational diabetes and one had pulmonary embolism after the delivery. Two patients were found to be heterozygous for factor V Leiden mutation, four patients were heterozygous for G20210A prothrombin gene mutation, five patients showed protein S deficiency, four patients showed combined protein S (PS) deficiency and heterozygous factor V Leiden mutation while only one showed antithrombin (AT) deficiency. Seven patients showing at least two major risk factors (Story of DVT/thromboembolism, AT deficiency, factor V Leiden mutation, G20210A prothrombin gene mutation, PS deficiency) were treated with low molecular weight heparin while twenty nine ones monthly underwent laboratory coagulation tests only. All pregnancies resulted in the delivery of a live newborn. Treatment of thrombophilia is an emerging problem in hematology, more solid studies are needed to establish international guidelines to help clinicians in the wide sea of screening and monitoring tests, patients to treat and treatment options.

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P119

SUDDEN SENSORINEURAL HEARING LOSS AND THROMBOPHILIA

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Sudden neurosensorial hearing loss (SSHL), or hypoacusis, is a highly invalidating symptom, with or without associated tinnitus, characterized by acute onset, variable duration, and an incidence of about 1-2/10,000 individuals/year. The most likely pathogenic mechanism appears to be related to abnormalities of the microcirculation in the labyrinth and/or in the cerebral cortex. Some studies have found a positive correlation between SSHL, the C677T polymorphism in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene and the Leiden mutation (G1691A) in the factor V gene. *Aim of the study*. Aim of our study was to verify whether acute onset hypoacusis, with without tinnitus, could be associated with a laboratory pattern characteristic of thrombophilia. *Materials and Methods.* Thirty-five patients (15 males and 21 females; age10-76, average 47), affected by acute hypoacusis with or without tinnitus, were screened for cardiovascular risk factors, comorbility, pharmacological treatment, and evaluated with MRI of the brain and petrous portion of the temporal bones (rocca petrosa), transthoracic (TT) echocardiography, ecocolordoppler of the supraaortic trunks (TSA), and laboratory screening for thrombophilia that included measuring PT, aPTT, fibrinogen, factor VII, factor VIII, antithrombin III, protein C, protein S, activated protein C resistance (RAPC), homocysteine levels before and after methionine load, vitamine B12, folic acid, LAC, anticardiolipin IgG e IgM, FV Leiden mutation, G20210A prothrombin mutation, and C677T MTHFR polymorphism. The patient group was compared with a control group including 61 individuals without previous vascular problems. *Results.* The occurrence of SSHL was correlated with increased homocysteine levels post-methionine load (χ^2=5.18, p=0.023; for the increment: χ^2=4.97; p=0.026), homozygous C677T MTHFR polymorphism (9.68% *vs.* 6.45% in controls; χ^2=7.87; p=0.019) and RAPC (χ^2=3.01; p=0.082). Moreover, 60% of the patients had high factor VIII levels (χ^2=13.2; p=0.001). All other parameters were not significantly different in patients and controls. *Conclusions.* Our results do not confirm the correlation between SSHL and a laboratory pattern of genetic thrombophilia. Nonetheless, the difference in homocysteine levels, along with the increased frequency of RAPC and elevated factor VIII levels in the absence of a concomitant fibrinogen and factor VII increase, suggest the existence of a hypercoagulable state not correlated to an inflammatory condition. Thus, anticoagulant therapy could be helpful in these patients.

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SUCCESSFUL PROTEIN C CONCENTRATE ADMINISTRATION DURING INITIATION OF ORAL ANTICOAGULATION IN ADULTS PATIENTS WITH SEVERE CONGENITAL PROTEIN C DEFICIENCY: REPORT OF TWO CASES

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Protein C (PC) is a vitamin K-dependent proenzyme with anticoagulant activity and congenital PC deficiency is a well known condition at high risk for thrombotic episodes. In patients with PC deficiency starting treatment with oral anticoagulant drugs is associated with a transient hypercoagulable state and clinically overt thromboembolic complications before reaching a full anticoagulant effect. We describe the successful supplementation with PC concentrate in two adult patients with moderately severe PC deficiency during the initiation of oral anticoagulation and a course of therapeutic dose of low-molecular-weight heparin (LMWH) for acute venous thromboembolism (VTE). Plasma PC through levels above 50% were observed in both patients and mainteined during the entire supplementation treatment period with PC concentrate, until a stable therapeutic anticoagulation level has been reached. These results have been obtained within a short time, thus allowing a safe administration of a loading dose of warfarin. No adverse reactions to the PC concentrate were seen; in details no skin necrosis and other thromboembolic complications, bleedings or allergic reactions were observed. In conclusion, PC concentrate seems to be effective for the prevention of thromboembolic complications and safe in patients with congenital PC deficiency while initiating oral anticoagulants.

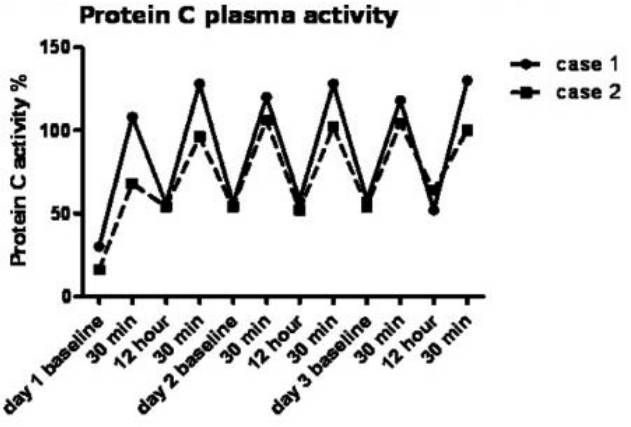

Figure 1. Plasma levels of protein C activity during administration of PC concentrate.

Antithrombotic Therapy: Clinical and Laboratory Issues

P121

PLATELET FUNCTION DRIVEN ANTIPLATELET THERAPY IN PATIENTS WITH ACUTE CORONARY SYNDROMES UNDERGOING PCI: RATIONALE AND DESIGN FOR THE DUAL ANTIPLATELET TAILORED THERAPY BASED ON THE EXTENT OF PLATELET INHIBITION (DANTE TRIAL)

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Background. Dual antiplatelet therapy with aspirin and clopidogrel has become a cornerstone of the medical regimen for prevention of ischaemic events in patients with acute coronary syndromes (ACS) undergoing PCI. A large variability in the platelet inhibition obtained with clopidogrel had been found and Residual Platelet Reactivity (RPR) on clopidogrel therapy (loading dose 600 mg followed by 75 mg daily) has been demonstrated to be an independent predictor of stent thrombosis and cardiac death in patients with ACS. *Study Design*. DANTE is a randomized, parallel-groups, prospective clinical trial. Approximately 450 ACS patients with RPR by ADP (measured by a point of care assay) will be randomized to clopidogrel 150 mg daily or clopidogrel 75 mg daily for the duration of the dual antiplatelet therapy according to the current guidelines. Inclusion criteria will be: NSTEMI and STEMI patients undergoing PCI treated with a clopidogrel loading dose of 600 mg. Exclusion criteria will be: bleeding diathesis; history of TIA/stroke; platelet count of less than 100000/mm^3; PT-INR >1.5; hemoglobin <10 g/dL at the time of the screening; body weight less than 60 Kg; creatinine levels ≥4 mg/dL, recent (within 3 weeks) major trauma/surgery, OAT, pregnancy, severe hepatic disease, active peptic ulcer.The primary end-point will be the incidence of major aderse cardiac events (MACE) at 6 and 12 months of follow up. Major safety end points will include TIMI major and minor bleeding. *Conclusions.* This trial of ACS patients with RPR by ADP on clopidogrel therapy will allow determination of the value of a strategy of a platelet-function driven therapy with clopidogrel.

P122

THROMBOELASTOMETRY AS POINT OF CARE IN HEART SURGERY: APPLICATIONS AND CLINICAL VALUE

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Background. The thromboelastometry (TEM) is a rapid registration of *ex vivo* global hemostasis. The apparatus was widespread in all laboratories of blood coagulation unit until the 1970. Because the introduction of partial thromboplastin time (PTT) together with the old prothrombin time (PT), the automated platelet count and the diluited von Clauss thrombin time for fibrinogen, the TEM was practically abandoned. Recently the TEM renewed, because of new tecnology of the apparatus, the speed and accuracy of the data showed, as point of care. Moreover the TEM has the ability of monitoring changes of blood coagulation waves induced by new anticoagulants which do not prolong the PTT or PT as LMWH or Fondaparinux or oral direct thrombin or recombinant factor Xa inhibitors, or on the opposite new pan-hemostatic agent, as Factor VII. Moreover the TEM has been considered to be useful as point of care in emergency department or critical care area in heart surgery and transplantation unit as valid tool in rapid diagnosis and possible treatement. *Aim.* The aim of the study was to evaluate the utility of TEM as regards to routine hemostasis test as ACT, PTT, PT, TT, FBG, PLT count in heart surgery. *Material and Methods.* The observation was conducted along one year (from January 2006 to January 2007). We perform TEM in 280 out of 700 s., submitted to heart surgery, because of aortocoronary by-pass, mechanical or biological valve prostheses replacement the patient were selected by surgeon because of previous altered hemostatic screening to test or PLT abnomalities or risk of bleeding or hypercoagulability or thinking of HIT the TEM was performed before, during and after the surgery and if necessary, the FOLLOW -up

was every day for 2-3 days.*Results.* The TEM was superior to ACT in predict the excess of circulating heparin and its better correction monitored by protamine sulphate in near 15% of the cases. The TEM was also able to show the acquired deficiency of plasmatic coagulation factors and its correction with 10-15 mL of FFP, the fall of PLTS and the thinking of the possible HIT from the 3th -5th day after LMWH treatement. In fact 10 patients were diagnosed as HIT, two of them were treated with lepirudin with success in one and the amputation in the other. Moreover the TEM turned out to be crucial to decide whether to perform salvage surgery. In conclusion, the TEM has been resulted an useful point of care for a quick and accurate diagnosis and adeguate treatement, sparing time and cost.

P123

RESIDUAL PLATELET REACTIVITY ON DUAL ANTIPLATELET THERAPY IN THE ACUTE AND SUBACUTE PHASE OF ACUTE CORONARY SYNDROME

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Dual antiplatelet therapy with aspirin and clopidogrel has become a cornerstone of the medical regimen for prevention of ischaemic events in patients with acute coronary syndromes (ACS) undergoing PCI. Our group and others have demonstrated that a single measurement of platelet function in the acute phase of ACS is an independent predictor of total recurrent ischemic events and in particular of stent thrombosis. On the other hand, it has been found that RPR is partially related to the inflammatory state present in the acute phase of the disease. Aim of this study i sto investigate platelet reacitvity according to the different timing from an acute ischemic event. In 191 ACS [147 M/ 44 F; age: 66 (28-91) yrs] patients undergoing PCI, we measured platelet function by LTA with different agonists (2 and 10 μM ADP, 1 mM arachidonic acid and 2 microg/ml collagen) within 24 hrs from clopidogrel loading and PCI (T1) and after 72 hrs from T1 (T2). RPR has been defined as maximal platelet aggregation by 1 mM AA≥20%, 2 and 10 microM ADP ≥70% and 2 μg/mL collagen ≥56%. No significant differences have been detected in the mean values of maximal platelet ggregation induced by all agonists at T1 and T2 (Table 1). According to the definition of RPR, 161/191 (84.2%) had concordant results by 10 μM ADP at T1 and T2, 141/191 (73.8%) by 1 mM AA, 155/183 (81.1%) by 2 μg/mL collagen. 18/34 (53%) patients with RPR by 10 μM ADP at T1, 26/64 (41%) RPR patients by AA at T1 and 19/35 (54.5%) RPR patients by collagen at T1, had no RPR at T2. At T2 12 patients (with no RPR at T1) (7.6%) showed RPR by 10 μM ADP, 24 (11.2%) RPR by AA and 24 (10.1%) RPR by collagen. Our results show that in about 80% of patients, platelet reactivity is similar in the acute and subacute phases of ACS. Nevertheless, in a portion of patients platelet hyperreactivity documented in the acute phase recedes in the subacute phase, suggesting the role of the acute inflammatory status in determining RPR.

Table 1.

	T1	*T2*	*p*
2 μM ADP	33.9±20,2	35.7±18,0	ns
10 μM ADP	51.8±21.3	53.5±17.1	ns
2 μg/mL Collagen	33.6±23.3	33.3±20.3	ns
1 mM Arachidonic Acid	21.7±20.9	20.2±14.9	ns

P124

QUALITY ASSURANCE SCHEME BASED ON LEVEY JENNINGS ANALYSIS AND WESTGARD RULES APPLICATION FOR POC-INR MONITORING

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Background. INR testing performed with Point Of Care (POC) coagulation monitors is a reliable and efficient option for Oral Anticoagulant Treatment (OAT) monitoring. Quality Control Programs for these monitors are widely performed, mostly evaluating the correlation between capillary and venous PT-INR. Manufacturers provide two levels of Quality Control material, for normal and pathologic INR values. For every batch of control material, acceptability ranges are declared by the manufacturer. *Aim of the study.* We retrospectively performed range recalculations on and applied Westgard rules analysis to real CQ data in order to: 1) validate usefulness of control material, 2) validate manufacturer's ranges, 3) suggest optimal data analysis scheme. *Materials and Methods*. Coagulometers (ProTime) and control material (Direct Check Low - DCL and High - DCH) were provided by Instrumentation Laboratory, Milan. The 17 ProTime monitors used in 12 outpatients services of the Umbria OAT-management-network underwent a quality control program, assessing correlation with venous PT-INR (R squared >0.85), four times a year. Contextually INR of DCL and DCH was measured on every monitor. Results were collected over one year period and analysed on Shewart control charts with Levey-Jennings analysis and Westgard Rules application. The following rules were applied according to Westgard multirule CQ procedure: 1:2s→4:1s→10:x or 1:2s→2:2s→R:4s→4:1s→10:x. Single batches of respectively DCL and DCH were used during the whole year. Recalculated ranges, based on actual results of the first 20 values, were adopted to perform the analysis upon subsequently obtained results. *Results.* The recalculated ranges were found to be narrow than the declared ones: 1.1-1.9 (declared) vs 1.23-1.88 (recalculated) and 2,1-3,7(declared) vs 2,44-3,38 (recalculated), for DCL and DCH, respectively. Graphical display of Levey Jennings analysis is given for DCH results in the Figure 1. No Westgard Rule violation was detected for DCL and DCH and both for declared and actual ranges. Only a warning result was found by the application of 1:2s Westgard rule on recalculated ranges and it was not followed by other violations. *Conclusions*. These results confirm beneficial application of POC-INR monitoring and demonstrate that Quality Assurance with control material provided by manufacturer can be considered feasible and reliable, provided that the same batch is used as long as possible.

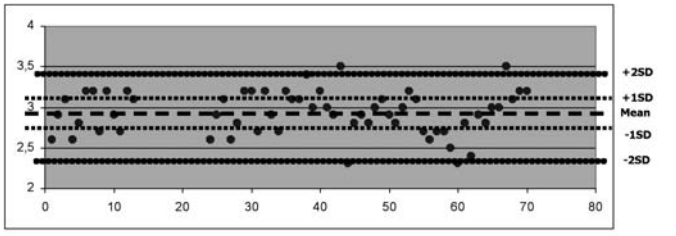

Figure 1.

P125

A NEW LABORATORY APPROACH FOR PATIENTS WITH VENOUS THROMBOEMBOLIC DISEASE

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Introduction. In a relatively high percentage of patients who develop venous thromboembolism (VTE), the mechanism of these events remains unknown. Protein C (PC)/Protein S (PS) pathway plays a crucial role as an anticoagulant physiological mechanism and several acquired and/or congenital alterations are known. *Aim.* To evaluate the performance of a new global assay assessing PC/PS pathway in patients with VTE and to assess the capability of this method to recognize a prothrombotic state stemming from conditions different from those already known. *Method.* HemosIL Thrombopath test (ThP, Instrumentation Laboratory) investigates possible dysfunctions of the PC/PS anticoagulant pathway by measuring the % inhibition of endogenous thrombin generation induced by Protac (PiCi%). Results of this assay are expressed as PiCi (%) = [(OD ThP B - OD ThP A)/OD ThP B)] x 100, where ThP A is the sample aliquot incubated with Protac and ThP B is the aliquot incubated without Protac. Thrombophilic tests were done according to routine laboratory methods. *Patients.* 150 healthy donors, 57 patients with idiopathic VTE and with thrombophilic risk factors [PC/PS deficiency (n=20), FV Leiden (n=34), Prothrombin G20210A polymorphism (n=3)] (group A) and 50 patients with idiopathic VTE and without known thrombophilic risk factors (group B) were evaluated. *Results.* No patient had AT deficiency or positivity for antiphospholipid antibodies The mean value of PiCi%, obtained in healthy blood donors, was 87.03±

4.84, with a cut-off of 77.35%. The PiCi% values found in group A patients were significantly different from healthy subjects ($p<0.0001$) with a sensitivity of 82% and a specificity of 95%. In group B patients the PiCi% values differed significantly ($p<0.0001$) from the normal subjects, but not from group A patients, with a sensitivity of 72% and a specificity of 94%. *Discussion.* Results obtained up to now allow us to suggest that Thrombopath can enter as a part of the algorithm for the prothrombotic screening, However, further applications in larger series of patients are necessary for a more in-depth knowledge of its interaction with the major thrombophilic risk factors and for its potential role in identifying also patients with other yet unknown risk factors.

P126

CLOSE MONITORING OF HEMOSTASIS BY THOMBOELASTOMETRY DURING EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO)

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The hemostatic system represents a main problem in ECMO as the foreign surface of the extracorporeal circuit activates platelets and the clotting system. Bleeding and/or thrombosis are frequent complications in ECMO patients that necessitate specific treatment. Purpose of this study was to investigate platelet function and clotting system, assessed by modified thrombelastography (TEG) and by aPTT point-of-care (POC) device, in adult patients undergoing ECMO. Five male patients (median age: 60 yrs, 23-72 yrs) were rescued by ECMO emergently. Four patients suffered from severe cardiac shock (3 due to acute coronary syndrome and 1 due to congenital heart disease); the fifth suffered from severe respiratory distress. The ECMO mean duration was 9.2±6.0 days. In all patients the whole blood levels of lactate was <2 mmol/L. Anticoagulation was accomplished with unfractionated heparin and was titrated to an aPTT of 50-60 s. Modified TEG was performed by a 4-channel analyzer (RoTEM, DASIT, Milan, Italy). For each session 4 tests were performed: native blood (recalcification alone, NaTEM assay), extrinsic and intrinsic activity (ExTEM and InTEM assays) and the assessment of heparin therapy (ExTEM in the presence of heparinase, EpTEM). The onset of coagulation (coagulation time, CT), kinetics of clot formation (CF), maximum clot firmness (MCF), and α-angle were measured. APTT was measured in ward by using POC coagulometer (Hemochron, ITC- Cremascoli & Iris, Milan, Italy). The comparison between first (1 to 3 hours after the beginning of ECMO) and last (3 hours after the end of ECMO) measurements of 4 RoTEM assays showed a restoring of normal hemostatic profile in all parameters examined in the 3 survivors at discharge of ECMO. On the contrary, in the 2 patients who died during ECMO overall alterations of hemostasis observed at the beginning of ECMO persisted. These data indicate that a close monitoring of hemostasis by the combination of aPTT and modified TEG in adult patients on ECMO could be useful to early identify hemostatic complications.

P127

IMMUNOASSAYS FOR HEPARIN-INDUCED THROMBOCYTOPENIA (TYPE II): HOW RELIABLE?

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A diagnosis of heparin-induced thrombocytopenia (type II, HIT) is made when HIT antibody formation is accompanied by an otherwise unexplained platelet count fall (usually ≥50% fall, even if the platelet count nadir remains >150×10^9/L). Only a subset of high-titer, IgG anti-platelet factor 4/heparin antibodies activates platelets. A number of assays for the detection of HIT-antibodies (HIT-Abs) are available but their specificity has been questioned. Over a period of one year, we tested for HIT-Abs 22 patients with suspected HIT using 3 immunoassays (Asserachrom HPIA, Stago; Zymutest HIA IgG, Hyphen BioMed; ID-PaGIA heparin/PF4 antibody test, DiaMed) and one functional assay (HIPA, Thromb Haemost 1991; 66:734). Results obtained with the two ELISAs were graded as negative (below cut-off OD, -), positive (above cut-off OD, but below the positive control OD, +) and strongly positive (above the positive control OD, ++). Negative,+, and ++ ID-PaGIA test results were adjudicated by a single operator on undiluted serum samples. With the HIPA test a sample was considered + for HIT-Abs according to Eichler et al (Thromb Haemost 1999; 81:625), or otherwise -. All but one patient with suspected HIT had undergone recent surgery (heart surgery in 14 patients). They all presented with fast-onset thrombocytopenia on UFH/LMWH treatment to a nadir of 55×10^9/L (30±13% of presumed baseline levels), associated with thrombosis (HITT) in 2 patients. Presence (+ or ++) or absence (-) of HIT-Abs was observed with all the immunoassays in 9 and 6 patients respectively. In the remaining 7 patients, results were all negative with Zymutest IgG, but they were -,+ and ++ in 1,2 and 4 patients with HPIA and in 0, 6 and 1 patient with ID-PaGIA. Positive results were thus observed in 16/22 patients with ID-PaGIA (++ in 5), in 16/22 patients with HPIA (++ in 8), and in 9/16 patients with Zymutest (++ in 8). With the HIPA test, 5 samples were positive, 4 from patients who were positive with all the immunoassays (one with HITT), and one from a HITT patient negative with Zymutest, + with ID-PaGIA, and ++ with HPIA. Heparin administration was interrupted in 9 patients – shifted to Fondaparinux - , 3 positive with 2 immunoassays and 6 positive with all. The increase in platelet counts from nadir values was faster in patients shifted to Fondaparinux (>100% of presumed baseline values by day 4 *vs.* 50% by day 5, p=0.004), irrespective of HIPA results.

P128

ON-LINE LEARNING IN MEDICINE: ANTICOAGULATION MANAGEMENT CERTIFICATE PROGRAM FOR GENERAL PRACTITIONERS

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Effective oral anticoagulant therapy requires accurate patient care management by OAT. Since the use of anticoagulant therapy has increased in the last few years, the need for a decentralized delivery system coupled with advanced educational programs in patient care management has emerged. The purpose of this continuing educational program organized by the Parma ASL is to provide an opportunity for General Practitioners to increase their knowledge in managing patients receiving OAT. The program addresses the following professional competences (learning objectives): - to describe the pathophysiology, risk factors, and presenting signs and symptoms of thrombotic disorders and haemorrhagic events; - to design, recommend, monitor, and evaluate patient specific anticoagulant regimens that incorporate the principles of evidence-based medicine; - to identify appropriate goals and outcomes of pharmacologic treatments of selected thrombotic conditions, common drug adverse effects, drug interactions, and impact of diet on drug anticoagulant effect; - to use effective oral and written communication skills to communicate with patients, caregivers, and other health care professionals regarding safe and optimal anticoagulant therapy. At the conclusion of the program the participant will be able to: 1) To know the pathophysiology of the more common thrombotic disorders. 2) To discuss the clinical assessment and laboratory monitoring of the patient receiving anticoagulant therapy. 3) To discuss the indications, dosing, adverse effects, and monitoring of anticoagulant drugs. 4) To use a dedicated Computerized Decision Support System (P:A.R.M.A. Program vers 5.7) to prescribe anticoagulant therapy. 5) To prescribe OAT in a appropriate way to patients. Participants completing this certificate program will have a clear understanding of the professional competency areas listed above. To assess the outcomes of the educational process, the following assessments are performed. Participants must complete 20 hours of continuing education using distance-learning technology (to have access to the next session GP must resolve 75% or grater of a five-questions exam), prior to participating in the experimental component of the program. This consists of identifying and resolving ten interactive clinical problems available on-line. Moreover, a 20 questions questionnaire will test the participant's knowledge and competence. To receive continuing education credit, participants must work through all cases and resolve at least 75% of questions. Participants will also be required to attend a one-day interactive anticoagulation management workshop led by faculty preceptors.

P129

THE EFFECTIVENESS, COST-EFFECTIVENESS AND ORGANISATION IMPACT OF AN ALTERNATIVE MODEL FOR DELIVERING ORAL ANTICOAGULANT THERAPY (OAT): INTERNET NETWORK CONNECTION BETWEEN GENERAL PRACTICE AND ANTICOAGULATION CLINICS IN THE PARMA AREA

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The constant increase of patients receiving OAT and their pressure on the thrombosis centres had led to the development of alternative models for delivery OAT: Primary Care, General Practitioner, Patient self testing and self management. Computerized Decision Support System (CDSS) has demonstrated to be able to improve treatment quality. In a future scenario of oral anticoagulant management, CDSS may have a pivot role in the decentralisation process. One effective model, that provides increased efficiency while maintaining the standards of clinical care, with relatively few resource implications, is a decentralized model employing a telemedicine system, whereby patients have blood drawn in the General Practice (GP) office, but analysis and prescription is still performed by the Anticoagulation Clinic (AC). In September 2007 the PARMA ASL completed an internet based network model connecting GPs (n= 7) and ACs (n=3), and through this model about 3000 patients in OAT have been managed. At first, a central data base was constructed collecting clinical and laboratory data of all patients in OAT. Then, a web supported program (PARMA System-IL, Milano and ITACA-Noemalife, Bologna), connected with central data base, was implemented in ACs and in participating GPs' offices. This model imply full informatization of the necessary steps for a good OAT management. In summary: 1) patient has his/her blood drawn in GP's office; 2) blood specimen is electronically identified and appointment electronically and automatically scheduled to laboratory; 3) INR is performed in Central Laboratory and results sent, via web, to CDSS (PARMA program); 4) Physician gives appropriate dose adjustment by CDSS (PARMA prog) and sends, in real time, OAT report, electronically signed, to data base (ITACA); 5) A nurse, in GP's office, prints through ITACA prog, the electronically signed OAT report and gives it directly to patient. This model of decentralization provides several advantages: 1) Direct communication between AC and GP; 2) Reduction of clerical errors, in comparison with previous *manual* managing model; 3) Nurse, Laboratory Technician, administrative Personnel and Physician work time saving; 4) Improvement of patients' quality of life; 5) Reduction of patients' travelling, time spent, and expenses

P130

COMPARISON OF A COMPUTER-BASED DOSING PROGRAM WITH A MANUAL SYSTEM TO MONITOR ORAL ANTICOAGULANT THERAPY: A RETROSPECTIVE COHORT STUDY

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The increasing number of patients receiving oral anticoagulant therapy (OAT) and the necessity for a careful monitoring of PT/INR results and oral anticoagulant (OA) drugs promoted a development of computerized programs to manage OAT. On October 2006 the computer program Prometeo by Siemens was provided with the algorithm which suggests the doses of OA and the schedule of follow-up according to a difference of previous PT/INR values in patients on long-term OAT. Out of 2890 patients who are on OAT at Haematology, Dipartimento di Biotecnologie Cellulari ed Ematologia, Università La Sapienza Roma, 1968 patients (males=1021,females=947; median age: 69 years, range:23-92) were already enrolled two years before the introduction of the algorithm. During two different periods of nine months, we analysed the OAT-quality according to the time spent in range, warfarin-dose, number of controls and out-therapy time: the first period of observation was from March to November 2005, when OAT was assigned by three expert physicians (manual dose group), the second period was from March to November 2007, when computer-based program has been utilised (computer adjusted group). Paired samples t-test and variance ratio test (F-test) were used for the statistical analysis. Out of the 1968 patients, 310(15,75%) underwent OAT for deep vein thrombosis, 584(29,67%) for atrial fibrillation, 863(43,85%) after heart valve replacement and 201(10,21%) for cerebrovascular accident. An overall number of 24,141 follow-ups has been considered: 11,845 recorded on the 2005 period and 13,296 recorded on the 2007 period (p=0.80). The analysis, comparing matched data, showed that the rate (60,7%) of the time spent within the therapeutic range during 2007 period was significantly (p<0.001) greater than that (49.8%) observed in the 2005 period. The mean INR value was higher in the manual dose group than in the computer adjusted one(3,04 *vs.* 2,72), (p<0.001); the mean INR variation, difference between targeted value and observed value, was significantly lower in the computer adjusted group than in the manual dose one(0,04 *vs.* 0,35; p<0.001). A mean drug dosage suggested by the computer algorithm was lower than that assigned by physicians (23,31 *vs.* 25.25; p<0.05). The average test interval was greater in manual dose group than in the other one(19,5 *vs.* 17,3 days; p<0.001). The results of our study are a further proof of the validity and clinical utility of the computerized programme to improve OAT.

P131

ASSESSMENT OF QUALITY CONTROL AND ACCURACY OF PORTABLE COAGULOMETERS FOR MONITORING ORAL ANTICOAGULANT TREATMENT

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Oral anticoagulant treatment (OAT) is prescribed to an increasing number of patients. A close monitoring of OAT measuring international normalized ratio (INR) by prothrombin time (PT) is needed to accomplish the narrow therapeutic range. INR measurement by capillary noncitrated blood samples has been developed to obtain rapid results without the discomfort due to venipuncture, which is frequently a troublesome for patients who need repeated PT-INR determination. To ensure appropriate patient care, the clinical safety of PT measurements is based on achievement of accurate and reliable results. This report was aimed to evaluate the routine quality control (QC) testing and tracking, as a part of a comprehensive quality assurance program, for INR evaluation with Thrombotrack, performed in the Anticoagulation Clinic of the University of Florence in Careggi Hospital. In this Anticoagulation Clinic about 550 patients per week refer for the control of OAT and PT, expressed as INR, is determined by capillary blood test (Thrombotest, Sentinel, Milan, Italy) in more than 28,000 analysis/year by using 4 instruments A protocol of laboratory staff for assessment of PT was designed to evaluate the proper use of these devices in the real world. To practice instrument, specific and repeated training courses were held to the hospital nurse staff. Storage and reconstitution of Thrombotest reagent, QCs – coefficient of variation (CV) for precision test - for each instrument and for each new lot of reagent were performed by the laboratory staff according to manufacturer's instructions. PT CVs from normal, abnormal and anticoagulated control reagents were respectively: within-day, 3%, 5% and 4%; day-to-day, 5%, 6% and 4%. *In vivo* duplicate testing values, performed by the nurses, showed significant correlations: <4 INR, n=180, r=0.99, p<0.001; ≥4 INR (n=178) r=0.98, p<0.001. Significant relationships were found between INRs obtained by Thrombotrack and those by laboratory on plasma samples (RecombiPlasTin, HemosIL, Milan, Italy): <4 INR, n=230, r=0.97, p<0.001; ≥4 INR (n=178) r=0.94, p<0.001. The quality of anticoagulation, expressed as percentage of time spent at different INR levels was: 60%, (within therapeutic range); 23% (above) and 17% (below). The use of this point-of-care method for INR determination by nurses in a strict co-operation with the laboratory staff for the continuous QC evaluation, provides accurate results for the OAT monitoring.

P132

RELIABILITY OF ROUTINE COAGULATION TEST IN PATIENTS WITH LOW FIBRINOGEN LEVELS

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Background. Tests for routine haemostatic evaluations, such as PT and aPTT, are extremely sensitive to hypofibrinogenemia, hyperlipidemia and haemolysis. Hypofibrinogenemia may result from impaired hepatic synthesis (hepatic failure, L-asparaginase and valproic acid therapy), increased turnover of fibrinogen (disseminated intravascular coagula-

tion) and congenital abnormal fibrinogen molecules (afibrinogenemias and dysfibrinogenemias). *Aim of the study*. To assess the reliability of routine coagulation test (PT, aPTT) in hypofibrinogenic samples (<150 mg/dL) comparing two different methods: magneto-mechanical versus photo-optical. *Methods.* We tested PT, aPTT and fibrinogen on 106 consecutive fresh plasma samples from Haematology, Oncology and Gastroenterology departments with the photo-optical analyzer BCS-Dade Behring using Dade-Behring reagents: Thromborel S, Pathromtin SL and Multifibren respectively. Hypofibrinogenemia was detected in 44 samples: they were then re-tested for PT and aPTT on the magneto-mechanical KC1 Coagulation Analyser from Amelung-Trinity Biotech using the same reagents. We applied Bland and Altman statistical method to compare results. *Results.* The Bland-Altman plot analysis revealed very low agreement between the two methods and an overestimation (e.g. positive bias) of the coagulation times when measured with the photo-optical analyzer. Upper and lower concordance limits for PT ratio were 2.02 and 1.45 respectively: therefore, the photo-optical method gave results greater than 2.02 and lower than 1.45 compared to the magneto-mechanical. Upper and lower concordance limits for aPTT ratio were 1.46 and 1.32 respectively: thus, the photo-optical method provided results greater than 1.46 and lower than 1.32 when compared to the magneto-mechanical. *Conclusions.* When fibrinogen is below 150 mg/dL, results of routine coagulation tests become totally method-dependent, therefore they do not provision reliable assessment of patient's haemostatic status. Antiblastic therapy with L-Asparaginase might induce very low fibrinogen levels but it is also a risk factor for thromboembolic events: on the basis of this study, we urgently need more accurate and reliable results of routine coagulation tests for safely monitoring haemostasis in our patients.

P133

NEONATAL CAVAL VEIN THROMBOSIS IN A CARRIER OF PROTHROMBIN G20210A MUTATION AND ANTITHROMBIN DEFICIENCY

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We report a case of a spontaneous neonatal caval thrombosis due to antithrombin deficiency and heterozygosis for the G20210A prothrombin mutation (FII G20210A). The same risk factors were previously identified in the mother because of a strong familiary history of thromboembolic disease and miscarriages related to thrombophilia; she had other two pregnancies: in the first she delivered successfully a healthy baby, carrier of antithrombin deficiency; her second gestation was interrupted at 20th week for foetal malformations. The mother, in good general health during all her third pregnancy, received prophylactic administration of Nadroparin (Seleparina, Italfarmaco) 5700 IU s.c. daily, from 10 weeks gestation until the post-partum. A caesarian section was performed at 36th week: the woman gave birth to a healthy baby. Before the delivery the mother was also treated with antithrombin concentrate (AT) (Antitrombina III, Baxter) (2000 IU I.V. daily) from 2 days before delivery until the 3rd day after the child birth. Laboratory testing for thrombophilia at birth revealed in the infant deficiency of Antithrombin (25%), low level of Protein C and heterozygosis for the FII G20210A. On day 3 after birth he developed oedema of the legs. A doppler ultrasonography evidenced a thrombosis of the inferior vena cava (IVC): the infant received enoxaparin (Clexane, Aventis Pharma) (80 IU/Kg s.c daily), AT concentrate (60 IU/Kg); he was also treated with protein C concentrate (Ceprotin, Baxter), because of the lower plasma protein C level, for two days until the oedema disappeared. The AT infusion was stopped after two weeks; the therapeutic dosage of enoxaparin was continued for 4 months then reduced and stopped after a control ultrasonography that showed the complete IVC recanalisation. If it is well-established that the prophylaxis treatment is needed to prevent pregnancy or postpartum complications in women with thrombophilia, further larger studies are necessary to define the management of the antithrombotic approach to prevent vascular diseases in infants with of one or more prothrombotic risk factors, evaluating the risk-benefits ratio of these treatments. In the meantime our case report seems to confirm that early diagnosis and an accurate antithrombotic therapy (heparin and AT and Protein C concentrates infusion, when necessary) can rapidly lead to the recanalisation in case of recent neonatal venous thrombosis and may prevent the post-thrombotic syndrome.

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DOSE ADJUSTMENT OF LOW MOLECULAR WEIGHT HEPARINS (LMWH) IMPROVE PREGNANCY OUTCOME IN THROMBOPHILIC WOMEN.

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Background. Acquired and inherited thrombophilias are known to be associated with unfavourable pregnancy outcome, including recurrent fetal loss. Heparin can significantly reduce pregnancy complications but there is no consensus on the dose of LMWH for thromboprophylaxis in these women, based on conflicting results suggesting fixed dosage throughout the pregnancy or dosage adapted to the gestational age. We show the results of LMWH dose adjustment by monitoring heparin level with anti F-Xa activity. *Methods.* 35 consecutive patients with history of fetal loss or intrauterine growth restriction (IUGR) or deep vein thrombosis during pregnancy were treated using adapted doses of LMWH monitored with anti-Xa activity chromogenic test (Instrumentation Laboratory) related to the specific molecule. In all women the starting dose was a fixed administration of 4000 UI daily. Monitoring started after the first two weeks of treatment and all further dosages were determined solely on the basis of anti-Xa activity values, which were determined every 2-3 weeks. As soon as these values were considered suboptimal (prophylaxis range >0.4IU/mL and <0.8 IU/mL anti-Xa value) the dose of LMWH was adjusted. In our experience the dose of LMWH had to be adjusted at least once over the course of the pregnancy and the mean daily dose increased from 4.000 to 8.000 UI between the 6th and 40th week of gestation. After 20th and 28th week of pregnancy Doppler evaluation of uteroplacental blood flow was performed. All patients had a good outcome of pregnancy with normal fetal growth and no thromboembolic event. Adverse effects including bleedings, thrombocytopenia and local complications did not occur. *Conclusions.* LMWH thromboprophylaxis in pregnancy enables modulation of systemic haemostatic parameters via inhibition of factor Xa and increase in plasmatic total and free TFPI levels. However, it remains to be debated if the patients require adjusted doses of LMWH and if the measured anti-Xa activity reflects anticoagulation *in vivo*. Some authors reported a 10% incidence of obstetric complications in women treated with LMWH fixed doses. In our experience adjusted doses prevent these unfavourable events and improve fetal growth.

Pharmacological Therapy: Anticoagulants, Antiplatelets and Thrombolytics

P135

ACTIVATION OF BLOOD PLATELETS AND COAGULATION IN ATRIAL FIBRILLATION: EFFECT OF PARNAPARIN, A LOW MOLECULAR WEIGHT HEPARIN

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Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice. Platelet abnormalities, as well as inflammation,may increase thromboembolic risk in AF. To investigate the role of platelets in AF and the possible association with inflammation, 11 patients (66.8±2.1 yrs) with persistent AF were recruited and compared with 22 matched healthy controls (61±0.5 yrs) in sinus rhythm. Patients received parnaparin (a low molecular weight heparin; 170 aXa IU/kg b.w.) twice daily and tested at day 0, 14, 15 (before and after ECV, respectively) and 21 of treatment. Platelet P-selectin, mixed platelet-leukocyte conjugates and leukocyte activation markers (CD11b, MPO) were measured by flow-cytometry in whole blood in basal condition and after *in vitro* ADP/collagen challenge. D-dimer, soluble P-selectin, IL-6 and ICAM-1 were measured in plasma by ELISA. Statistical analyses were performed by Mann-Whitney test for intergroup differences or Repeated Measures ANOVA for variations over time. In AF patients before parnaparin, the expression of platelet P-selectin was significantly lower, while both soluble P-selectin and D-dimer were significantly higher than in controls. Platelet count and platelet-leukocyte conjugates were also lower; leukocyte activation markers and soluble cytokines were unchanged (Table 1). Parnaparin significantly reduced D-dimer, in concomitance with increased Xa activity; on the other hand, cell activation and inflammatory markers were unaffected. Circulating platelets were activated in AF, but appeared to be less reactive *in vitro*, presumably due to previous *in vivo* activation; both leukocyte activation markers and soluble molecules were unmodified. In conclusion, parnaparin is an effective anticoagulant therapy in AF, while it does not appear to affect either platelet or leukocyte activation. No increase in inflammatory markers was found to be associated with AF.

Table 1. Platelet and coagulation markers in AF patients and controls.

	AF patients		*Controls*	
	Basal	*ADP/Collagen*	*Basal*	*ADP/Collagen*
Platelet P-selectin (%)	2±0.8*	18.3±3.4	4.3±0.8	23.6±2.0
Soluble P-selectin (ng/mL)	97.8±24.1**	29.2±2.5		
D-dimer (ng/mL)	250.5±13.8*	220.0±34.2		
Platelet count (103/µL)	186.5±11.6**	247.0±9.7		
Platelet-PMN conjugates (%)	2.5±0.3	5.1±1.2*	10.6±3.3	25.1±5.0
Platelet-monocyte conjugates (%)	4.0±0.8	13.6±3.2	14.3±4.0	28.3±6.2

*Data are expressed as mean ± SEM; *p<0.05, **p<0.01 from control group by Mann-Whitney test.*

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SOCIAL COSTS OF ANTICOAGULANT TREATMENT FOR ATRIAL FIBRILLATION IN PATIENTS MANAGED IN ANTICOAGULATION CLINICS OR USUAL MEDICAL CARE SETTINGS

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Introduction. Atrial fibrillation (AF) is an independent risk factor for stroke and requires chronic anticoagulant treatment in medium/high-risk patients. Few studies have evaluated the social costs (non-healthcare and productivity losses) of oral anticoagulant treatment (OAT) in patients with AF. The aim of this study was to evaluate social costs associated to OAT in patients with AF managed in anticoagulation clinics (ACCs) or in usual medical care (UMCs) settings. *Methods.* This observational prospective multicenter study was performed in three ACCs and two UMC settings in Umbria. Consecutive AF patients were included in a 3-month study. Social costs included travelling to the site of monitoring, parking fee and costs related to the loss of productivity. Social costs were collected by interviewing patients and/or caregivers and were monetised according to prevalent prices and estimated unitary costs; loss of productivity was estimated according to the human capital approach. The costs were analyzed from the patient perspective. *Results.* Overall, 152 patients were included in the study: 100 managed in ACCs and 52 in UMCs settings. Mean patient age was 73 years (44-92). A caregiver was associated with the patient in 40% of the cases. The estimated average number of controls was 17.6/year. The mean time spent for the control of anticoagulation was 2.1 hours/each control. The overall social costs were estimated to be €16.4 per patient/each INR control and €274.5 per patient/year. Number of controls, time spent for monitoring and social costs (with their components) for patients managed in ACCs or in UMCs settings are shown in the Table 1. More INR control were required in patients managed in UMC settings. However, no difference in social costs were observed among ACCs and UMCs in OAT management as cost for individual control was higher in patients managed in ACCs. This extr-expensive were mainly related to travel costs. *Conclusions.* Social costs associated with monitoring on OAT for AF patients are not a negligible issue and should be considered when assessing the total cost of the care in these patients.

Table 1. Resources consumption, volumes and costs.

	ALL	*UMC*	*ACC*	*p*
Estimated INR tests per year, No (±SD)	17.6 (5.2)	19.9 (7.5)	16.4 (2.8)	**
OAM time per INR test – house to house (h)	2.3 (2.6)	3.4 (4.1)	1.7 (1.1)	**
Time in hospital per INR test (h)	1.5 (2.1)	2.4 (3.4)	1.0 (0.8)	**
Time extra hospital per INR test (h)	0.8 (1.1)	1.0 (1.79	0.7 (0.7)	
OAM 3 months cost, Euro (±SD)	76.0 (89.1)	68.7 (77.4)	79.7 (94.6)	
Travel	37.8 (50.9)	21.7 (23.2)	45.9 (58.7)	**
Out-of-pocket	2.0 (4.3)	1.6 (3.7)	2.1 (4.6)	
Informal care	32.1 (66.4)	40.9 (73.9)	27.7 (62.1)	
Productivity losses	4.2 (23.1)	4.5 (22.7)	4.0 (23.3)	
Average cost per INR test, Euro (±SD)	16.4 (21)	15.1 (19.8)	17.1 (21.6)	
Estimated OAM yearly cost, Euro (±SD)	274.5 (312.4)	265.4 (289.1)	279.1 (324.8)	

OAM, oral anticoagulation management; (**) p-value <0.01.

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FONDAPARINUX RELATED THROMBOCYOTPENIA IN A PREVIOUS LOW- MOLECULAR WEIGHT HEPARIN INDUCED HEPARIN INDUCED THROMBOCYTOPENIA

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Fondaparinux has been effective in both the prophylaxis and therapy of venous thromboembolism and shows a low cross-reactivity with heparin-induced thrombocytopenia (HIT antibodies *in vitro*. A anti PF4/heparin antibodies can be generated in Fondaparinux-treated patients who undergo orthopaedic surgery. Fondaparinux associated antibodies have been demontrated to exhibit a low ability to react against Fondaparinux/PF4 complexes. We describe the first case of a recurrent episode of HIT, triggered by the use of Fondaparinux thromboprophylaxis, that occurred in a patient with a preceding history of LMWH (Nadroparin) induced HIT. A 71 year old female developed a HIT in 2004 during prophylaxis with Nadroparin. In April 2007 she undergo a total hip replacement. According to the scheduled strategy 2,5 mg of subcoutaneous Fondaparinux was administered six hours after surgery. Warfarin was started on day six and Fondaparinux was withdrawn on day 8.The platelet count decreased, reaching the Nadir on postoperative day 11 (50,000/mm^3) with no evidence of DVT or pulmonary embolism. A strong positivity for anti PF4/heparin antibodies was found by ELISA test. The laboratory tests indicated that there was a fondaparinux-related immunogenic response associated with the thrombocytopenia, which we called fondaparinux-related HIT. We have excluded other cause of Thrombocytopenia and familial or acquired thrombophilic condition. The platelet count increased to a normal value on 22 day. Some caution should be taken before using Fondaparinux in patients with a previous history of LMWH-induced HIT.

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PROLONGED COAGULOPATHY RELATED TO ANTICOAGULANT SUPERWARFARIN RODENTICIDE OVERDOSE

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A 88-year-old man with history of hypertension and gastric low grade lymphoma presented to our emergency department with massive hematuria. At the admission, laboratory tests showed: Hb=7.3 g/dL, Hct=20.2%, WBC=11.21x10^3/mmc, PLT=124x10^3/mmc, creatinine=2.77 mg/dL. The prothrombin time (PT) and activated partial thromboplastin time (aPTT) were markedly prolonged, respectively PT INR >15, PTT Ratio=6.31, while thrombin time and D-dimer were in normal range (TT Ratio=1.0, D-dimer=0.22 μg/mL) and fibrinogen was elevated (=512 mg/dL).Results of liver function studies were normal. Mix tests (1:1 mix of patient plasma with normal plasma) fully corrected the PT and aPTT. The patient received blood transfusion, fresh frozen plasma and vitamin K to correct the coagulopathy and anemia. Despite multiple doses of vitamin K, after an incomplete correction, the PT resulted significantly elevated. The diagnosis of rodenticide ingestion was suspected on severe and prolonged coagulopathy and on the absence of likely alternatives, after exclusion of common causes of acquired coagulation disorders (liver disease, vitamin K deficiency, DIC, circulating inhibitors). On questioning, patient's daughter discovered tablets of rodenticide hidden in the patient's bedside table. Tablets and patient's blood samples were sent to the poisons centre to identify the substance ingested and the suspicion of superwarfarin poisoning was confirmed with highly toxic serum levels of brodifacum. The patient was hospitalized until day 20, requiring high doses of vitamin K e.v. (30 mg daily) because of ineffectiveness of vitamin K oral administration. After discharge, he required vitamin K s.c. 30 mg daily for 3 weeks, 20 mg daily for two weeks, and 10 mg daily for other two weeks. INR and Hemoglobin were monitored weekly. The complete INR normalization was obtained in about three months. In conclusion, the ingestion of long-acting warfarin-like rodenticide has to be suspected based on the markedly prolonged coagulation tests, the partial response to therapy, the long duration of the coagulopathy and the absence of likely alternatives. Actually the most common used rodenticides are the 4-hydroxicoumarin derivatives brodifacum and difenacoum, created to overcome acquired warfarin resistance in rats. They act by the same mechanism as warfarin but are 100 times more potent and have much longer serum and tissue half-lives and high lipid solubility depositing in fatty tissue and liver.

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TWO YEARS PROSPECTIVE OBSERVATION OF HEPARIN-INDUCED THROMBOCYTOPAENIA IN MORE THAN THREE THOUSAND PATIENTS REVCEIVING CARDIOVASCULAR SURGERY: EXPERIENCE OF TWO CENTRES

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Background. The prevalence of thrombocytopaenia (HIT) is controversial in subjects (s) undergoing cardiovascular surgery and prophylaxis with UFH or LMWH. *Aim.* in order to determine the prevalence of HIT in these patients, we promoteed a two years prospective evaluation in two distinct Centres in the same town. *Methods.* globally 3222 patients were observed. In all patients Platelet (Plt) count, PT, APTT, Fibrinogen, D-dimers were evaluated preoperatively and postoperatively. Presence of Heparin-PF4 (H-PF4) antibodies, detected by ELISA-GTI, were evaluated only in presence of severe thrombocytopenia (less 50×10^9/L or drop of 50% of the Plt count in the first two days after surgery). In one center (1220 patients) postoperative thrombosis prophylaxis was prevalently performed using UFH, in the other (2002 patients) LMWH was used. *Results.* HIT (documented by severe thrombocytopenia and positive anti Heparin-PF4 antibodies) occurred in 18 patients. In 5 cases arterial or venous thrombosis occurred (1 DVT, 1 arterial limb thrombosis requiring amputation, 1 arterial limb thrombosis requiring femoral artery thrombectomy, 1 intra-cardiac thrombosis and massive femoral arterial emboli requiring thrombectomy, 1 arterial and venous thrombosis terminated with death). The remaining 13 patients had no evidence of macro-or micro-thrombosis associated with thrombocytopenia and positive anti Heparin-PF4 antibodies. No difference in incidence of HIT with or without thrombosis was evident based on type of heparin prophylaxis. In all cases heparin treatment was promptly arrested, Lepirudin treatment was succesfully administered in 7 patients. *Conclusions.* thinking and carefull monitoring for HIT has accounted for a low incidence of severe appearance of thrombo-embolic events associated with this syndrome. Prevalence of HIT was equally ditributed in patients prevalently treated with UFH or LMWH for postoperative prophylaxis.

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NO NEED OF BLOOD TESTS (ANTI-XA, APTT) FOR MONITORING SUBMINISTRATION OF PROPHYLACTIC DOSE OF NADROPARINE IN ELDERLY WOMAN WITH RENAL FAILURE

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A limitation in the use of LMWH is the presence of renal failure, that is a frequent finding in elderly patients, often with serum creatinine in range (Brophy, 2001; Becker, 2002). We've studied 16 cases, women, consecutives, patients of the Clinica Geriatrica, in prophylactic treatment with Nadroparine (3800 ui/1 die), answering the inclusion criteria of the study, indicated as: being women, receiving fixed dose of LMWH for at least 3 days, not presenting an acute illness or a congestive heart failure, not showing a weight's variation of ±5% in the previous week's determination. During the observational period (15 days) none of the patients developed venous thromboembolism, pulmonary embolism, haemorrhage or heparin-induced thrombocytopenia. The examined parameters are those listed in the attached Table 1: age, weight, serum creatinine, acquired from the unit's data; PT-INR, aPTT, Anti-Xa activity measured from samples taken by the examiner and analyzed by the Laboratorio Urgenze using Dade Behring's Berichrom Eparina test. The results were subsequently divided according to the 3 levels of CrCl proposed by the KDIGO to evaluate CKD (The CARI Guidelines, Evaluation of Renal Function, 2005), using the CG formula to obtain the calculated CrCl. For statistical needs, Anti-Xa activity values lower than the inferior limit were equalled to that limit. The goal was to compare Anti-Xa activity levels and the risk or major bleeding in patients with a CrCl <30 mL/min and those with 30 mL/min<CrCl<60 mL/min, testing the necessity of a major control in elderly women with renal failure. The results showed a not significant variation of the anti-Xa activity in the different groups, and a p=0,1 in the comparison of the mean aPTT (t test). *Conclusions.* Even in patients with low levels of CrCl, it's not necessary monitoring the haemocoagulative parameters.

Table 1. Prophylactic Nadroparine in hospitalized elderly women

Age	*Weight (Kg) Creatinine- Cr_S (mg/100 mL) (0,6-1,1)*	*Serum creatinine clearance* CrCl (mL/min)*	*Calculated (0,88-1-13)*	*PT-INR (23-37)*	*aPTT (sec) (20-1500)*	*Anti-Xa activity*
			CrCl 15-29(mLmin)			
92	66,3	2,12	17,68	1,31	31	20
102	60,8	1,53	17,74	0,98	28	160
88	53	1,34	24,19	1,13	32	50
92	44,2	0,97	25,77	1,1	31	20
89	48,2	1,03	28,22	1,17	30	31
93	72	1,42	28,91	1,18	27	111
92,6±4,9	57,4±10,8	1,4±0,4	23,8±4,9	1,1±0,1	29,8±1,9	65,3±57,7
			CrCl 30-59(mLmin)			
84	62	1,23	33,27	1,08	29	20
94	89	1,42	33,94	0,97	29	220
84	57,3	1,05	36,05	1,27	28	39
90	59,5	0,81	42,07	-	28	20
88	51,1	0,73	42,71	1,15	25	20
87	61	0,75	50,41	1,11	27	20
83	61,7	0,72	57,41	1,15	26	20
86	63,5	0,70	57,78	1,13	30	66
87,0±3,7	63,1±11	0,92±0,3	44,2±9,9	1,1±0,9	28,0±1,7	49,1±61,8
			CrCl > 60(mL/min)			
81	62,5	0,67	65,29	1,14	25	20
77	60,3	0,49	90,20	1,12	30	33
79±2,8	61,4±1,5	0,58±12,7	77,7±17,6	1,13±1,4	27,5±3,5	26,5±9,2

**calculated with Cockcroft-Gault formula: [(140-age)x weight]/(Cr_S x72)x0,85*

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PFA-100® CLOSURE TIME TO PREDICT CARDIOVASCULAR EVENTS IN ASPIRIN-TREATED CARDIOVASCULAR PATIENTS: A META-ANALYSIS OF 19 STUDIES COMPRISING 3,003 PATIENTS

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Introduction. Monitoring of platelet function during antiplatelet treatment could improve clinical outcomes in cardiovascular patients. However, so far few studies only have correlated measures of interindividual variability in platelet response to aspirin, as assessed by laboratory tests, with the occurrence of clinical events; therefore no definite conclusion has been reached. Four recent systematic reviews, including different studies, independently reached the conclusion that laboratory tests may predict clinical recurrences in cardiovascular patients under aspirin treatment (Crescente *et al.*, TH 2008; Reny *et al.*, JTH 2008; Krasopoulos *et al.* BMJ 2008; Snoep *et al.*, Arch Int Med 2007). We decided to pool all the studies included in at least one of the aforementioned systematic reviews to more powerfully estimate the risk of vascular events in *aspirin non-responders*, as detected by the Platelet Function Analyzer (PFA)-100, one of the most employed tests to monitor aspirin response. *Methods.* For this purpose, fixed-effects pooled measures were calculated as the inverse variance weighted mean of the log ORs. Heterogeneity among studies was assessed using Cochran's Q test and the I2 statistics. Nineteen studies, including 3,003 patients, were identified (Figure 1). *Results.* The overall fixed-effects OR was 2.35 (95% CI: 1.96-2.83), with a modest evidence of heterogeneity (I2= 41.4%, p= 0.03). The pooled OR for 9 non prospective studies was significantly higher than that for prospective ones (3.12, 95% CI: 2.40-4.06 vs 1.75, 95% CI: 1.35-2.28, p=0.005). Treatment with aspirin either alone or plus clopidogrel did not influence the risk (2.13, 95% CI: 1.72-2.64 *vs.* 3.21, 95% CI: 2.23-4.61, p=0.19). As the median value of PFA-100 closure time cut-off was 170 sec, we compared the pooled OR from studies using a cut-off ≤170 sec (*low cut-off value*) with that of studies using cut-off >170 sec (*high cut-off value*): no significant difference was found (2.43, 95% CI: 1.83-3.22 *vs.* 2.30, 95% CI: 1.79- 2.94, p=0.80). Residual heterogeneity was apparent within the latter subgroup (I2=56.1%, p=0.01). *Conclusions.* The present analysis confirms the significant association between *aspirin non-response* measured by a platelet laboratory test and the recurrence of vascular events and highlights the need for prospective trials to firmly establish whether laboratory tests are useful to predict adverse vascular events and to optimize individual antiplatelet therapy.

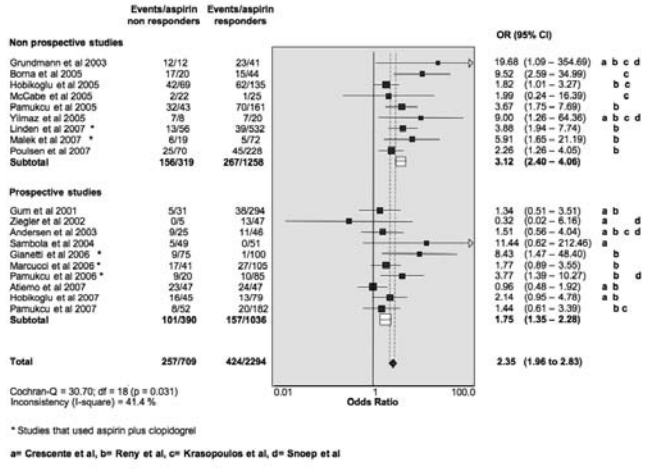

Figure 1.

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PHARMACOKINETICS AND PHARMACODYNAMICS OF PRASUGREL (CS-747, LY640315), A NOVEL THIENOPYRIDINE P2Y12 RECEPTOR ANTAGONIST, IN HEALTHY SUBJECTS

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Purpose. Prasugrel (CS-747, LY640315), a novel P2Y12 ADP receptor antagonist, provided more potent inhibition of platelet aggregation (IPA) versus clopidogrel in preclinical studies. This phase 1 study investigated the antiplatelet effects and pharmacokinetics of prasugrel during 21 days of repeat dosing. *Methods*. Thirty-two subjects were randomly assigned to 1 of 3 prasugrel oral dosing regimens with a loading dose (LD) on day 1 and a maintenance dose (MD) on days between 2th and 21th: 1) prasugrel 40 milligrams LD and 7.5 milligrams MD, 2) prasugrel 60 milligrams LD and 15 milligrams MD, 3) placebo LD and placebo MD. IPA was assessed by turbidometric aggregometry using 20 micromolar ADP as agonist. Pharmacokinetics of 3 inactive metabolites were measured at preselected time points between days 1 and day 26. *Results*. Both LDs of prasugrel were effective in rapidly and consistently inhibiting ADP-induced platelet aggregation and achieved substantial levels of IPA (median 65 percent). The differences in median IPA between both prasugrel groups and placebo were statistically significant within the first hour following the LDs and throughout the MD phase. The prasugrel 15 milligrams MD regimen was able to maintain the high level of median IPA achieved by the LD; the median IPA with the 7.5 milligrams MD decreased to approximately 40 percent by day 14, becoming statistically different to that of the 15 milligrams MD at day 21. The pharmacokinetics of 3 inactive metabolites of prasugrel were well described by an integrated population pharmacokinetic model. From the pharmacokinetic behavior of these metabolites, it can be inferred that prasugrel was absorbed rapidly, demonstrating only modest intra- and inter-subject variability. The pharmacokinetic parameters for prasugrel metabolites appeared consistent across dose. Among all subjects, the most frequently reported (major or equal 10 events) drug-related adverse events were headache, hematoma (procedural related), and dizziness. *Conclusions.* In healthy subjects, both prasugrel dosing regimens (60 milligrams LD and 15 milligrams MD; 40 milligrams LD and 7.5 milligrams MD) demonstrated consistently high levels of platelet inhibition and were safe and well tolerated. Pharmacokinetics for 3 inactive metabolites of prasugrel indicate that prasugrel was absorbed rapidly and metabolized in all subjects with modest variability.

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ANTI-INFLAMMATORY EFFECTS OF CLOPIDOGREL IN A MOUSE

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Activated platelets (Plts) interact with leukocytes and signal inflammatory response. Plts-leukocyte interactions are fundamental not only for atherothrombosis but also for the innate immune response to invading bacteria, such as occurs in sepsis, a complex clinical syndrome characterized by widespread tissue damage, intravascular coagulation and multiple organ failure. We explored the effect of clopidogrel on inflammatory responses in mice challenged by endotoxin (10 mg/kg, i.p.). CD1 male mice were treated orally with 25 mg/kgx2/day, for 2 days, plus a 25 mg/kg 2 hours before experiments. Plt/granulocyte(PMN) conjugates and Mac-1 expression in PMN were analysed by whole blood flow cytometry. TNF-alpha and IL-1beta were measured in plasma by ELISA. TNF-alpha and IL-1beta mRNAs were analysed in spleens and hearts by real time RT-PCR. Data (means±SD, n=3-6), were analysed by ANOVA or by Student t test. Endotoxin increased the percentage of circulating Plts expressing p-selectin (4,67±0,76% *vs.* 2,45±0,39% in treated *vs.* untreated animals, p<0.05) and up-regulated P-selectin expression induced by thrombin *ex vivo*. Clopidogrel abolished *in vivo* and *ex vivo* P-selectin up-regulation. At 30 and 60 minutes after endotoxin challenge, 45±17% and 29±13.3%, respectively, of circulating PMNs carried adherent Plts; Plts/PMN conjugates decreased to 6±0.8% and 11.6±2.9 (p<0.05) in clopidogrel-treated mice. At these time points, clopidogrel reduced

(p<0.05) up-regulation of Mac-1 in PMN (from 227.9±1.6 and 388.9±3.1 to 151.2±17.3 and 201.0±9.8, MFI. Basal values were 72.2±7.0 MFI). Endotoxin increased TNF-alpha and IL-1beta in plasma, reaching a maximum, 620±28,3 and 2110±101 pg/mL, at 120 and 60 minutes after challenge, respectively. Clopidogrel virtually abolished IL-1beta and significantly reduced TNF-alpha in plasma as well as IL-1beta mRNA in spleen and heart. In contrast with the inhibition of leukocyte activation and cytokines production elicited in wild type mice, clopidogrel did not modify Mac-1 upregulation in P-selectin-/- mice challenged by endotoxin. Similarly, clopidogrel reduced IL-1beta in wild type or Mac-1-/- mice but barely affected IL-1beta in P-selectin-/- mice. The efficacy of clopidogrel on inflammatory cellular reactions in a mouse model that reproduces many of the components of the innate immune response that are normally concerned with sepsis, suggests that it might be also effective against armful and damaging host response to systemic infections.

P144

ANTIPLATELET DRUGS FOR POLYCYTHAEMIA VERA AND ESSENTIAL THROMBOCYTHAEMIA: A COCHRANE META-ANALYSIS

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Background. Polycythaemia vera and essential thrombocythaemia are chronic Philadelphia-negative myeloproliferative disorders with an increased risk of arterial and venous thrombosis as well as bleeding. In association with different available therapeutics strategies, aspirin is often used to prevent platelet aggregation. Main objective of this systematic review is to quantify the benefit and harm of antiplatelet drugs for long-term primary and secondary prophylaxis of arterial and venous thrombotic events in patients with polycythaemia vera or essential thrombocythaemia. *Methods.* CENTRAL, MEDLINE and EMBASE databases were searched. All randomised controlled trials (RCTs) comparing long term (>6 months) use of an antiplatelet drug versus placebo or no treatment in patients with polycythaemia vera or essential thrombocythaemia, diagnosed by established international criteria, with data for at least one of the outcomes, were included. Using a predefined extraction form, the following data were collected and analysed where appropriate: mortality from arterial and venous thrombotic events, mortality from bleeding episodes, fatal and non-fatal arterial thrombotic events, fatal and non-fatal venous trombotic events, major and minor bleeding episodes, all-cause mortality, any adverse events. Quantitative analysis of outcome was based on an intention-to-treat principle. The overall treatment effect was estimated by the pooled odds ratio (OR) with 95% confidence interval (CI) using a fixed-effect model (Mantel-Haenszel). *Results.* Two RCTs (GISP and ECLAP) that investigated 630 patients with an established diagnosis of polycythaemia vera with no clear indication or contraindication to aspirin therapy were included in this review. The use of aspirin, compared with placebo, was associated with lower risk of fatal thrombotic events, although this benefit was not statistically significant (OR 0.20, 95% CI 0.03 to 1.14), without an increased risk of major bleeding (OR 0.99, 95% CI 0.23 to 4.36). No RCTs have been published in patients with essential thrombocythaemia and with other antiplatelet drugs. *Conclusions*. The available evidence suggests that the use of aspirin is associated with a not statistically significant reduction in the risk of fatal thrombotic events without an increased risk of major bleeding compared with no treatment in patients polycytaemia vera and with no clear indication or contraindication to aspirin therapy.

P145

PLATELET ASPIRIN TRANSPORT THROUGH MRP4 IS RESPONSIBLE FOR DRUG REDUCTION ACTIVITY IN CORONARY ARTERY BY-PASS SURGERY PATIENTS

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Aspirin is the most useful drug to prevent cardio and cerebro-vascular complications in high-risk patients. It has been recently reported that patients at 5 days after coronary artery by-pass surgery (CABS) presented a high residual platelet activation despite in-vivo and in-vitro aspirin treatment. Multidrug resistance protein-4 (MRP4) is expressed in human platelets and it is an organic anion unidirectional transporter. as aspirin, at physiologically pH, becomes an organic anion, it may be a target for this protein. Here we investigated the role of MRP4 in the reduction of aspirin sensitivity in CABS patients. The results obtained showed an increased of aspirin entrapment in platelets from healthy volunteers, reducing the MRP4 mediated transport by inhibitors (dipyridamole or MK571) as well as an increased aspirin effect on COX-1. In fact, thrombin induced thromboxane-A2 (TxA2) production is reduced when platelets were pre-treated with dipyridamole or MK-571 before aspirin addition. Platelets derived from megakariocytes transfected with MRP4.siRNA, showed a reduction of MRP4 expression and have a higher aspirin and salicylic acid entrapment and lower TxA2 production compared to platelets derived from control cultures. We confirmed a residual platelet COX-1 activity in CABG patients despite *in vivo* and *in vitro* aspirin treatment that was reduced after inhibition of MRP4 mediated trasport by dipyridamole as thrombin induced TxA2 formation is reduced by inhibiting MRP4 transport. In such patients we also found a higher MRP4 genomic (evaluated by MRP4 platelet mRNA) and proteomic (evaluated by immunoblot-analysis and immunogold-electromicrographs) expressions compared to healthy volunteers. In conclusion, we first demonstrated that the residual COX-1 activity in CABS patients is due to higher expression of MRP4 that enhances aspirin extrusion from platelet cytosol leading to a residual platelet COX-1 activity.

P146

REDUCED ERYTHROCYTE DEFORMABILITY AND ANEMIA MAY INFLUENCE RESIDUAL PLATELET REACTIVITY IN PATIENTS WITH ACUTE CORONARY SYNDROMES ON CLOPIDOGREL TREATMENT

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Background. Previous studies explored the association between hemorheological alterations and residual platelet reactivity in patients on aspirin treatment. Aim of this study was to evaluate the association between hemorheological variables and platelet reactivity in patients with acute coronary syndromes (ACS) submitted to percutaneous coronary intervention (PCI) on dual antiplatelet therapy. *Methods.* The study population included 528 ACS patients undergoing PCI. Hemorheological studies were performed by assessing whole blood viscosity (at shear rates of 0.512 s-1 and 94.5 s-1) and plasma viscosity (Rotational Viscosimeter LS 30) and erythrocyte deformability index by Myrenne filtrometer. Patients were assessed for post-treatment platelet reactivity after a loading dose of 600 mg of clopidogrel; the presence of residual platelet reactivity was defined as platelet aggregation by 10 μmol adenosine 5'-diphosphate ≥70%. *Results.* Significantly higher values of whole blood viscosity at 94.5 s-1 as well as lower values of hematocrit and erythrocyte deformability index were found in patients with high residual platelet reactivity. Mild but significant correlations between platelet aggregation by 10 μmol adenosine 5'-diphosphate and hematocrit (r=-0.26; p=0.000), erythrocyte deformability index (r=-0.36; p=0.000), whole blood viscosity at 94.5 s-1 (r=0.17; p=0.000), plasma viscosity (r=0.16; p=0.000) as well as fibrinogen (r = 0.15; p=0.000) were observed. At multivariate analysis (adjusted for age, gender, traditional cardiovascular risk factors, renal dysfunction, previous coronary artery disease and therapy), lower values of hematocrit and erythrocyte deformability index as well as higher values of whole blood viscosity and leukocytes resulted independently associated with residual platelet reactivity (Model 1 analysis) also after simultaneous adjustment for hematocrit, leukocyte count and erythrocyte deformability index (Model 2 analysis). *Conclusions.* These results demonstrate an independent association between reduced erythrocyte deformability and residual platelet reactivity and suggest a possible role of anemia in influencing residual platelet reactivity to adenosine 5'-diphosphate thus explaining the higher incidence of thrombotic events during follow up of anaemic patients. Our results suggest that these variables could be used as possible targets to optimize antithrombotic therapy in these patients.

P147

EVALUATION OF PLATELET FUNCTION TESTS AS PREDICTORS OF THROMBOTIC EVENTS IN HIGH RISK PATIENTS

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Higher rate of clinical events in poor clopidogrel and/or aspirin responders was documented by using different methods to measure platelet function, but no conclusive data about the appropriate methodology to explore platelet reactivity are available. In 746 patients successfully receiving drug-eluting stents we evaluated the residual platelet reactivity (RPR) in platelet-rich plasma by 10 μM ADP, 1 mM AA and 2 gramms/mL collagen and in whole blood by PFA-100 system. At 6-month follow-up, RPR by two stimuli (ADP and AA or ADP and collagen) and by three stimuli (ADP, AA and collagen) is significantly associated with higher percentage of primary (definite or probable stent thrombosis) and secondary (cardiac mortality and stent thrombosis) end-points than RPR by ADP, AA, collagen and PFA-100 system. According to the primary and secondary end points, the specificity values for RPR identified by two (ADP and AA: 94%; ADP and collagen: 97%) and three stimuli were higher with respect to RPR by ADP (88%), or RPR by AA (83%) or RPR by collagen (90%). The positive likelihood ratio values of RPR by three stimuli (9.55) or of RPR by ADP and collagen (8.08) were higher than those of RPR by ADP (2.59), by AA (2.05), by collagen (4.73), or by PFA-100 (2.63). This prospective study documents that the evaluation of platelet reactivity addressed to identify patients at risk of thrombotic events on dual antiplatelet treatment has to be carried out by methods able to explore different pathways.

P148

COMPLIANCE IS A MAJOR DETERMINANT OF ASPIRIN RESISTANCE IN PATIENTS WITH STABLE CARDIOVASCULAR DISEASE

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Background. Poor compliance may be implicated in aspirin resistance but its prevalence as well as the underlying mechanism are unclear. *Methods*. In twenty atherosclerotic patients daily monitored for aspirin(100 mg) intake, arachidonic-acid (AA)- induced platelet aggregation was measured with or without *in vitro* addition of aspirin. This exploratory study showed that platelet aggregation <20% was associated with a) inhibition of platelet thromboxane (Tx)A2 , that was not reduced further by *in vitro* addition of aspirin, and b) serum TxB2 <10 ng/mL. Then, we studied one hundred consecutive aspirin-treated patients with stable atherosclerosis. Patients with platelet aggregation >20% were considered non responders and entered a 7-day follow-up of 100 mg/day aspirin intake monitoring (first follow-up). After analysis of platelet aggregation, patients who still had aggregation>20% entered a second 7-day follow-up of 325 mg aspirin daily monitoring with repetition of platelet aggregation at the end of the second follow-up. *Results*. Among one hundred patients of the prospective study, 69% with platelet aggregation <20% and serum TxB2<10 ng/mL were considered responders, while 31% were non responders. Among the 31 patients 26 became responders after the first follow-up and were considered non compliant; the remaining 5 were still non responders after the second follow-up and considered resistant. Logistic regression analysis adjusted for age, sex, cardiovascular risk factors, and anti-atherosclerotic drugs demonstrated that the number of daily pills was an independent predictor of poor compliance (O.R.: 2.509; 95% C.I.: 1.551-4.058; $p<0.001$). *Conclusions*. Multiple antiatherosclerotic treatments may cause poor compliance and apparent aspirin resistance. True resistance seems to occur in 5% of patients.

P149

SUCCESSFUL LOCO-REGIONAL THROMBOLYTIC TREATMENT OF THE AORTIC CONDUIT IN A PATIENT WITH ORTHOTOPIC LIVER TRANSPLANTATION: A CASE REPORT

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Alternatives to the hepatic artery (HA) anastomosis are needed in liver transplantation when the native HA cannot be used or when HA complications develop. Aortic conduits, that use donor iliac artery interposed between the recipient infrarenal aorta and the HA of the graft, represent a reliable technique for graft revascularization in orthotopic liver transplantation (OLT). Unfortunately, the use of aortic conduits in arterial reconstruction is associated with higher risk of hepatic artery thrombosis (HAT), compared to the usual arterial anastomosis, and is a significant cause of graft loss. The thrombotic obstruction of an arterial conduit has severe consequences and may require re-transplantation. Little is known about its treatment. We describe the case of a 65 years old male who underwent OLT in 2002 due to HCV-related liver cirrhosis. Due to intimal dissection of the recipient HA it was impossible to perform the usual termino-terminal anastomosis, and an infra-renal aorto-hepatic artery conduit was made utilizing the common and the external iliac artery of the donor. On March 2007, during a follow-up visit, a decrease in blood flow in the conduit was detected. During arteriography, which detected a stenosis of the conduit, a small thrombus was mobilized inside the conduit. The patient was treated with anticoagulant doses of low-molecular weight heparin (LMWH) and warfarin with a target INR of 2.5 (more than 80% of time in range). In April 2008, to treat the stenosis which caused a further decrease of blood flow through the conduit, a stent (Astron Pulsar 5 mm, Biotronik, Germany) was placed. During this manoeuvre a large thrombus inside the conduit was detected. The patient received therapeutic doses of LMWH in combination with aspirin 100 mg/day. After a few days, the patient started complaining of diarrhoea and was admitted to our Hospital. Doppler ultrasonography and arteriography showed a complete obstruction of the conduit. A loco-regional thrombolytic treatment with recombinant tissue plasminogen activator (5 mg bolus, followed by 1 mg/h for 24 hours) was administered. After 24 hours arteriography showed a complete patency of the conduit. A combination of antiaggregation therapy of clopidogrel 75 mg/day and aspirin 100 mg/day, plus therapeutic doses of LMWH were given. After ten days the patient is in good clinical conditions and the patency of the conduit is maintained. To our knowledge, this is the first report of successful thrombolysis of an arterial conduit.

Platelets: Qualitative and Quantitative Alterations and Study Methods

P150

COMPARATIVE TRANSCRIPT PROFILING OF HUMAN PLATELETS FROM PATIENTS WITH STABLE ANGINA AND ACUTE CORONARY SYNDROMES

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Platelets play a key role in atherothrombosis and coronary artery disease. Platelets have the capacity of de novo protein synthesis through translation of pre-existing megakaryocyte-derived mRNAs, raising the possibility that these mRNAs may influence physiological and pathological platelet functions. Indeed, recent reports describe that platelet mRNAs may vary in clinical conditions such as systemic lupus erythematosus, sickle cell disease, and ST-elevation myocardial infarction. *Objective.* To screen platelets from patients with stable angina (SA) and non-ST elevation acute coronary syndromes (ACS) for differentially expressed transcripts that may modulate the platelet pro-thrombotic potential. Methods. Venous blood samples were withdrawn from 14 SA and 15 ACS patients. Total RNA was then extracted from washed platelets virtually free of leukocyte contamination, pooled (three pools of total RNA for each group of patients) and used for comparative transcriptome analysis by means of microarray technology. Confirmatory PCR and immunoblot analyses were performed in platelet preparations obtained from independent subjects (26 SA and 17 ACS patients in total). *Results.* We detected and validated differential expression of seven genes in SA and ACS platelets on mRNA level (BAIAP2/IRSp53: BAI1-associated protein-2/insulin receptor substrate p53; CLTA: clathrin light-chain; GP1BB: platelet glycoprotein-1b β-chain; MRPL4: mitochondrial ribosomal protein-L4; PKIG: protein kinase inhibitor-γ, SELPLG/PSGL1: P-selectin ligand; VPS72: vacuolar protein sorting-72 homolog; Fold differences ACS/SA=0.1-8.7; p=0.002-0.04). Of these, differential expression of platelet CLTA and GP1BB was observed also on protein level, as was that of CALB2: calbindin-2/calretinin (CALB2: fold difference=1.6, p=0.02; CLTA: fold difference=1.7, p=0.001; GP1BB: fold difference=2.4, p=0.00005). *Conclusions.* Comparison of the platelet transcriptome of SA and ACS patients identified differentially expressed genes that may modulate platelet reactivity in coronary artery disease. These findings highlight a set of platelet genes and pathways already known in the context of coronary atherothrombosis and indicate novel ones, thereby suggesting directions for further research in this clinically relevant area.

P151

PLATELET ACTIVATION IN PATIENTS UNDERGOING CAROTID ARTERY STENTING PROCEDURE

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Background. Carotid Artery Stenting (CAS) is an evolving method to treat carotid stenosis. A limitation of CAS is the periprocedural risk of temporary or permanent ischemic neurological deficits resulting from local thrombosis and distal embolization. Platelet activation in CAS occurs as a result of vessel wall damage and subendothelium exposure. The dual antiplatelet regimen (aspirin+thienopyridine) has a significant impact on reducing adverse neurological outcomes. No data on platelet activation during dual antiantiplatelet therapy and after thienopyridine discontinuation have been examined in CAS. *Aim.* To analyze platelet activation during dual antiplatelet therapy and two months after thienopyridine discontinuation in patients who underwent CAS procedure. Methods. We enrolled 40 patients with high-grade carotid artery stenosis (>70% NASCET criteria). Blood was withdrawn 1 day before (T0), 1 (T1) and 30 days (T2) after CAS and 2 months after thienopyridine discontinuation (T3). Platelet activation markers (PAC-1, CD62P and Tissue Factor [TF] and the percentage of monocyte-platelet aggregates [MPA]) were assessed by whole blood flow cytometry in resting conditions and upon *in vitro* ADP stimulation and reported as %UR. As a comparison, platelet activation in 16 healthy subjects was also analyzed (Controls). *Results.* In resting conditions, CD62P and PAC-1 positive platelets and MPA were constant throughout the time points examined (average T0-T3: 0.22±0.02, 0.16±0.01, 9.51±0.68 respectively) and similar to those found in Controls. *In vitro* ADP-induced CD62P, PAC-1 and MPA levels were significantly higher at T3 (13.36±2.69, 59.78±10.51, 80.16±4.73 respectively) when thienopyridine was discontinued compared to T0, T1 and T2 (average T0-T2: 5.57±0.51, 46.15±2.09, 72.02±1.86 respectively) and similar to that observed in Controls. Levels of TF positive platelets were constant, in resting conditions, throughout the time points examined (average T0-T3: 5.04±0.43), but 3 fold higher that those found in Controls (1.95±0.56, p<0.01). Drug treatment does not inhibit ADP-induced TF expression being the values similar to those found in Controls. *Conclusions.* Significant higher levels, compared to Controls, of TF-positive platelets circulate in peripheral blood of CAS patients. Dual antiplatelet therapy inhibits the expression of ADP-induced platelet activation markers, but TF. This prothrombotic platelet phenotype may have implications for CAS complications.

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APPROPRIATE HOSPITAL MANAGEMENT FOR IMMUNE THROMBOCYTOPENIC PURPURA IN ADULT MEN AND NON-PREGNANT WOMEN: RESULTS OF A RETROSPECTIVE ANALYSIS CARRIED OUT AT THREE NORTH ITALIAN INSTITUTIONS

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Aim of the study. The present study was aimed at verifying the practicability of American guidelines in non-pregnant adults hospitalized due to immune thrombocytopenic purpura (ITP). *Material and Methods.* We analyzed retrospectively the computerized discharge diagnosis records (HDF) of a 3 year period (2000-2002) at 3 North Italian Hospitals. All coded 287.3 (ICD-9-CM) HDF were collected. In available hospital charts of non-pregnant adults we examined the appropriateness of ITP diagnosis and treatment according to American guidelines. *Results.* ITP diagnosis was verified in 120 (71%) of 169 available hospital charts of non-pregnant adults with 287.3 code (the total number of hospitalizations with 287.3 code was 242) (Figure 1).

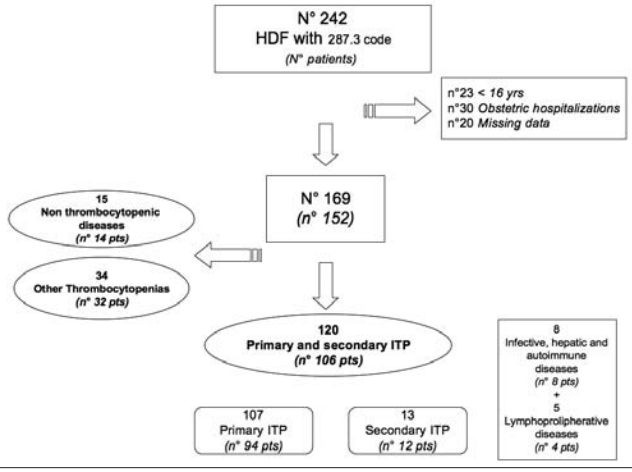

Figure 1.

In these 120 hospitalizations, hospital admission was due to: elective splenectomy (19 HDF; 15.8%), medical or surgical treatment of diseases associated with ITP (37 HDF; 30.8%), medical treatment for ITP that could not be deferred (62 HDF; 51.7%), and requirement of hospital diagnosis or observation for ITP (2 HDF; 1.7%). In the last two conditions (64 HDF), hospital admission resulted appropriate 42 times (65.6%). The appropriateness of different interventions ranged from 100% for standard glucocorticoid treatment to 19% for prophylaxis against bacterial infections before splenectomy, while it was 86.4 and 40.9% in indication for splenectomy and prophylaxis against bleeding before splenectomy. For High Dose (HD) glucocorticoid treatment and

HD immunoglobulins (Ig), the appropriateness was 33.3 and 47.4%, but it increased to 80 and 66% when our modifications, based on data observations, were included in available guidelines. Moreover, through these modifications, guideline applicability for HDIg use increased from 57.6 to 100% (χ^2=17.8; p<0.0001). For platelet transfusions as medical treatment for bleeding, even if the appropriateness, evaluated through our modifications, increased only from 20 to 24%, a group of uncertain appropriateness (44%), not previously provided, could be recognized. This group reduced the inappropriateness of platelet transfusions from 80 to 32% (χ^2=11.7; p<0.001). *Considerations.* Our data substantially confirm the practicability of available American guidelines on ITP. For HDIg, HD steroids, and platelet transfusions they do not allow definite considerations. However, particularly for HDIg and platelet transfusions, our modifications of available American guidelines could be considered in formulating new guidelines.

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SAFETY OF R-HIRUDIN USE FOR HEPARIN-INDUCED THROMBOCYTOPENIA TREATMENT IN A CEREBRAL VEIN THROMBOSIS PATIENT: A CASE REPORT

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A 49-year-old woman was admitted to our department after partial seizure, described as paresthesia and tonic-clonic movements of the left hemisoma. On admission the neurological examination evidenced left arm and facial weakness. A cerebral computed tomography (CT) scan showed two small cortical haemorrhages on the right hemisphere. A cerebral magnetic resonance-angiography (MRA) showed a cerebral vein thrombosis (CVT) with total occlusion of the superior sagittal sinus, of the left lateral sinus and a reduced perfusion of the rectum sinus. Unfractionated heparin (UFH) was started and replaced after 48 hours by low molecular weight heparin (LMWH). A CT-scan performed after 8 days showed a reduction of the hemorrhagic lesions. On the 10th day after the beginning of heparin treatment, a decrease in the platelet count (from a baseline value of 250.000/mmc to 72.000/mmc) was found. On the same day the patient had a severe headache, no new neurological signs, no variations on cerebral CT-scan. LMWH was stopped. The ELISA test for heparin-induced thrombocytopenia (HIT) resulted positive. We started dermatan sulphate (DS) at the dosage of 12 mg/kg/die incrementing of 4 mg/kg/die to a maximum of 24 mg/kg/die, and an aPTT Ratio between 1.3-1.7 was reached after 12 hours. The platelet count continued to fall down to 25.000/mmc two days after the beginning of DS. The patient had a new partial tonic-clonic seizure. The functional test for heparin induced anti-platelet antibodies (Heparin Induced Platelet Aggregation, HIPA) showed a cross-reactivity of DS to anti platelet factor 4 (PF4) antibodies. Administration of DS was stopped and standard dose r-hirudin was started (bolus of 400 micrograms/kg, followed by continuous intravenous infusion of 150 micrograms/kg, adjusted to maintain an aPTT Ratio of 1.5-2.5 times the baseline value). The platelet count started to increased the day after. When platelet count reached 70.000/mmc, warfarin was started over and r-hirudin was discontinued when INR was stably in the therapeutic range. The patient presented an excellent complete clinical recovery and the follow-up MRA performed after 14 days from diagnosis showed a restored venous blood flow in the superior sagittal sinus and in the rectum sinus (Figure 1). On discharge the platelet count was 151.000/mmc and the INR 2.45. The patient continued on oral anticoagulation and phenytoin for at least 6 months. Up to ten months the patients has no residual neurological defects.

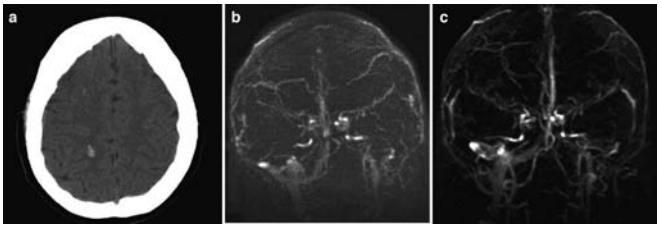

Figure 1. a. Cerebral CT: two small cortical haemorrhages on the right hemisphere. b. Cerebral MRA: total occlusion of the superior sagittal sinus, of the left lateral sinus and reduced perfusion of the rectum sinus. c. Follow-up cerebral MRA, performed 14 days after diagnosis restored venous blood flow in the superior sagittal sinus and in the rectum sinus.

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PERFORMANCE OF TWO NEW EIA ASSAYS FOR DETECTION OF IGG ANTI-PF4/HEPARIN ANTIBODIES IN PATIENTS SUSPECTED OF HAVING HEPARIN-INDUCED THROMBOCYTOPENIA

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Heparin-induced thrombocytopenia (HIT), an immune thrombocytopenia caused by IgG Abs to PF4 which become antigenic after interacting with heparin, is associated with a high risk of thrombosis. HIT can not be diagnosed on clinical criteria alone and no single laboratory test has 100% sensitivity and specificity. The functional assays are either technical-demanding and of limited availability (serotonine-release assay) or poorly sensitive though highly specific (platelet aggregometry assay). Conversely, the antigenic assays are widely available and highly sensitive but, detecting all anti PF4/heparin Abs (IgG, IgM and IgA), they are lees specific than functional assays. The results of these antigenic tests should therefore be interpreted in the appropriate clinical context. A pretest clinical score system (*4Ts*), based on clinical criteria, provides classification of patients into 3 groups with different probability of HIT. We evaluated two recently introduced EIA assays (PF4 IgG, GTI Diagnostics and Zymutest HIA IgG, Hyphen BioMed) that are expected to have a higher specificity since they detect only IgG anti PF4/heparin Abs. From Nov 2007 to Mar 2008, 107 patients (48 females; mean age 65 y, range 3-96 y) were referred to our lab for suspected HIT. Confirmation/exclusion of HIT was based on the flow chart proposed by Pouplard *et al.* (J Thromb Haemost 2007). Briefly, a particle gel immunoassay (H/PF4 PaGIA) was immediately performed in all patients; the probability of HIT was estimated by the *4Ts* clinical score. In patients with a positive H/PF4 PaGIA test or in those with a negative H/PF4 PaGIA but with high pretest clinical probability a platelet aggregation test was also performed [1 and 100 IU/mL of unfractionated heparin (UHF)]. The presence of HIT was confirmed if the aggregation was > 20% with 1 IU/mL and completely inhibited or <20% with 100 IU/mL UHF. HIT was diagnosed in 8 patients (7.5%). Using the cut-off levels recommended by the manufacturers, the GTI assay sensitivity (cut-off = 0.400 OD) was 100% but the specificity was very low (34.3%; 95% CI 25.1-44.6); on the contrary, only 6/8 patients with HIT were correctly identified by the Hyphen assay (cut-off = 0.300 OD; sensitivity: 75.0%, 95%CI 34.9-96.8) though the specificity was very high (97.0%; 95%CI 91.4-99.4). The results of both tests slightly improved by using specifically calculated cut-offs. Our results do not seem to confirm the high expected sensitivity/specificity of these new assays that are declared to be specific for IgG anti PF4/heparin Abs.

P155

PLATELET BEHAVIOUR IN PATIENTS WITH DRUG-ELUTING STENTS AFTER THIENOPYRIDINE TREATMENT DISCONTINUATION

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Background. Dug-eluting stents (DES) are the treatment of choice in percutaneous coronary interventions (PCI). Concerns have been raised, however, that DES may be associated with an increased late stent thrombosis rate. Dual antiplatelet therapy with aspirin and clopidogrel is standard of care following DES implantation. No data are available on platelet behaviour after stopping thienopyridine treatment. Aim. To assess platelet activation during dual antiplatelet therapy (T0) and one month after thienopyridine discontinuation (T1) in patients treated with DES. Methods. We enrolled 37 stable angina (SA) patients treated with DES (Cypher n=12, Taxus n=13, Endeavor n=12). The expression of platelet activation markers (Glycoprotein IIb/IIIa activated complex [PAC-1], Tissue Factor [TF] and P-selectin [CD62P] and the percentage of total and TF-positive monocyte-platelet aggregates) was assessed by whole blood flow cytometry and reported as percentage of positive events (%UR). As a comparison, platelet activation in 20 medically treated SA patients was analyzed. *Results.* During dual antiplatelet therapy after PCI (mean 88±77 days), basal levels of platelet-associated PAC-1 (0.20±0.02), TF (7.80±1.73) and CD62P (0.47±0.06) were significantly higher than medically treated SA patients (1.5, 2.5 and 2.2 fold respectively, p<0.02). Monocyte-platelet aggregates (20.52±3.00) and TF-positive monocyte-platelet aggregates (4.48±0.85) were more that two- and

six-fold higher respectively. One month after thienopyridine discontinuation, platelet-associated TF and PAC-1 expression was further upregulated (almost two fold compared to T0). Levels of CD62P, total and TF-positive monocyte-platelet aggregates did not change significantly (0.42±0.07; 22.51±3.65 and 5.29±1.45 respectively). Conclusion. During dual antiplatelet therapy, platelet activation in DES patients is higher than in medically treated SA patients and further increases after thienopyridine discontinuation. This platelet behaviour may have implications for late DES thrombosis.

P156

MODIFICATIONS OF RETICULOCYTES AND RETICULATED PLATELETS IN SEDENTARY HEALTHY MEN AFTER AN ACUTE EPISODE OF STRENUOUS EXERCISE

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Introduction. Exercise is considered a physiological stimulus for cells' release by the bone marrow. In particular, maximal exercise, carried out under hypoxic conditions, has been reported to determine reticulocytes' release, probably due to the augmented levels of erythropoietin. We aimed to investigate whether physical exercise can determine, together with reticulocytes, also the release of reticulated platelets (RP). *Methods.* Haematological parameters (red blood count, white blood count, haematocrit, haemoglobin, platelets), reticulocytes, and RP were measured in 20 healthy sedentary men (median age: 34 years) by using the Sysmex XE-2100 haematology analyzer (Sysmex, Kobe, Japan). Reticulocytes and RP were counted according to the measurement of scatter and RNA content was analyzed using oxazine. The reticulocytes' fractions with low (L), medium (M), and high (H) RNA contents were assessed, the M and H fraction being immature reticulocytes. All subjects performed a maximal incremental graded treadmill test and blood samples were drawn before (T0), at the end (T1), and 30 minutes after the test (T2). *Results*. All the haematological parameters showed a significant (p=0.002) increase at T1 with respect to T0, by returning similar to baseline at T2. Reticulocytes demonstrated a significant trend of increase at T1 with respect to T0 [52,650 (35,900-99,500) *vs.* 51,000 (26,800-105,000) ret/μL; p<0.05], with a decrease in L fraction and a significant increase in H fraction [0.65(0.3-1.3) *vs.* 0.4(0-0.8); p=0.01]. At T2 these parameters showed a trend of decrease, by reaching similar values with respect to baseline. Similar to the pattern of the reticulocytes' modifications, significant (p=0.01) higher levels of RP were observed at T1 with respect to T0 [9,550(7,300-24,000) *vs.* 8,250 (5,000-20,400) plt/μL]. At T2, however, RP values returned to similar values with respect to baseline. *Conclusions.* In conclusion, we documented that a maximal physical exercise can induce, in healthy sedentary men, a release of reticulocytes, mainly of the H immature fraction. Furthermore, we are able to report for the first time that, similarly to the effect on reticulocytes, a strenuous exercise is able to induce also a release of immature RPs which are known to have a greater prothrombotic potential and higher levels of intracellular thromboxane A2, so potentially contributing to the prothrombotic state after a strenuous exercise.

P157

RETICULATED PLATELETS AND PLATELET REACTIVITY IN RENAL TRASPLANT RECIPIENTS ON ANTIPLATELET THERAPY

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Introduction. Renal transplant recipients (RTRs) patients are at increased risk of cardiovascular morbidity and mortality. On this epidemiological finding is based the rationale of using antiplatelets drugs in these patients. Scarce data are available about the platelet function reactivity in RTRs patients and no data, at our knowledge, are present about the possible role of reticulated platelets (RP), which are immature platelets with a greater prothrombotic potential with respect to smaller platelets.*Aim.* To asses the platelet reactivity and RP in RTRs patients. *Methods.* We evaluated 150 renal transplant recipients (M 98, F 52) with a median age of 50 (17-75) years [66/150 (44.0%) were on ASA 100 mg treatment] and 60 healthy blood donors, comparable for age and sex. RP were measured by using the Sysmex XE-2100 haematology analyzer (Sysmex, Kobe, Japan). RP were expressed as the percentage of RP of the total optical platelet count (immature platelet fraction; IPF), as the percentage of RP highly fluorescent (H-IPF) and as the absolute number of RP (IPF#). Platelet function was assessed by optical aggregometry (PA) on platelet-rich-plasma induced by 1 mmol arachidonic acid (AA-PA) and 2 μg/mL collagen (Coll-PA). *Results.* A significant difference for IPF, H-IPF and IPF# between RTRs patients and controls was observed [IPF 3.6 (0.9-15.1) *vs.* 2.8 (0.9-5.6) % p=0.002; H-IPF 1.0 (0.2-5.6) *vs.* 0.9 (0.2-2.0) p<0.05; IPF# 7300 (1700-22900) *vs.* 6150 (2400-12800) p<0.05]. In addition, a higher percentage of RP was found in patients not on aspirin therapy with respect to patients on aspirin [IPF: 3.9 (1.1-15.1) *vs.* 3.0 (0.9-7.5)% p=0.005; H-IPF 1.0 (0.3-5.6) *vs.* 0.8(0.2-2.0) p=0.002; IPF# 8050 (2000-22900) *vs.* 6750 (1700-15200) p=0.019]. A significant positive correlation between reticulated platelets and PA by collagen and arachidonic acid was observed [IPF and Coll-PA r=0.19 p=0.02; H-IPF and Coll-PA r=0.18 p=0.03, IPF# and Coll-PA r= 0.21 p=0.009; IPF and AA-PA r=0.15 p<0.05; H-IPF and AA-PA r=0.16 p<0.05]. At a multiple linear regression analysis adjusted for age, gender, hypertension, hypercholesterolemia, diabetes, smoking habit, aspirin and cyclosporine treatment, IPF and IPF# were significantly and positively related to collagen-PA [MEAN±SE (95%CI): Coll-PA and IPF 7,2±3,0 (1.17-13.2) p=0.020; Coll-PA and IPF# 9.03±2.9 p=0.002]. At multivariate logistic regression analysis, adjusted for age, sex, traditional cardiovascular risk factors, aspirin use and maximal platelet aggregation by collagen and AA, IPF and IPF# were significantly associated with renal transplantation [OR (95%CI): IPF and renal disease 1.68 (1.15-2.44) p=0.007; IPF# and renal disease 1.17 (0.99-1.37) p<0.05]. *Conclusions*. We documented a higher levels of RP in RTRs patients with respect to controls. Furthermore, RP were found to be associated with renal transplantation independently of age, sex, cardiovascular risk factors and antiaggregating therapy and with platelet reactivity and in particular to collagen-evoked platelet response, independently of the use of ASA treatment.

P158

HEPARIN-INDUCED THROMBOCYTOPENIA: WHEN AND HOW OFTEN MUST WE FEAR THROMBOSIS?

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Heparin-induced thrombocytopenia (HIT) is a prothrombotic adverse reaction of heparin (H) administration, characterized by the occurrence of anti-heparin/pF4 antibodies (antiHIT-Ab); it is considered a life-threatening condition, since it can be complicated by severe thromboembolic events (TE) which may be lethal if H is not promptly withheld. Incidence of TE in the course of HIT has been reported between 11% and 75%. We studied a cohort of 85 consecutive patients with HIT, aiming to 1. establish the role of unfractionated H (UFH) and low-molecular-weight H (LMWH) in HIT-related thrombosis; 2. find any correlation between HIT and dosage/duration of H administration; 3. identify any possible risk factor for thrombosis with antiHIT-Ab. 26 patients had received H for major abdominal/cardiovascular surgery, 17 for nephropathy, 9 for neoplasia, 10 for ischemic heart disease, 6 for deep venous TE, 3 for polytrauma, 4 for sepsis, the remaining ones for other pathologies including autoimmune disorders. 25 subjects had arteriopathy/diabetes as comorbidity. 16 patients had received UFH, 56% at prophylactic dosage (PD,100-200U/kg/d), the remaining ones at therapeutic dosage (TD,384U/kg/d); 50 had received LMWH, 72% at PD (50-100U/kg/d), the remaining ones at TD (200U/kg/d); 19 subjects had received UFH first, then LMWH: PD of UFH had been administered in 42% and PD of LMWH in 74% of subjects. We observed 10 thromboses (11.7%), of which none in the UFH group, 6 in the LMWH group and 4 in the LMWH+UFH group; of these 4, 2 occurred in patients receiving LMWH at PD; overall, 8 TE over 10 (80%) developed in patients who had received LMWH as prophylaxis. Most thromboses occurred in arteriopathic/diabetic subjects (5/25;20%), surgical patients (4/26,15.4%), or septic/polytraumatized patients (1/4, 25% and 2/3, 66.7% respectively) Duration of administration directly correlated with the incidence of thrombosis: 80% of TE occurred in patients who had received LMWH at PD for more than 6 days. We conclude that in patients with HIT the chance of developing thrombosis is lower with UFH and increases with prolonged administration of prophylactic LMWH. Alternative prophy-

lactic strategies with innovative drugs may help overcome this problem. The probability of TE in these patients seems to correlate with the thrombotic risk of the underlying disease and the presence of arteriopathy. Our data suggest a possible role of platelet/endothelial activation in precipitating a TE in HIT patients.

P159

PLATELET CLUMPS IN SUBJECTS WITH NORMAL PLATELET NUMBER: NEED FOR CONCERN?

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Abnormal platelet clumping is a well-known, time-dependent artefactual interference, due to anticoagulant -triggered autoantibodies reactive against platelet membrane glycoproteins and causing a false low number of platelets that must be diagnosed as pseudothrombocytopenia, in order to avoid unnecessary, expensive and even harmful investigational procedures in quest of nothing. The sophisticated analysis methods of modern blood cell counters are able to detect interference due to platelets clumping or the presence of macrothrombocytes and produce appropriate flags. We have studied cases in which these flags occurred in presence of normal platelet counts. Samples were the daily load of in- and out-patients for routine complete blood count and differential (CBC-D) analyzed with blood cell counter between 8 am and 11 am (T1). The cases were selected among the complete blood count results not validated by the expert system operating in our lab with appropriate rule. Selection criteria were: a) presence of the alert flags *platelet clumps* and/or *giant platelet*; b) signs of interference in white blood cells graph; c) normal platelet number. Each sample's blood film was examined after M-GG staining and a second CBC-D was performed 4-6 hours later (T2). For each sample the observation at microscope of platelets aggregates and/or macrothrombocytes was registered. Statistical analysis was run by paired T-test with a level of significance <0.01. According to the selection criteria, in a 9 days survey 25 cases were found out of 2526 complete blood counts corresponding to a prevalence of 0.99%. Blood film examination disclosed the presence of macrothrombocytes in 13 cases (52%) and platelet clumps in 12 cases (48%).The mean platelet number in macro group is 211.000/ microliter (SD 60.000) at T1 and 218.000/ microliter (SD 65.000) at T2 (difference not statistically significant); the mean platelet number in platelet clumps group is 203.000/ microliter (SD 59.000) at T1 and 158.000/ microliter (SD 48.000) at T2 (p= 0.000179) (Figure 1). Our data show that platelet clumping occurs also in cases with normal platelet number, making it somehow unreliable considering quantitative platelet parameters only. This may have importance: a) in the determination of *normal reference ranges* for platelet number; b) in the evaluation of border-high platelet number cases, being present the risk of missing and not investigating a situation of possible thrombocytosis.

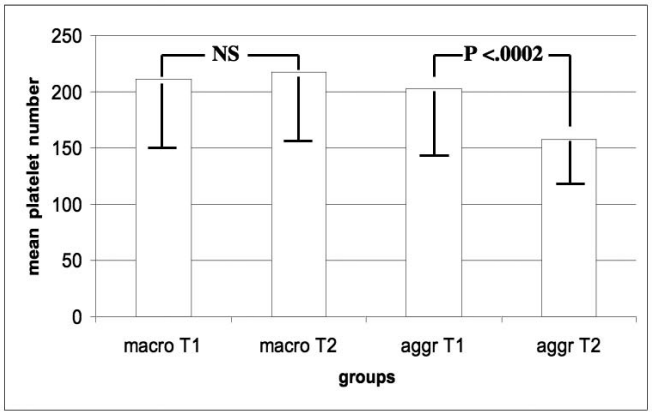

Figure 1.

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PLATELET PROTEIN S ANTIGEN LEVELS IN TYPE I AND III PROTEIN S DEFICIENT PATIENTS

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Background. Protein S (PS) is a vitamin K-dependent plasma glycoprotein with a molecular weight of 70 kDa. In human plasma, 40% of PS circulates in a free form and the remaining 60% in complex with C4b-binding protein. PS is contained inside platelets (Plts) in the alfa granules. Free PS acts as a cofactor of activated protein C (APC) for the inactivation of coagulation factor Va and factor VIIIa. Intra-Plts PS, released upon Plts stimulation, plays a crucial role in regulating thrombin generation and therefore controlling procoagulant activity. PS deficiency is inherited as an autosomal dominant disorder and is classified in three types: I) reduced plasma levels of total and free PS antigen (PSAg); II) normal concentration of total and free PSAg but with low cofactor activity; III) low free PSAg and normal total PSAg. *Aim of the study.* To evaluate the intra-Plts PSAg levels in 27 carriers of type I and in 15 of type III PS deficiency. *Patients and methods.* After informed consent, 20 mL of blood was collected in 3.8% sodium citrate solution (1:9 vol/vol) from 27 type I and 15 type III deficient PS subjects and from 22 normal individuals. In all subjects, we determined plasma total and free PSAg, PS activity and intra-Plts PSAg. *Results.* In type I subjects total and free plasma PSAg levels were 62±7% and 37±12%, respectively. In carriers of type III defect total and free PSAg levels were 85±13% and 41±13%, respectively. Intra-Plts PSAg levels in type I and III were 66±32% and 80±37%. In subgroup of healthy individuals total, free and intra-Plts PSAg levels were 119±17%, 110±17% and 101±30%, respectively. *Conclusions.* Our study demonstrate a strict correlation between total and free plasma PS and intra-Plts PS. The reduction of intra-Plts PS mirrors the reduced levels of free and total PSAg present in plasma of carriers of the defect even through PS levels in Plts appears unexpectedly higher than the free PS counterpart. Which of the total or free PS is the more relevant in determining intra-Plts PS levels is unknown. Further studies are needed to evaluate the relationship between these two (plasma/intra-Plts) compartments and to understand the pathophysiological role of the participation of platelets in the PC/PS pathway.

P161

PLATELET ACTIVATION BY THROMBIN IS INCREASED IN RETINAL VEIN OCCLUSION

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Retinal vein occlusion (RVO) is the most common retinal vascular disorder second to diabetic retinopathy. The main risk factors in patients with RVO are hypertension, diabetes, hyperlipidemia, increased blood viscosity and glaucoma. The pathogenesis of RVO has not yet been clarified. In these events platelets could play a very important role. We have recently demonstrated that platelets of patients were more responsive to low concentrations of collagen or ADP than healthy subjects. In addition in platelets of patients stimulated with collagen increased phosphorylation of p72syk, of phospholipase Cγ2 and intracellular calcium rise were found, while a lower basal level of nitric oxide compared to healthy subjects was measured [Leoncini *et al.*, Thromb Haemost 2007, 97, 218-227]. The aim of this study was to verify the response to thrombin of platelets from RVO patients compared to healthy subjects and to clarify the molecular mechanisms involved. In both groups of platelets aggregation, p38MAPK phosphorylation status, cytosolic phospholipase A_2 activation and thromboxane B_2 formation induced by thrombin have been measured. Results have shown that platelets of patients were more responsive to thrombin than healthy subjects and the response was significantly different (p<0.0005) at low concentrations of the agonist. Moreover in resting or in thrombin-stimulated platelets from RVO patients the p38MAPK phosphorylation and cytosolic phospholipase A_2 activation were increased compared to platelets from healthy subjects, being the difference significant at low concentrations of thrombin. In addition the thrombin-induced thromboxane B2 production was significantly higher in RVO patients than in healthy subjects. Oxidative stress

is an important mediator of abnormal platelet function. Superoxide anion is an important source of oxidative stress and limits the biological activity of NO. Thus we have tested superoxide anion levels in resting or stimulated platelets of patients and healthy subjects. Significantly higher levels of superoxide anion in patients that in healthy subjects have been measured. Altogether these results stress that platelet hyperaggregability of patients, contributing to the thrombogenic effects, could have a pivotal role in the development of RVO.

P162

PLATELET REACTIVITY IN STABLE ATHEROSCLEROTIC DISEASE PATIENTS ON CHRONIC ANTIPLATELET TREATMENT

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In stable atherosclerotic disease patients on aspirin treatment (100-325 mg/die), the prevalence of residual platelet reactivity (RPR) measured by different platelet function tests varies widely and the extent of such phenomenon over long-term follow-up is uncertain. The prevalence of RPR was detected in 85 patients on ASA therapy (n=85). Platelet function was determined in all patients at 6 (T1) months, and at 12 (T2) and 18 (T3) months in 43 patients. Collagen and arachidonic acid (AA) induced light transmission platelet aggregation (LTA) and collagen/epinephrine closure time (CEPI CT) by PFA-100 (Dade Behring, Milan, Italy) were evaluated. Results of LTA profile and CEPI CT are showed in table. No significant differences among measurements were observed. At T1 the prevalence of RPR patients was: AA-LTA, 19%, Collagen-LTA, 19% and CEPI CT, 22%.No significant change of prevalence of RPR patients were found at T2 and T3. This study shows that in stable atherosclerotic disease patients on long-term ASA treatment platelet function profile remains similar during the follow-up period and the prevalence of RPR remains unchanged.

Table 1. LTA profile and CEPI-CT PFA-100 results.

	T1 (6 months)	*T2 (12 months)*	*T3 (18 months)*
1 mmol/L AA-LTA	14% (4-91%)	15% (5-93%)	14% (6-85%)
2 microg/mL collagen-LTA	55% (11-97%)	52% (12-89%)	54% (6-86%)
CEPI-CT PFA-100	300 sec (85-300 s)	217 sec (97-300 s)	300 sec (94-300 s)

P163

UP-REGULATION OF PLATELET GP 91PHOX IN PATIENTS WITH HYPERCHOLESTEROLEMIA. RELATIONSHIP WITH URINARY ISOPROSTANES

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Background. Enhanced formation of reactive oxidant species (ROS) has been observed in hypercholesterolemic patients but the underlying mechanism is still unclear. *Methods and Results.* Urinary excretion of isoprostanes, a marker of oxidative stress, platelet expression of gp91phox, the catalytic unit of NADPH oxidase, and platelet production of O2- were measured in 50 children and 50 adults with normal or high serum cholesterol. The above reported variables were also measured in 4 patients with hereditary deficiency of gp91phox. Hypercholesterolemic adults had higher platelet gp91phox expression and O2- formation compared to controls. Similar findings were obtained in hypercholesterolemic children compared to controls. Patients with hereditary deficiency of gp91phox expressed a significantly lower gp91phox on platelet surface and lower production of platelet O2- compared to healthy subjects. Adults with hypercholesterolemia had significantly higher urinary excretion of isoprostanes compared to controls. Similar findings were obtained in hypercholesterolemic children compared to controls. Patients with hereditary deficiency of gp91phox showed a significantly lower excretion of urinary isoprostanes compared to healthy subjects. Platelet expression of gp91phox significantly correlated with platelet production of O2- and urinary excretion of isoprostanes in adults and children. *Conclusions.* The study provides the first evidence that in hypercholesterolemia platelet gp91phox is up-regulated and may be responsible for enhanced production of isoprostanes.

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ASSESSMENT OF PLATELET AGGREGATION IN CORONARY ARTERY DISEASE PATIENTS BY USING DIFFERENT CONCENTRATIONS OF ADP IN COMPARISON WITH A POC SYSTEM

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Clopidogrel, daily administered in CAD patients, antagonizes platelet surface P2Y12 receptors for ADP, inhibiting platelet aggregation. Light transmission aggregation (LTA) induced by ADP is the reference method to assess residual platelet reactivity (RPR) in clopidogrel-treated patients. A point-of-care (POC) device, VerifyNow system, has been suggested as a rapid tool to identify patients who present RPR. Aim of this study was to compare LTA induced by 10 and 20 μmol/L ADP with VerifyNow P2Y12 Assay tests (expressed as P2Y12 Reaction Units - PRU-), in 180 CAD receiving dual antiplatelet therapy. The extent of LTA was defined by the maximal (peak) and late percentage of aggregation. Treated patients were considered to have a RPR when their platelets showed an aggregation peak and late >70% and a PRU value >264. The prevalence of patients with RPR was: 24.9% (peak), 24.8% (late) and 29.3% (peak), 28.5% (late) with 10 and 20 μmol/L ADP-LTA respectively; 26.3% with VerifyNow P2Y12 assay. Significant correlations between 10 and 20 micromol/L ADP-LTA values were observed [rho=0.90 (peak), rho=0.91 (late), both $p<0.001$]. LTA induced by 10 μmol/L ADP and VerifyNow P2Y12 assay showed a significant correlation [rho=0.68 (peak), and rho=0.67 (late), $p<0.001$], with significant agreements of k=0.45 for peak and k=0.35 for late, $p<0.001$. Similar relationships were observed between 20 μmol/L ADP LTA and VerifyNow system [rho=0.67 (peak), and rho=0.65 (late), $p<0.001$], with significant agreements of k=0.41 for peak and k=0.31 for late, $p<0.001$. Concerning the agreement between the two methods, similar behaviour was found both between 10 and 20 micromol/L ADP LTA and POC system.

P165

LOW-DOSE ASPIRIN THERAPY AFTER KIDNEY TRANSPLANTATION

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Aspirin therapy has been found to be beneficial after myocardial infarction even in patient who have chronic kidney disease (CKD); in addition aspirin therapy was demonstrated to be associated with improved allograft function and survival. Current guidelines recommend the use of aspirin for primary and secondary prevention in renal transplant recipients (RTR) with ischemic heart disease, diabetes or other high risk factors for cardiovascular disease (CVD) such as hypertension, obesity, smoking habitus. The aim of our study was to evaluate residual platelet reactivity (RPR) by using the light transmission aggregometry (LTA) in 102 patients stable at least one year after kidney transplantation. All patients (67 M, 35 F, ,mean age 50.5±11.9 years) were on aspirin therapy with low dosage (100 mg/d). Aggregation studies were performed by an APACT-4 aggregometer Labitec (Germany), arachidonic acid (AA) 1 mmol/L final concentration- was used as agonist. RPR was found in 38/102 patients (37%). RPR was more frequently found in patients with impaired allograft function (Creatinine plasma levels >1.5 mg/dl) respect to patients with normal function (48% vs 27% respectively; $p=0.05$). Our data indicate the association between impaired allograft function and reduced inhibition of platelet aggregability by ASA. The persistence of platelet hyperactivity in relation to allograft function may play a role in the CVD risk in these patients.

P166

POINT OF CARE METHODS TO EVALUATE RESIDUAL PLATELET REACTIVITY IN HIGH RISK PATIENTS WITH ISCHEMIC HEART DISEASE

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Inhibitory effect of platelet function by dual antiplatelet therapy in patients undergoing percutaneous coronary intervention (PCI) is not consistent and the presence of residual platelet reactivity (RPR), measured by light transmission aggregometry (LTA), in acute coronary syndrome (ACS) patients is associated with a higher prevalence of clinical adverse events. Point-of-care (POC) methods such as impedance platelet aggregometry (IPA) in whole blood by using a new device (Multiple electrode platelet aggregometry - Multiplate analyzer, DASIT, Milan, Italy), and PFA-100 system (Dade Behring, Milan Italy) have been suggested as rapid tools to evaluate RPR. We compared LTA with Multiplate IPA by using 2 and 10 μmol/ L ADP, 1 mmol/ L arachidonic acid (AA) and 2 micrograms/mL collagen and PFA-100 Closure Times by collagen/epinephrine (CEPI CT) in 95 ACS patients undergoing PCI receiving dual antiplatelet therapy and in 15 control subjects. Aggregation with Multiplate IPA was quantified as area under the curve of arbitrary units (AU*min). Good significant correlations between LTA and IPA were observed for 2 and 10 μmol/L ADP (rho=0.73, and rho=0.72, respectively, $p<0.0001$). Moderate but significant correlations were observed between LTA and Multiplate IPA (1 mmol/L AA: rho=0.37; 2 μg/mL collagen: rho=0.59, $p<0.0001$ respectively) and between LTA and PFA-100 CEPI CT (rho=-0.63, $p<0.0001$ for AA and collagen). Between the two POC systems significant correlations were observed (1 mmol/L AA: rho=-0.38; 2 micrograms/mL Coll: rho=-0.43, $p<0.0001$ respectively). These preliminary results indicate that Multiplate IPA is a POC method which provides information of platelet function inhibition comparable with those obtained by LTA. Cut-off values for POC methods need to be defined for a clinical use.

P167

PLATELET AGGREGATION IN WHOLE BLOOD: SODIUM CITRATE OR HIRUDIN AS ANTICOAGULANT?

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Many efforts tend to assess platelet function in high risk patients on dual antiplatelet therapy by using a fast, standardized, and easy to use method. Recently, a method that implements the principle of impedance platelet aggregometry (IPA) in whole blood by using a new device to measure platelet aggregation (Multiple electrode platelet aggregometry - Multiplate analyzer, DASIT, Milan, Italy), has been developed. Aim of this study was to evaluate the influence of different anticoagulants on platelet function in patients on dual antiplatelet therapy and in controls by using Multiplate IPA. Blood samples from 68 patients were withdrawn and from 10 healthy subjects by using as anticoagulants both 3.2% sodium citrate (1/10, V/V) and hirudin (25 μg/mL). As agonists 10 micromol/L adenosine diphosphate (ADP), 1 mmol/ L arachidonic acid (AA) and 2 μg/mL collagen were used. Results of Multiplate IPA are showed in Table 1.

Table 1. Multiplate impedance platelet aggregation by using the two anticoagulants.

	*Citrate whole blood controls (AU*min)*	*Hirudin whole blood controls (AU*min)*	*Citrate whole blood patients (AU*min)*	*Hirudin whole patients blood (AU*min)*
10 μmol/L ADP-IPA	70.4±25.2	73.8±3	23.1±16.1	16.1±17.3
1 mmol/L AA- IPA	43.0±20.6	43.0±20.6	11.0±12.1*	13.7±18.9
2 μg/mL Coll- IPA	67.5±16.5	70.1±23.3	25.2±19.2	24.8±23.6

$p<0.05$ vs. hirudin.

Statistical difference was found only between the two anticoagulants in ACS patients for 1 mmol/ L AA-IPA Significant correlations between citrate and hirudin (all inducers: at least r=0.82, $p<0.0001$ for all) were found. These data show a good correlation between the two anticoagulants used. Further clinical studies are needed to assess the normal range value of whole blood aggregation obtained with both anticoagulants. Multiplate impedance platelet aggregation by using the two anticoagulants.

P168

EFFECT ON LIGHT TRANSMITTANCE AGGREGATION OF ADJUSTMENT OF PLATELET COUNT IN PLATELET-RICH PLASMA IN PATIENTS ON DUAL ANTIPLATELET THERAPY

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Standardization of light transmission aggregation (LTA) requires many challenges in ensuring accurate results. Previous study reported controversy concerning platelet count adjustment by mixing platelet-rich plasma (PRP) with autologous platelet-poor plasma (PPP) to achieve platelet counts between 200,000 and 300,000 plt/μL. Mixing procedure itself could influence aggregation results and PPP may contain substances affecting platelet function. Aim of this study was to compare LTA in 10 healthy subjects and in 20 acute coronary syndrome (ACS) patients on dual antiplatelet therapy, performed in non-adjusted (400,000-700,000 plt/μL) and adjusted (200,000-300,000 plt/μL) platelet count PRP. LTA was assessed by using arachidonic acid (1 mmol/L AA), adenosine diphosphate (2 and 10 μmol/L ADP) and collagen (2 μg/mL) as agonists. In healthy subjects no difference was found in the overall platelet aggregation response before and after dilutions by all inducers. In ACS patients 2 and 10 μmol/L ADP-LTA and 2 microg/mL collagen-LTA performed in adjusted PRP samples (200,000-300,000 plt/μL) showed a significant decrease respect to non-adjusted PRP samples: 2 and 10 micromol/L ADP-LTA moving from 41±18% to 32±13%, ($p=0.02$) and from 62±20% to 52±15%, ($p=0.05$), respectively; 2 μg/mL collagen-LTA moving from 45±20% to 30±16% ($p=0.02$). No difference was observed in 1 mmol/L AA-LTA between adjusted and non-adjusted PRP samples. Present findings indicate that the activating PPP plasma effect is no detectable when LTA was performed in PRP from ACS patient on antiplatelet therapy.

Hemophilia, and other Hemorrhagic Disorders

P169

INHERITED BLEEDING DISORDERS: THE EMILIA ROMAGNA REGION REGISTRY, 2007 UPDATE

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Since 2003 the Health Authority of Emilia-Romagna Region (RER), a northern Italy region with 4,5 millions of inhabitants, founded a completely web-based registry designed to monitor epidemiology and improve the quality of health care. The 8 Haemophilia Centres (HC) of the RER adopted a computerized clinical record (EmocardÒ) and the Centre of Parma extracted, processed and published relevant data in a dedicated web-site (www.registroemofiliarer.it), in respect of Italian privacy law. Since 2006 a new web-based clinical record (xl'EmofiliaÒ) was built and adopted by all HC; it shares database with registry, so data extraction is totally automated and in *real time*. Great efforts are made to ensure high quality of data collection. At December 2007 data on 672 individuals are included in this registry: 259 have haemophilia A(HA), 68 haemophilia B(HB), 192 von Willebrand's disease(vWD), 93 rare bleeding disorders, 7 platelet disorders and 53 carriers of haemophilia. 375 patients have been screened for mutations: 228 were genotyped. In 2006 among 204 patients tested for inhibitors, 14 were found to be affected: 13 severe HA while 1 moderate HA. On 2007 3 children were in ITI; 4 HA, 1HB and 1 FXIII defeciency were in primary prophylaxis; 41 HA, 9 HB 1 vWD and 2 FXIII deficiency were in secondary prophylaxis. Compared to 2003 there was an increase of enrolled patients and more bleeding episodes and concentrates consumption recorded. The RER Registry can improve the quality of care by means of detailed epidemiological reporting, quality of assistance monitoring and analyzing the quality of life and treatment costs.

P170

INCREASED THROMBIN GENERATION IN SEVERE HEMOPHILIACS WITH MILD CLINICAL PHENOTYPE

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Introduction. Some severe hemophiliacs (FVIII/FIX<1%) exhibit a mild bleeding tendency, but the basis for this clinical heterogeneity is poorly understood. This study investigated the relationship between the values of endogenous thrombin potential (ETP) and clinical phenotype in severe hemophiliacs. The impact of FVIII/FIX gene mutations and thrombophilic polymorphisms was also evaluated.

Table 1.

	SB (#22)	*IB (#28)*	*MB (#22)*	*p*
Age (yr)	38 (21-76)	38 (23-62)	32 (22-73)	NS
Age 1st bleed (yr)	1 (0-4)	2 (0-6)	3 (1-10)	<0.005
Bleeding episodes/yr	36 (25-60)	10 (3-20)	0 (0-2)	<0.0005
Factor use (IU/Kg/yr)	2207 (2040-8696)	1068 (207-2400)	60 (25-487)	<0.0005
Clinical score	18 (10-35)	10 (0-34)	3 (0-17)	<0.005
Petterson score	44 (14-62)	28 (0-48)	17 (3-40)	<0.0005
Null mutations (%)	59	70	6	<0.005
PTG20210A (%)	0	7	5	NS
FV Leiden (%)	5	7	0	NS
Median ETP (nM)	414	478	850	<0.05

Methods. severe hemophiliacs older than 18 years without inhibitor history and treated on demand were eligible. Mild bleeders (MB) and severe bleeders (SB) were defined as follows: spontaneous bleeding episodes per year <2 (MB) or >25 (SB) and concentrate consumption <500 (MB) or >2000 (SB) IU/Kg/year. Patients who did not fit these criteria were considered as intermediate bleeders (IB). FVIII was measured by chromogenic assay and ETP was measured in platelet-rich plasma after addition of tissue factor. *Results.* 22MB, 22SB and 28IB were enrolled. MB had lower clinical and radiological scores when compared with both IB and SB (p<0.005). MB showed an older age at first bleed compared to SB (p<0.005) and p for trend among the 3 groups was also significant (p<0.05). The prevalence of severe FVIII/FIX gene defects (null mutations) was lower and ETP values were higher in MB compared with both IB and SB (p<0.05). *Conclusions.* our results indicate an extremely low prevalence of null mutations in severe hemophiliacs with mild bleeding diathesis. The measurement of thrombin generation in platelet-rich plasma may allow to identify this subgroup of patients, not otherwise distinguishable by conventional functional assays.

P171

MARKERS OF ANGIOGENESIS IN PATIENTS WITH VON WILLEBRAND DISEASE

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Background and Objectives. Von Willebrand disease (VWD) is an inherited bleeding disorder characterized by a deficiency or abnormality of von Willebrand factor (VWF), the multimeric adhesive glycoprotein that plays a major role in platelet-adhesion and platelet-platelet interactions under high-shear stress conditions. VWF is synthetized in endothelium and megakaryocytes: it circulates in plasma and is present in subendothelium and platetets. Cross-talks between vascular endothelium and hematopoietic stem cells are important for the organization of the bone marrow niche. Moreover, endothelial progenitor cells (EPCs) and circulating endothelial cells (CECs) can be considered markers of vessel wall formation and damage. VWF defects may therefore play a role at both bone marrow and peripheral blood sites. To explore the role of VWF as marker of angiogenesis, we have evaluated the number of EPCs, CECs and various serum cytokines in a cohort of 72 patients with different forms of VWD. *Patients and Methods.* Seventy-two VWD patients were diagnosed according to recommendations of the Scientific Standardization Committee on VWF of the ISTH. CECs ($CD146^+$, $CD31^+$, $CD45^-$) and EPCs ($CD34^+$, $CD133^+$, $CD45^-$) were evaluated by flow cytometry. Serum VEGF, TPO, sCD62E and EPO were determined by ELISA. *Results.* VWD cohort was composed by the following VWD types: 1 (n=22), 2A (n=10), 2B (n=19), 2M (n=15), 3 (n=6). Additionally we examined 20 healthy controls. VWD population had higher CECs (p=0.0008) and lower EPCs (p=0.001) than normal controls. Moreover VWD patients had high VEGF (p=0.003), sCD62E (p=0.001) and EPO (p=0.0006) serum levels, while they showed normal TPO concentration. As far as VWD types, type 1 VWD patients were characterized by slightly high CECs (p=0.03), normal sCD62E and TPO, markedly low EPCs (p=0.0005) and high VEGF (p=0.03); VWD type 2A and 2M grouped together showed high CECs (p=0.03) and low EPCs (p=0.007) as VWD type 1; high VEGF (p=0.006), TPO (p=0.04) and markedly higher sCD62E (p<0.0001). Conversely, VWD type 2B by high CECs (p=0.0002), normal EPCs and elevated sCD62E (p=0.002). *Conclusions.* VWD patients showed abnormal levels of EPCs, high VEGF and EPO. VWD types characterized by reduced platelet-VWF interactions (Type 1, 2A and 2M) seem to involve bone marrow progenitors as shown by a marked decrease of EPCs. Conversely, VWD 2B with enhanced interaction with platelet glycoproteins seem to involve primary the vascular cell compartments, as shown by increased CECs.

P172

THE COAGULOPATHY OF CIRRHOSIS ASSESSED BY THROMBOELASTOGRAPHY AND ITS CORRELATION WITH CONVENTIONAL COAGULATION PARAMETERS

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Background. Thromboelastography allows continuous registration of the blood viscoelastic changes upon activation by cephaline or tissue-factor in the presence of calcium-chloride. The technique is used as a near-patient-testing devise to guide transfusion in patients undergoing cardiac surgery or liver transplantation and much less to investigate hemostasis in comparison with traditional coagulation tests in other clinical conditions. *Aim.* We report on the investigation of stable cirrhosis in comparison with conventional coagulation parameters. The thromboelastographic parameters investigated were the coagulation-time (CT), clot-formation-time (CFT) and maximum-clot- firmness (MCF). *Results.* Relatively few patients [14/51 (27%)] were identified as abnormal by the CT; in contrast, a greater proportion were identified as abnormal by the CFT [41/51 (80%)] or MCF [39/51 (76%)]. Both CFT and MCF were correlated with the platelet count, antithrombin and fibrinogen. PT-ratio was correlated with CFT and MCF. None of the coagulation parameters (except PT-ratio) were correlated with CT. The correlation of the Child-Pugh-score versus MCF or PT-ratio was 0.457 (p<0.001) or 0.484 (p<0.001), suggesting that MCF could be a suitable prognostic index. CFT and MCF, but not CT, obtained ROC curves that were useful to distinguish patients from controls. *Conclusions.* Thromboelastography, currently used to investigate cirrhosis during liver transplantation might also be suitable for investigating stable cirrhosis. CFT and MCF, but not CT are the most interesting parameters to consider. However, the role played by this laboratory tool as well as the advantages that thromboelastography may have over conventional coagulation in the setting of stable cirrhosis should be investigated by appropriate clinical trials.

P0173

RECURRENT MUTATIONS IDENTIFIED ON LMAN1 AND COAGULATION FACTORS VII, X, XIII GENES

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Rare Bleeding Disorders (RBDs) as Factor (F)V, FV+FVIII, FVII, FX, FXI and FXIII deficiencies, afibrinogenemia and hypoprothrombinemia, are transmitted as autosomal recessive traits. RBDs are relatively rare in Europe (1:0.5-2 millions), but their frequency is increasing due to the high rate of immigration from the Middle East and North Africa where the incidence is significantly higher. In 2004 an International RBDs Database (RBDD: www.rbdd.org), structured to collect and to report phenotype, genotype, clinical and therapeutic information on each single disorder, was developed. This database contains data on 310 not related patients from all over of the world. Of them, 250 have been genetically characterized, with only 6% (16 patients) lacking a gene mutation in the coding region and 400bp of 5'-3'UTR. Out of 167 identified mutations, 52% (86) were missense, 12% (21) nonsense, 12% (20) splicing site, 21% (35) insertion/deletion and 3% (5) were located at the 5'UTR. A careful analysis of RBDD showed common mutations specific to some geographical areas: -p.Gln160Arg (originally reported as Gln100Arg) mutation on FVII gene was confirmed to be present only in Europeans, being found only in Italians (5 families out of 18, 28%) and in one Swedish although 59 families coming from different countries (Table 1) were characterized; -p.Arg40Thr(Arg-1Thr) mutation, on FX gene, was found only in Iranians (4 families out of 21, 19%), although 31 families coming from different countries (Table 1) were characterized; -p.Met1Thr mutation on LMAN1 gene was confirmed to be present only in Italians (4 families out of 7, 57%), although 25 families coming from different countries (Table 1) were characterized; -p.Gly216Arg (Gly215Arg) and p.Arg78His(Arg77His) mutations on FXIII gene, were found respectively only in Serbians (100% of studied families) and in Iranians (6 families out of 18, 33%), although 28 families coming from different countries (Table 1) were characterized. The haplotype analysis in future will help to explain more on each genetic mutation distribution in different ethnic groups and eventual *founder* effect. Our results suggest the existence of recurrent mutations in specific geographic areas which could help for prevention of these disorders through prenatal diagnosis in families with already one severe affected child, particularly in those countries with low economic resources.

Table 1.

GENE	Recurrent mutations	N° characterized families	Countries	N° families in which the mutation was found and country of origin	% of families from the same geographical region with the same mutation
FVII	p.Gln160Arg (Gln100Arg)	18	Italy	5 Italians	28%
		24	Iran	1 Swedish	-
		4	Turkey		
		1	Sweden		
		1	Hong Kong		
		3	India		
		3	Serbia		
		1	Greece		
		1	Pakistan		
		1	Romania		
		1	Arabia		
		1	USA		
FX	p.Arg40Thr (Arg-1Thr)	5	Italy	4 Iranians	19%
		21	Iran		
		2	Turkey		
		1	France		
		1	India		
		1	UK		
LMAN1	p.Met1Thr	7	Italy	4 Italians	57%
		2	Iran		
		2	Turkey		
		13	India		
		1	Serbia		
FXIII	p.Gly216Arg (Gly215Arg)	3	Italy	3 Serbians	100%
		18	Iran		
		2	Lebanon		
	p.Arg78His (Arg77His)	1	India	6 Iranians	32%
		3	Serbia		
		1	Greece		

P174

RANDOMISED, OPEN, PROSPECTIVE, MULTICENTER PILOT STUDY TO EVALUATE THE EFFICACY AND SAFETY OF ACTIVATED RECOMBINANT FACTOR VIIA (NOVOSEVEN) IN ACUTE INTRACEREBRAL HAEMORRHAGE IN PATIENTS TREATED WITH ORAL ANTICOAGULANT OR ANTIPLATELET AGENTS

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Introduction. Intracerebral hemorrhage (ICH) is the deadliest form of stroke with a mortality rate between 30% and 55%, increasing to as high as 67% in patients receiving vitamin K inhibitors (VKAs). Recombinant factor VIIa (rFVIIa) has been successfully used to control ICH in patients with hemophilia or other coagulation disorders, and it has been recently shown that it limits the growth of the hematoma, potentially reduces mortality, and improves three-month functional outcome if administered within four hours of ICH in non VKAs patients. rFVIIa has been shown to promptly and safely reverse VKAs effect in healthy volunteers and patients. The aim of this study is to analyze efficacy and safety of rFVIIa in preventing haematoma growth in spontaneous ICH in high thrombotic risk patients treated with VKAs or antiplatelets. *Methods*.

This study is an open label, prospective, multicenter, pilot trial to enroll 32 patients on a competitive basis. Subject included in the trial are adult patients on VKAs or antiplatelets affected by spontaneous ICH documented by CT scan within 24 hours of symptoms onset for patients treated with VKAs and within 3 hours for patients treated with antiplatelets. Centralized randomization is obtained by phone. Randomization is stratified for ASA and OA patients. Outcome evaluation (CT scans and clinical follow up) is blinded. rFVIIa or standard therapy should be administrated within one hour from the diagnostic CT and 24 hours from symtoms onset. All the oral anticoagulants treated patients will be administered with Vit K at the dose of 10 mg iv (by slow infusion which can be repeated after 12 h), independently of the randomization group. The treatment for control group is left to the caring physician (activated prothrombin complex concentrates are suggested). The treatment group are receives a single bolus of rFVII (80 mcg/Kg). *Results*. The trial started in 2005 and 12 centres are actively enrolling. Up to date 105 patients were screened, 13 of which were included in the trial and 8 treated with rFVIIa. Three patients (1 VKAs and 2 antiplatelets) died for non treatment related events. *Conclusions.* Feasibility and overall safety of the trial are up to now provided. The study is still open to centre recruitment (study protocol available at www.clinicaltrial.gov or from iorioa@unipg.it)

P175

AUTOMATED APTT CYCLE FOR RAPID PREKALLIKREIN (PK) DEFICIENCY IDENTIFICATION

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Introduction. Very prolonged Activated Partial Thromboplastin Times (APTT) with normal level of coagulation factors, in the absence of lupus anticoagulant (LA), suggest abnormalities in the contact phase system. In Prekallikrein (PK) deficiency the severely prolonged APTT is normalized by increasing up to 20 min the preincubation time of plasma with reagent. Such long-incubation-APTT is not commonly available on automated coagulometers; furthermore, the manual tilting-tube method is not feasible in all laboratories. We report on an APTT cycle (APTT-PK) implemented on the ACL Elite coagulometer (Instrumentation Laboratory, IL) that allows the easy identification of PK deficiencies through the simultaneous determination of APTT with standard (5 min) and prolonged (20 min) preincubation times of reagent with citrated plasma. *Methods.* Blood from normal subjects and from patients with LA or PK, factor VIII, IX, XI, XII deficiency, and from patients on oral anticoagulant (OAT) or heparin therapy were collected into tubes containing 1/9 vol of 0.109 M sodium citrate. Plasma was prepared by centrifugation at 2000xg for 30 min. A lyophilized High Molecular Weight Kininogen (HMWK) deficient plasma was tested too. APTT-PK cycle, carried out on the ACL Elite using APTT SP reagent (IL), consists of the simultaneous preincubation of each sample with the activator-cephalin reagent for 5 min and as well as for 20 min before adding calcium chloride. *Results.* Representative results are shown in Figure 1 (dashed line: upper limit of the reference range).

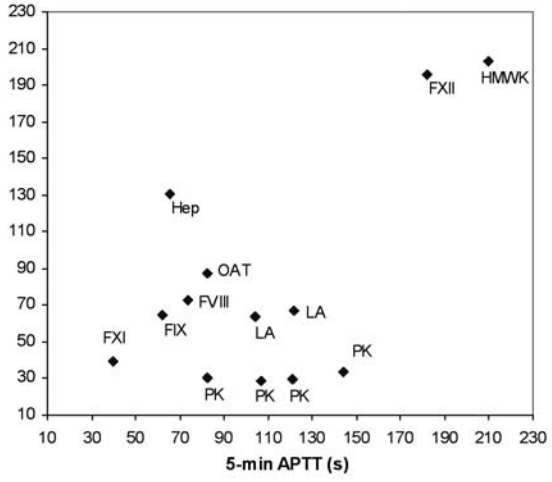

Figure 1.

The APTT-PK test showed: correction to normal of the markedly prolonged APTTs of PK deficient samples; slight shortening, although still prolonged, of APTTs in LA samples; no significant change in all congenital (FVIII, FIX, FXI, HMWK) or acquired (OAT therapy) factor deficiencies; slight prolongation of the APTTs of FXII deficient samples; marked prolongation of the APTT of the heparinized plasma. *Comments.* A simple APTT cycle, that allows the simultaneous determination of APTT with plasma recalcification after 5-min and 20-min preincubations, was implemented on the automated coagulometer ACL Elite. Normalization of prolonged APTTs with 20-min preincubation time was specific for PK deficient plasmas. Neither intrinsic factors deficient plasmas nor LA plasmas showed the same behaviour. This modified APTT cycle could represent a rapid, easy and specific screening test for PK deficiency in the workup of severely prolonged APTTs.

P176

INACCURACY IN APTT DETERMINATION DUE TO SAMPLE CARRYOVER

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Introduction. Activated Partial Thromboplastin Time (APTT) is a screening test that is prolonged in coagulation factor deficiencies or in the presence of acquired inhibitors like lupus anticoagulant (LA). It is also used to monitor heparin and replacement therapies. Accuracy and reproducibility of results are essential for patients clinical management. We report on a relevant sample carryover (CO) that caused inaccurate results in APTT determination on ACL coagulometer. *Methods.* Blood, collected into vacuum tubes containing 0.109 M sodium citrate (Becton Dickinson), was centrifuged at 2000 x g for 10 min. APTT determination was carried out in duplicate on 1028 consecutive plasma samples using ACL 7000 (Instrumentation Laboratory - IL) and APTT SP reagent (IL). Carryover, evidenced by discrepant duplicate results (difference >10%), was investigated by performing APTT with the sample scheme "sensitive/contaminant/sensitive" (one replicate each), and increasing up to three the number of the washing cycles for the sample needle during plasma loading. CO% calculation: [(APTT2–APTT1)/APTT1]*100, where APTT1 is the basal value of the sensitive sample tested before the contaminant plasma and APTT2 is the value of the first replicate of the sensitive sample analysed just after the contaminating plasma. *Results*. Sample carryover affected APTT test in 17 cases resulting in: - shorter than expected coagulation times for severe coagulation factors deficiency samples (11 cases): CO range=12.7-56.2%; - falsely prolonged coagulation times due to LA presence (6 cases): CO range=13.0-49.8%. Increasing the washing steps between plasma sampling from one to three reduced CO to 5.2-13.8% (factor deficiency) and 12.5-21.6% (contamination by LA). *Comments.* A significant sample carryover in APTT determination was observed on an automated coagulometer. Sample carryover is unpredictable in a random sequence of unknown samples and has to be considered as a possible cause of inaccurate APTT results. The heaviest and clinically most relevant CO effect in APTT was due to contamination by LA plasmas that was not corrected by sample-needle extra-washing and was striclty related to the sample own characteristics. APTT single determination on ACL coagulometers demands the definition of appropriate operating conditions and a careful validation procedure of results to ensure correct diagnosis and proper clinical management.

P177

ANTIVIRAL TREATMENT FOR CHRONIC HEPATITIS IN PATIENTS HIV-INFECTED

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Introduction. Up to 1985, hemophilia patients treated with non-virus-inactivated concentrates were prone to virus infection. According to the Italian Registry of Congenital Coagulopathies, at least 7% of hemophilia patients shows HIV-HCV co-infection. Concerns about increased hemorrhagic risk refrained from use in these patients of HCV antiviral therapies shown to be effective in non-HIV non-hemophilia patients. Aim of this systematic review is to investigate efficacy and safety of HCV antiviral treatment in HCV-HIV coinfected patients. *Methods.* The electronic searches included the Cochrane Library, the Cochrane Hepato-Biliary Group Controlled Trials Register, MEDLINE, and EMBASE. Two reviewers extracted data independently. The primary efficacy out-

come was the 6 months sustained virological response (S-VR). Trials comparing similar treatment regimens were combined in random effect meta-analyses. Intention-to-treat analyses including all patients randomised were performed. Results are presented as relative risks (RR) with 95% confidence intervals (CI) in different groups of treatment. Safety outcomes were mortality, withdrawals (W), total adverse events (T-AE). *Results.* Among the 83 references identified in the literature search, we identified 13 RCT that fulfilled our inclusion criteria. Efficacy outcomes showed advantage of combination therapy, use of PEG interferon and high-dose regimens (overall, RR ranged between 1.76 to 3.36). The most frequently used regimens were 180 mcg or 1.5 mg/kg/oiw for PEG, 3 milion U/tiw for interferon, and 800 mg/oid for RIBA for 24 or 48 w depending on the HCV genotype. In 4 trials, 1214 treatment naive patients were randomised to PEG plus RIBA vs interferon plus RIBA. The proportion of patients with SV-R was significantly higher among patients randomised to PEG (36% vs 15%, RR 2.35, 95% CI 1.41 to 3.94). There was no significant difference in mortality (RR 2.11, 95% CI 0.72 to 6.18). No significant difference was found in the number of W and T-AE, while PEG increased the risk of anaemia or flu-like symptoms. Three trials compared PEG plus RIBA vs PEG alone in 786 patients that were treatment naive or did not have a VR after 3 months treatment (33% versus 16%, RR 2.04, 95% CI 1.58 to 2.65). *Conclusions.* Peginterferon plus ribavirin should be considered the treatment of choice for HIV/HCV coinfected patients. The risk of T is considerable and require careful monitoring.

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THE GOOD USE OF PLASMA. A CRITICAL ANALYSIS OF FIVE INTERNATIONAL GUIDELINES

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Background. The clinical use of fresh-frozen plasma (FFP) is progressively increasing both nationally and internationally, despite the fact that many studies have shown the weaknesses of the indications for its use. Guidelines on the good use of plasma have been adopted in various countries. The aim of the present study was to analyse some of the existing guidelines on the good use of plasma, applying a scientifically validated method. *Methods.* A bibliographic search (1990-2006) was conducted in databases, websites, and the archives of scientific societies. The references were blindly screened in duplicate and relevant articles were recovered in full. The selected guidelines were evaluated using the AGREE instrument, which assesses the completeness and structural quality of the guidelines and, in some aspects, the contents of the recommendations. The project, co-ordinated by the Regional Centre for Co-ordination and Compensation (CRCC) and carried out by the four Services of Immunohaematology and Transfusion (SIT) in Umbria, was funded by the Region of Umbria and approved by the 4 health care institutions involved.

Table 1.

Domain	Item	Guidelines				
		British committee for standards in Haematology	*Agence Française de securité sanitaire de produits de sante*[20]	*Australasian society of blood transfusion*[19]	*Canadian members of the expert working group*	*American society of anesthesiologists*[18]
		2004	*2002*	*2001*	*1997*	*1996*
Scope and purpose	1-3	87%	78%	61%	80%	78%
Stakeholder involvement	4-7	25%	36%	69%	58%	40%
Rigour of development	8-14	38%	56%	45%	75%	62%
Clarity and presentation	15-18	67%	74%	81%	75%	63%
Applicability	19-21	31%	33%	50%	36%	39%
Editorial indipendence	22-23	10%	7%	22%	47%	38%
Overall assessment		(2)	(2)	(2)	(2)	(3)

Results. The bibliographic search yielded 3067 abstracts of which 239 were considered relevant. The analysis of these led to the recovery of 11 guidelines, among which five were selected: those from the British Committee for Standards in Haematology, the Agence Française de Securité Sanitaire de Produits de Sante, the Canadian Members of the Expert Working Group, the American Society of Anesthesiologists Task Force on Blood Component Therapy and the National Health and Medical Research Council (NHMRC)/Australasian Society of Blood Transfusion. The overall evaluation is shown in the Table 1. The standardised domain-specific score was obtained using the formula suggested in the AGREE instrument. For each domain, the guideline that was assigned the highest score is shown in bold; scores above 70% are underlined. The overall assessment score was assigned by formal consensus in response to the question: "Would you recommend the use of this guideline in clinical practice?": (1) Strongly; (2) I would; (3) No; (4) Not sure. *Conclusions.* None of the guidelines analysed obtained a score higher than 50% in all the domains of the AGREE score. There was no evidence of a tendency to improvement over time in the guidelines analysed. Objective evaluation of the guidelines analysed could provide the starting point for the subsequent production of similar documents.

P179

SUCCESSFUL OUTCOME OF AORTIC VALVE REPLACEMENT IN A PATIENT WITH NIEMANN-PICK DISEASE

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Niemann-Pick disease (NPD) is an inherited disorder characterised by accumulation of sphyngomielin within cells of all major organs due to deficiency of lysosomial hydrolase and sphyngomielinase. A haemostasis defect has not been described in NPD. We here report the case of a 34 year old patient presenting to our center with severe aortic stenosis with moderate degree regurgitation. Her functional class was NYHA III and she was complaining of shortness of breath for any kind of exertion. She was diagnosed in childhood with type B NPD. Her echocardiography showed a maximum transvalvular gradient of 93 mmHg and an aortic valve area of 0.79 cm^2. The left ventricular diameter in diastole was 5.4 cm with a 65% ejection fraction, and there was no pulmonary hypertension. Clinical evaluation showed a multi-system involvement by the NPD, with severe hepato-splenomegaly, diffuse interstitial lung disease and mild renal impairment. She also showed a major haemostatic disorder involving both platelets and the plasma components. She had a prolonged pro-thrombin (INR 1.8) and partially activated thromboplastin times (ratio 1.6), thrombocytopenia (99,000/mcl), and moderate level reduction in plasma levels of coagulation factors II, V, VII, IX, X, XI, XII. Her bleeding time was also prolonged (>15 minutes). Platelet aggregation was also impaired as shown by hypo-reactivity to ADP, arachidonate, collagen and epinephrine, while the platelet fluorocytometric analysis did not show significant alterations in the expression of the platelet membrane glycoproteins. Despite the bleeding diathesis, because of the severe heart failure, the patient was scheduled for aortic valve replacement with a porcine St. Jude bioprosthesis. The therapeutic protocol performed was as follows: since 12 hours before surgery, fresh-frozen plasma was administered at the dose of 20 ml/kg as continuous infusion; immediately before surgery, 1 unit/10 kg body weight platelet concentrates were given; at the end of the extracorporeal circulation and after 2 hours, activated eptacog alfa was administered at the dose of 70 mcg/kg as an iv bolus. With this protocol, an excellent control of bleeding was achieved. Indeed, patient post-operative course was uncomplicated without any sign of bleeding. No anticoagulation was given during the first 3 months after valve placement.

P180

ACQUIRED HEMOPHILIA SYNDROME: DIFFERENT CLINICAL OUTCOMES IN DIFFERENT IMMUNOSUPPRESSIVE APPROACHES

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Background. Acquired hemophilia syndrome (AHS) is characterized by the sudden onset of bleeding spontaneous or provoked by surgery or trauma. Its evolution is severe and sometimes fatal. The AHS incidence is about 1.6 per 1×10^6 subjects/year. The autoimmune nature is confirmed by the presence of serum specific autoantibodies directed against functional epitopes of factor VIII. AHS is frequently idiopathic (50% of the cases) but several clinical conditions might be associated to: autoimmune disorders, pregnancy and post-partum, malignancies or drug

hypersensitivity. *Aim.* to evaluate, by clinical and laboratory assessment, the follow-up and clinical outcome in patients with AHS who were treated with immunosuppressive therapy. *Methods.* in an observation period between March 1995 and April 2008 diagnosis of idiopathic AHS was established in two elderly patients (76-years old woman; 83-years old man) and in a 18-year old woman. *Results.* for all patients clinical findings at initial presentation were multiple and large ecchymoses; in the young patient, a spontaneous left ileo-psoas muscle haematoma was also present. The last two patients referred diffuse arthralgias and one of these had a clinical history of atraumatic hemarthrosis and thrombocytopenia. All patients were severly anaemic at diagnosis (two cases needed RBC transfusion. Activated partial thromboplastin time (APTT) was prolonged; the Factor VIII levels was <1% in all cases; the inhibitor titre was 32 and 13 BU in both old patients and 474 BU in the young patient. Only in young subject hemostatic therapy was started using rVIIa (Novoseven). At the first line, the immunosuppressive therapeutic cycle was established for all subjects administering prednisolone ranged to 1-2 mg/Kg/day gradually tapered in the following weeks. The old patients assumed cyclophosphamide too at 50 mg/day (man) and 50 mg/day, 5 days a week (woman). In the following months a reduction of immunosuppressor agents was made in regard to clinical and laboratory improvement. In the young patient the suspension of initial steroid therapy, after the Factor VIII inhibitor disappearance, provoked a gluteus and piriform muscles haematoma that required an immediate treatment with: i.v. IgG (30 g/day, 4 days a month), methylprednisolone (4 mg/day) and azathioprine (50 mg/day, 10 days a month). During the immunosuppressive monthly cycles, fluctuations of the inhibitor occurred, and patient experienced a relapse of gluteus haematoma. For this reason, a continuous treatment with azathioprine (50 mg/day, 4 days a week) combined with methylprednisolone (4 mg/day) was attempted. In the elderly woman the suspension of cyclophosphamide and steroid therapy, after the Factor VIII inhibitor disappearance, induced the onset of a severe picture of epistaxis, subglottic oedema with inspiratory dyspnoea. The patient attended the emergency department and therapy with Novoseven and antihemorrhagic treatment with tranexamic acid was started. The Factor VIII was <1 and the inhibitor titre was elevated. The cyclophosphamide (50 mg/day) and prednisolone (30 mg/day) therapy was restored and prolonged in the following months with a gradual reduction. The last patient followed a low-dose immunosuppressive therapy with a complete control of symptoms and coagulation factors. *Outcome.* Actually, the both elderly patients follow a maintenance immunosuppressive therapy with cyclophosphamide (50 mg/day, 3 days a week) with an optimal control of AHS and no side-effects. In young patient the immunosuppressive therapy (methylprednisolone and azathioprine) was stopped after a long-term treatment (4 years), despite the normalisation of the Factor VIII and its inhibitor occurred after only six months. She is in complete remission from 1999. *Discussion and Conclusions.* Immunosuppressive therapy is indicated for idiopathic, autoimmune and malignancy-related AHS pictures. The three AHS cases reported here demonstrate that a short-course therapy with immunosuppressive drugs is not indicated for the treatment of this syndrome. In fact, only a long-term protocol allowed a complete remission in young patient and an optimal control of disease in the last patients. The idiopathic AHS in the young patient represents an unique case because it rose up in absence of any predisposing factor. The juvenile and fertile age of young patient imposed the choice of azathioprine as suppressive agent instead of cyclophosphamide.

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RETROSPECTIVE EVALUATION OF DYSFIBRINOGENAEMIC PATIENTS AT A SINGLE CENTER: CLINICAL FEATURES AND LABORATORY FINDINGS

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Background. Dysfibrinogenaemia is a very rare bleeding disorder in which the clinical phenotype is unpredictable. The literature consists predominantly of collections of case reports. A relatively recent compilation of over 250 patients, revealed that 53% were asymptomatic, 26% had haemorrhage and 21% had thrombosis, some of whom also had haemorrhage. *Aims.* To retrospectively investigate clinical features and laboratory findings of our dysfibrinogenaemic patients and to compare our data to literature's. *Methods.* Over the last ten years, 17 dysfibrinogenaemic patients were diagnosed at our centre [7 males, 10 females, median age at diagnosis 46.6 years (6.1-80.9), 8 families]. The reasons for admission were: finding of a reduction of fibrinogen activity in 12 patients, familial study in 5. Laboratory findings are shown in the accompanying Table 1. *Results.* Ten/17 (59%) patients experienced hemorrhagic symptoms, mostly mild: traumatic cutaneous bleedings in 7 patients; gastro-intestinal bleeding in 2; epistaxis in 3; gum bleeding in 2. One/17 patient (6%) experienced a cerebral ischemia (concomitant disease: acleisto-cardia). Twelve patients underwent surgery, eleven underwent dental extractions. Tranexamic acid was used as prophylaxis treatment of surgery in 2 patients. Only 1 patient bled after dental extraction. Eleven spontaneous deliveries and 3 cesarian sections were performed in 9 women without any prophylaxis treatment. No hemorrhagic or thrombotic complications were reported. No spontaneous abortions occurred. None of the 17 patients was transfused. *Conclusions*. We confirm that dysfibrinogenemia is a rare coagulation disorder. The prevalence of asymptomatic patients is inferior to literature data (35% vs 53%). Just 1 patient (6%) had a thrombotic event. Hemorrhagic patients (59%) experienced mostly mild symptoms. No one of them needed transfusion therapy.

Table 1.

Patients	*Fibrinogen activity/antigen (mg/dL)*	*PT ratio (0.90-1.14)*	*PTT ratio (0.92-1.16)*
1	112/380	1.07	1.1
2	Undetectable/250	1.3	1.2
3	36/192	1.2	1.3
4	66/250	1.17	1.12
5	52/140	1.2	1.06
6	57/140	1.1	1.02
7	62/380	1.16	0.9
8	70/250	1.04	1.08
9	50/470	1.3	0.9
10	98/226	0.9	1.09
11	Undetectable/195	1.14	1.1
12	Undetectable/250	1.2	1.04
13	Undetectable/250	1.09	1.32
14	Undetectable/250	1.2	1.06
15	56/192	1.2	0.9
16	Undetectable/215	1.28	1.04
17	Undetectable/400	1.28	1.15

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CLINICAL AUDIT OF THE USE OF FRESH FROZEN PLASMA IN UMBRIA: STUDY DESIGN AND RESULTS OF THE PILOT PHASE

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Background. Fresh frozen plasma (FFP) is considered as elective treatment in the following conditions: acute hemorrhage in patients with abnormal clotting tests or disseminated intravascular coagulation; rare bleeding disorders when specific concentrates are not available and thrombotic thrombocytopenic purpura. However, several reports in the literature point out a high rate of inappropriate prescriptions. We report the results of a pilot clinical audit of FFP usage in Umbria. *Methods.* All the four ImmunoHematology Services (IHS) of the Umbria Region took part in the study. The requests for FFP issued in April 2006 were collected and analyzed. The clinical record forms (CRF) of the transfused patients were also evaluated. The following indicators were identified and analysed: request completeness, appropriateness for indication and dosage, CRF completeness, adverse events, mortality, efficacy (normalization of coagulation tests after FFP infusion) and the agreement between electronic database and paper request forms. All the data were extracted from the databases of the HIS, the original paper request forms and the CRF of the patients. Data analysis was performed with SPSS ver 13.0. *Results.* Two hundred-twenty-one requests (615 FFP units) referring to 109 patients (22.9% from medical, 51.4% from surgical and 25.7% from intensive care units) were collected. Ninety-three percent of the corresponding CRF were obtained and analysed. Only 50.7% of the

requests was scored as 3-4/4 for completeness (65-80% of fields filled-in). Appropriateness for indication was found to be 31.5% of evaluable requests (56.1% of the total), without any significant difference among issuing entity (medical, surgical or intensive care units). Appropriateness for dosage was found to be 62.7% of evaluable requests (62% of the total). The overall completeness of CRF reporting was around 70%. The evaluation of paired pre- and post-infusion coagulation tests showed a statistically significant correction for INR values only (from 2.4; 95% C.I. 1.75-3.05 to 1.69; 95% C.I. 1.53-1.84 p=0.02). The agreement between paper request forms and HIS databases was found to be: 99% for anagraphical data; 72.3% for dosage; 90% for coagulation tests results and for indication. *Conclusions.* The results of the clinical audit showed that major issues to manage and optimize the use of FFP were: appropriateness for indication, completeness of request filling and CRF reporting.

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THINKING TO ACQUIRED HEMOPHILIA SYNDROME: IT IS VERY RARE DISEASE? FIVE CASES IN ONE-YEAR OBSERVATION IN TWO REGIONS OF SOUTHERN IT-ALY (APULIA AND BASILICATA) FROM AD-HOC-STUDY GROUP

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Background. it is recognized that acquired hemophilia syndrome (AHS) is a very rare condition (about 1.6 per 1x106 subjects/year). *Aim.* in order to determine the prevalence of this disease, the characteristics of clinical manifestations, the first lines of hemostatic and immunosuppressive ther-apy, the possible inhibitor eradication and the follow-up, including the morbidity and mortality, we organized an ad-hoc-study-group on AHS in two regions of Southern Italy. Methods: the observation was made from February 2007 to January 2008. Diagnosis of AHS was established in five patients (p.): three very old men (aged 81, 84, 86 years) one with preneoplastic lesion, the other two idiopathic, and two women, one was 64 years old with history of SLE from 30 years, the last aged 30 years with post-partum AHS. *Results.* all the p. showed severe spontaneous musculo-skeletal and traumatic hemarthroses, one prolonged and persistent rectorrhagia and one post-partum menometrorrhagia. the Factor VIII levels ranged <1 to 7 % (mean 3.7); the inhibitor titre ranged 1 to 15 BU (mean 7.6). Each subject received RBC transfusion. In all p. but one, hemostatic therapy was started with rVIIa (Novoseven) at initial dose of 90 mcg/Kg per bolus every 2-3 hours, the treatment lasted from 1 to 3 days. The immusuppressive therapy was started immediately with prednisone ranged to 1-2 mg/Kg/die. In the p. with SLE cyclophosphamide was doubled (from 50 mg to 100 mg/die). Outcome: the immunosuppressive were stopped after the disappearance of inhibitor was achieved in two p. (one in post-partum AHS, the other in idiopathic p.); one p. died. In the last two, the immunosuppressive therapy was reduced in relation to behaviour of inhibitor. Conclusion: At present, all p., except one, are alive, without inhibitor.

P184

THROMBOELASTOMETRY IN HEART SURGERY: APPLICATIONS AND CLINICAL VALUE

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Introduction. The study, which lasted one year, was conducted on 280 (40%) of the about 700 patients who had been given myocardial revascularization surgery by either aortocoronary bypass or valve replacement with biological or mechanical prostheses. Actually, the thromboelastograph was used to assess blood clotting values of both patients at risk of hemorrhage (thrombocytopenia and platelet function anomalies) and patients with thrombophilia. *Results and Conclusions.* Thromboelastometry tracings were obtained preoperatively, intraoperatively, as well as postoperatively. What is more, thromboelastometry was resorted to in the presence of persisting bleeding immediately after surgery to detect conditions ascribable to coagulation deficit or to iatrogenic causes. In the absence of these latter thromboelastometry turned out to be crucial to decide whether to perform salvage surgery. A more rapid clinical solution, with important savings in terms of both time and costs, was obtained in all the cases under study thanks to a targeted use of either blood products or missing plasma factors. Thromboelastometry was used to monitor ten cases of HIT with positive anti-heparin/PF4 antibodies as a successful supplement to therapy as shown by its role in contributing to the positive clinical outcome of the cases under study.

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MOLECULAR BASES OF TYPE 3 VON WILLEBRAND DISEASE IN ITALY: REPORT ON 12 FAMILIES

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Type 3 von Willebrand disease (VWD) is characterized by recessive inheritance, moderate to severe bleeding history and severely reduced FVIII and von Willebrand factor (VWF) measurements. The elucidation of the molecular mechanisms underlying this subtype is of utmost importance since the risk of inhibitor development against exogenous VWF upon replacement therapy with potentially severe allergic reactions has been reported only for these patients. A large heterogeneity of the molecular basis responsible for this subtype has been reported, even though some mutations(e.g, R2305X, delC2435 in North Europe) are particularly frequent in selected geographical area. In Italy, apart from a very few patients with homozygous gene deletions associated with inhibitor occurrence, little is know on the molecular mechanisms underlying this subtype and the risk of inhibitor. In this study we report the molecular characterization of 12 families with type 3 VWD. Out of the 12 index cases enrolled, four index cases from families of South of Italy were homozygotes for a T deletion causing frameshift (c.6182 delT, 2061fs), two families were homozygotes for splice site mutations (IVS18-1 G>C and IVS46+1 G>T, and two families for stop codons (c.592 C>T Gln198X and c.3931 C>T Gln1311X). An additional patient had homozygous R854Y, with measurable FVIII/VWF levels in plasma. Compound heterozygosity was demonstrated in 3 cases: IVS13 splice site/Q77X, R365X/S1731T and delCTCTc.2016/I1343V. No inhibitor was evident in any of the investigated patients. The molecular bases of type 3 VWD in Italy shows a wide heterogeneity, apart from cases living in Campania region, in whom a common ancestor appears likely to be responsible for its high prevalence.

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SHORTER VON WILLEBRAND FACTOR SURVIVAL IDENTIFIED BY MEANS OF THE VWF PROPEPTIDE IN PATIENTS WITH TYPE 1 AND TYPE VICENZA VWD

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A shorter von Willebrand factor (VWF) survival is one of the pathogenic mechanisms behind von Willebrand disease (VWD). A reduction in the VWF propeptide to VWF:Ag ratio (VWFpp ratio) has recently been suggested to predict a shorter VWF half-life. We analyzed the VWF half-life in 22 type 1 and 14 type Vicenza VWD patients, comparing post-DDAVP VWF T1/2 elimination (T1/2 el) with the VWFpp ratio. Type Vicenza VWD patients had a considerably higher VWFpp ratio (13.02±0.49) than normal subjects (1.45±0.06), whereas the VWFpp ratio was normal (1.56±0.07) in type 1 VWD patients, except for 4 patients carrying the C1130F mutation (4.69±0.67). The VWF T1/2 el was very short (1.3±0.2 h) in type Vicenza VWD, while in all type 1 VWD patients it was similar to that of controls (11.6±1.4 h and 15.4±2.5 h, respectively), except for the patients carrying the C1130F mutation, whose VWF survival was significantly shorter (4.1±0.2 h). A significant inverse correlation was apparent between the VWFpp ratio and the VWF T1/2 el in both types of VWD patient and in normal subjects: the higher the VWFpp ratio, the lower the VWF half-life. The VWFpp ratio is therefore a simple and useful tool for assessing VWF survival, predicting a shorter VWF half-life and identifying the nature of the VWF defect in VWD patients.

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SEVERE VON WILLEBRAND DISEASE WITH INHIBITOR: SEARCHING THE BEST WAY TO TREAT

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Background. Patients (pts) with severe von Willebrand disease (vWD) and large deletion within the vWF gene are at high risk of developing precipitating alloantibodies against vWF, as a consequence of the replacement therapy, and may present severe post-transfusional anaphylactic reactions. In case of need the choice of hemostatic treatment is very hard and complex so that different therapeutic strategies often have been adopted. Experience: Over a period of 10 years (1996-2006) we experienced 8 critical bleedings, of which 3 lifethreatening events, in pts suffered from severe vWD with inhibitor: 3 dental extractions, 1 deep intra-pelvic hematoma, 1 large hematoma of hip, 1 widespread subcapsular renal hemorrhage, 1 severe persistent tonsillar bleeding, 1 very prolonged hemorrhage from the Vater's papilla. *Aim.* recombinant activated FVII (rFVIIa), recombinant F.VIII (rFVIII), plasma-derived F.VIII/vWF (p-d FVIII), antifibrinolytic drugs (tranexamic acid) and the local application of fibrin glue have been used respectively in relation to the severity and type of bleeding with the aim of obtaining the most effective clinical results. The dosage of rFVIIa ranged from 90 to 200 mcg/Kg b.w. given at 2-3-4 hour intervals and by continuous infusion (about 20 mcg/kg/h), rFVIII ranged from 50 to 100 I.U./Kg b.w. every 4-12 hours and by continuous infusion (1000 I.U./h), p-d FVIII/vWF ranged from 30 to 50 I.U./Kg b.w./8-12 h. *Results and Conclusions*. rFVIIa provided an effective and safe hemostasis in oral surgery and particularly in treating the lifethreatening hematomas which a rapid stop of bleeding needed. The continuous infusion of rFVIII proved of great help to maintain the hemostasis after the acute phase of bleeding. However in cases of failure of the combined therapy with rFVIIa and rFVIII the most effective treatment was p-dFVIII/vWF strictly monitored by an intensive care unit because of the high risk of immuno-allergic reactions. Tranexamic acid and local application of fibrin glue supported hemostasis in mucosal bleedings. Neither complications nor adverse events in all the pts occurred.

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REGRESSION OF ACQUIRED VON WILLEBRAND SYNDROME AFTER SUCCESSFUL TREATMENT OF LOW GRADE GASTRIC MALT LYMPHOMA

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Background. Acquired Von Willebrand syndrome (AVWS) is a rare bleeding disorder particularly frequent in lymphoproliferative or myeloproliferative diseases. *Clinical history*. A 48-year old woman with a previous diagnosis of Von Willebrand Disease (VWD) came to our attention in 2005 because of relapsing episodes of gastric bleeding. She had no personal history of bleeding before 2002, when muco-cutaneous symptoms occurred. Laboratory tests were as follows: aPTT ratio 1.15, PT ratio 0.90, BT >13 minutes (n.v. <9 minutes), VWF:RCo 20% (n.v. 50-150), FVIII:C 52% (n.v. 48.5-120%), VWF:Ag 34% (50-126%), CBA 19% (n.v. 50-146%), RIPA 1.4 mg/mL (n.v. 0.8-1.2 mg/mL), VWF:RCo/Ag ratio 0.6 (discrepant <0.7); the diagnosis of VWD subtype 2A was therefore confirmed. A gastric mapping endoscopy with multifocal biopsies was performed and it showed an infiltration of low grade gastric MALT lymphoma B cells, HP negative. A bone marrow biopsy showed a <10% involvement; CT scan excluded other involved sites. The patient was classified as stage IVE according to the Ann-Arbor classification of extranodal non-Hodgkin's lymphomas (NHL). The patient underwent Rituximab immunotherapy at a dose of 375 mg/m^2 once weekly for four weeks. Then, maintenance treatment with Rituximab 375 mg/m^2 every three months for four doses, was performed. *Results.* Restaging documented a complete remission. On July 2007, reassessment of VWD was performed. Laboratory tests showed these *Results.* aPTT ratio: 0.87, BT 3 minutes, VWF:RCo 106%, FVIII:C 158%, VWF:Ag 110%, VWF:RCo/Ag ratio 0.96. Data, were confirmed in October 2007: aPTT ratio 0.91, VWF:RCo 76%, FVIII:C 174 %, VWF:Ag 78%, VWF:RCo/Ag ratio 0.97. *Conclusions.* Normalization of the coagulative parameters in concomitance with a complete remission of the lymphoproliferative disorder, allowed us to reach a diagnosis of acquired VWD in association with a low grade gastric MALT lymphoma. The successful treatment of the underlying primary disease was followed by a regression of the VWD.

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POLYMORPHISMS IN GENES INVOLVED IN AUTOIMMUNE DISEASE AND THE RISK OF FVIII INHIBITORS DEVELOPMENT IN ITALIAN PATIENTS WITH HEMOPHILIA A

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Hemophilia A is a life-threatening haemorrhagic bleeding disorder that is caused by mutations in the factor VIII (FVIII) gene. About 20% of patients who receive replacement therapy with intravenous FVIII products develop neutralising antibodies (FVIII inhibitors) that inhibit the clotting activity of substituted FVIII and seriously affect health and quality of life. Both genetic and environmental factors influence the susceptibility of patients to develop inhibitors. The strongest relationship has been found with the causative FVIII gene mutation. However, several patients with high-risk mutations do not develop inhibitors and the reason for this is unknown. Therefore, it is likely that other critical genetic factors apart from the gene defect impact inhibitor expression. The objective of this study was to evaluate whether polymorphisms in different genes involved in the regulation of the immune system may confer susceptibility to inhibitor development in patients with haemophilia A. In the present study we analysed the distribution of polymorphisms in the CTLA4 (ex1 c.49A>G, p.Thr17Ala), PTPN22 (ex14 c.1858C>T, p.Arg620Trp) IL10 (promoter -1082G/A), TNFα (promoter -308 G/A), FOXP3 (ex12 c.1189C>T, p.Arg397Trp) and IRF5 (IVS1 +198G>T) genes that have been reported to be associated with a number of autoimmune disease. We focused on a cohort of Italian unrelated hemophilic patients with and without a history of inhibitors. Genotyping was carried out with standard methods for genetic analyses including RFLP, real time PCR and direct DNA sequencing.In this exploratory study our data show that, considering single nucleotide variations, genotype frequencies in patients with inhibitors were not significantly different from those observed in patients without inhibitors, suggesting a lack of association between these polymorphisms and the development of inhibitors. In particular, the results obtained for TNFα polymorphism are not consistent with previous studies where the association between the -308 A/A genotype and inhibitors has been shown in a subgroup of patients with severe haemophilia A enrolled in the Malmo International Brother Study. Inconsistencies with previous studies may depend on the different genetic background of the population examined. The development of inhibitors is a polygenic complex process that some extent may be influenced by environmental factors. Further studies may contribute to a clearer understanding of this process.

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PHARMACOKINETICS OF THE REFORMULATED B-DOMAIN DELETED RECOMBINANT FACTOR VIII CONCENTRATE USING CHROMOGENIC AND ONE-STAGE CLOTTING ASSAYS WITH POOLED NORMAL PLASMA AND REFACTO LABORATORY STANDARD

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Introduction. The use of ReFacto Laboratory Standard (RLS) in one-stage clotting assay was proposed to reduce underestimation of FVIII plasma concentration measurement after infusion of B Domain Deleted recombinant FVIII (BDD-rFVIII) in hemophilia A patients. Both BBD-rFVIII and RLS were recently reformulated. Aim of this study was to evaluate the performance of reformulated RLS in the measurement of FVIII plasma concentration after infusion of reformulated BDD-rFVIII. *Methods.* Haemophilia A patients volunteering for the study were injected intravenously with 25 UI/kg of BDD-rFVIII. A wash out of at least 3 days was carried out before the infusion. Patients were not on bleeding state and were negative for inhibitor. Venous blood samples were collected at 0.25, 0.5, 1, 3, 6, 9, 24, 28 and 32 h after the end of infusion. Cmax, Tmax, Half life, AUC, distribution volume (Vz), Clearance (Cl) Mean Residence Time (MRT) were calculated both for chromogenic (Chromo) and one-stage assays and both with Pooled Normal Plasma (PNP) and RLS. The pharmacokinetic (PK) analysis was performed

according to non-compartmental method on single patient data for each combination of assay and standard. As goodness-of-fit index of the PK analysis, we calculated the R squared and the number of points used to estimate Lambda(z). PK analysis was performed using WinNonlin ver 5.1 (Pharsight Corporation, US). An ANOVA model was built up on PK estimates for each single patient and each single assay with a fixed factor accounting for the assay and a random term accounting for inter-patient variability; post hoc contrasts were tested according to Tukey's HSD procedure; statistical analysis was performed with SPSS package, ver 13.0 for Windows (SPSS Inc, US). *Results.* Thirteen severe hemophilia patients were enrolled in the study. Their median age was 31.6 years (range 21-68.7). All patients were HIV seronegative; 12 were HCV seropositive without severe liver disease. The mean (±SD) actual dose infused was 24.7 (±0.6). Main results are shown in the Table 1. *Conclusions.* We confirm previous results about a better sensitivity of one-stage method for the lowest concentrations of FVIII, with a more accurate evaluation of terminal half life. Measured Cmax is slightly superior than expected values and independent from the assay and standard used. The clinical utility of RLS in the evaluation of FVIII concentration after infusion of BDD-rFVIII seems to be lower after reformulation of the product.

Table 1.

	Chromogenic Plasma std		*Chromogenic Refacto std*		*One-stage Plasma std*		*One-stage refacto std*		*ANOVA p value*
	Mean	*Std dev*	*Mean*	*Std dev*	*Mean*	*Std dev*	*Mean*	*Std dev*	
λ_z	.0020	.0009	.0015	.0008	.001	.0003	.0012	.0002	.001
HL_ λ_z	462.2	255.44	565.0	228.0	777.01	283.77	623.24	170.50	<.001
Tmax	18.00	6.324	17.14	5.44	16.500	4.743	15.00	.000	Ns
Cmax	.632	.0857	.631	.1394	.583	.1297	.597	.1302	Ns
AUC last	418.3	114.41	376.5	123.03	460.7	84.70	410.2	131.37	Ns
AUC inf	464.8	166.37	426.4	147.86	575.1	147.36	468.6	161.73	.010
Vz	34.9	11.41	50.76	26.23	48.79	11.83	52.9	20.20	Ns
Cl	.0600	.0203	.064	.0199	.046	.0153	.061	.0269	.033
MRT last	509.9	130.20	506.9	125.34	621.6	108.79	592.3	82.80	<.001
MRT inf	686.1	319.30	726.7	240.96	1079.7	438.68	856.9	257.78	<.001
Vss	36.22	7.515	44.49	14.570	46.59	12.11	49.57	17.54	.050
λ_z estimation									
R squared	.990	.019	.989	.009	.991	.0136	.973	.047	Ns
No of points	6.20	2.25	6.57	2.138	7.100	1.96	6.71	2.016	Ns

a Test = Chromo_P_std

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NINE NOVEL MUTATIONS IN FXI GENE IN PATIENTS WITH INHERITED FXI DEFICIENCY

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Background. Factor XI deficiency is a an autosomally inherited bleeding disorder characterized by an extreme heterogeneity as to bleeding tendency, often independent from circulating FXI levels. More than 150 mutations in FXI gene has been found so far (http://www.factorxi.com/) and dysfunctional molecules are rare. Two prevalent mutations (Glu117X and Phe283Leu), account for the majority of abnormal alleles in Jews, while large genetic heterogeneity is found in non-Jewish patients. We report 9 novel mutations in FXI gene in 9 patients with FXI deficiency, of wigh one was associated with a type II phenotype. *Methods.* The whole coding sequence and intron-exon boundaries of FXI gene were sequenced in three patients with severe FXI deficiency (FXI:C <1% to 3%; FXI:Ag 2-5%) and 6 with partial FXI deficiency (FXI:C 27-56%; FXI:Ag 39-125%). None was of apparent Jewish ancestry. *Results.* The three patients with severe deficiency resulted all compound heterozygotes (novel mutations with asterisk): Gln5X*/Glu117X, Lys252Ile/IVS 3+2 T>A*, Gly79AlaP/Glu117X). In the heterozygotes, six novel mutations were detected (His127Arg, Gly373Arg, Gly400>Ser, Asp556Gly, Gly578Cys, Tyr590His). The patient with His127Arg showed evidence of a type II defect, since the discrepant FXI:C and FXI:Ag results (27% and 125%, respectively. This pattern was confirmed in other family members. His127Arg is adjacent to Cys122-Cys128 disulfide pairing in Apple 2 domain responsible for substrate binding site in FXIa. *Conclusions.* This report confirms the wide allelic heterogeneity of FXI deficiency in non-Jewish patients. However, the common type II Glu117stop mutation detected in Jews appears to be largely present also in Italian and Czech patients with FXI deficiency. Furthermore, a rare type II mutation occurring in Apple 2 domain has also been identified.

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REPLACEMENT THERAPY WITH RECOMBINANT FIX. MULTICENTRIC EVALUATION OF CURRENT DOSING PRACTICE IN ITALY

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Introduction. Recombinant factor IX (r-FIX) is reported to have a lower *in vivo* recovery in comparison to plasma-derived products, with potential clinical implications in the its dosing. In practice, a conversion (augmentation) factor is suggested for dosage calculation of r-FIX, based on pharmacokinetic studies. *Aim of the study.* Assessment of the range of values for this conversion factor in usual clinical practice in Italy. *Methods.* The study was questionnaire-based and proposed to all Italian Haemophilia centres treating Haemophilia B (HB) patients. Age, weight, treatment regimen (prophylaxis (PR) versus on-demand (OD)) and dosage used in the last effective infusion were collected for severe HB patients treated with r-FIX in the participating centres. Mean, standard deviation, median and range were calculated for demographic and treatment data for the overall population and for PR and OD patients. The conversion factors relative to three different theoretical dosages (20, 40 and 60 U/Kg) were calculated and compared to the observed prescriptions. The 40 U/Kg dose was used to calculate mean estimators, while the 20 and 60 U/Kg doses were used to better approximate Confidence Interval (CI) values. *Results.* 108/195 (55%) patients from 28 centres resulted to be treated with r-FIX. The sample represents 80% of the severe Italian HB patients population. Age range was 0-72 years (median 15 for 49 PR and 23 for 54 OD patients). Weight range was 3-108 Kg (median 55 for PR 69 for OD patients). Mean dosage was 44.9 U/Kg, with no significant difference between PR and OD; a slight reduction in dosage was observed with increasing age (0.3 U/Kg/year). Mean value for the conversion factor was 1.12, 95% CI 0.67-1.85 (0.5-2.78 with the most conservative method). Main results are shown in the Table 1. *Discussion.* The Italian survey found a wide and rightly skewed distribution of the values. Results confirmed the routine use of an augmentation factor, which was found to be lower than expected (1.12 *vs.* 1.2).

Table 1.

	Whole population	*Prophyilaxis*	*On-demand*
n° of patients	*108*	*49*	*54*
Conversion factor versus theoretical dosage			
20			
Mean	1.49	1.55	1.41
Median	1.33	1.33	1.29
Range	0.67-3.70	0.67-3.70	0.68-2.78
40			
Mean	1.12	1.16	1.06
Median	1.00	1.00	0.97
Range	0.50-2.78	0.50-2.78	0.51-2.08
60			
Mean	0.74	0.77	0.70
Median	0.66	0.66	0.64
Range	0.33-1.85	0.33-1.85	0.34-1.39

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MANAGEMENT OF PATIENTS WITH HEMOPHILIA IN AN INTER-REGIONAL NETWORK IN CENTRAL ITALY

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Introduction. Since 2002 three Haemophilia Treatment Centres (HTC) in middle Italy (Macerata, Perugia, Pescara) started a shared network, setting up a clinical collaboration and building up an inter-regional database of patients records available through the web. The aim of this report is to describe the clinical functions of the database and to show main results about epidemiologic and treatment data. *Methods.* The web-available database is syncronyzed with the local databases of EmoCard, the

HTC management program promoted by the Italian Hemophilia Centres Association. Physicians and patients can access and update their records through a password-proteced system. Patients can authorize any treating physician to access their data and treaters can access updated records from everywhere in the network. The database is also used to prepare epidemiologic reports. *Results.* The database contains 386 records (6 duplicates), 19 of which relating to dead patients. The database collects records of the following alive patients: Hemophilia A: 55 severe (mean age = 32.66, range = 2.19-68.68), 26 moderate and 89 mild; Hemophilia B: 12 severe (mean age = 23.83, range 8.03-66.58), 14 moderate and 20 mild; vWD: 70 type1, 4 type 2 and 3 type 3. Factor VII deficiency 10, other rare deficiencies 29. Inhibitor patients were 18 (107 high responder, of which 0 undergoing ITI and 8 low responder, of which 8 transient. 91.7%, 81.6% and 65.2% of severe, moderate and mild haemophilia patients received replacement therapy on demand or prophylactically (20-40 IU kg–1 2-3 times week-1 were administered in 36.4% of severe and 15.0% of moderate patients). Recombinant factor VIII and IX concentrates were used in 66.7%, 82.5% and 87.4% of respectively severe, moderate and mild patients. Adverse events were observed in 3 of 41 treated Haemophilia B subjects. *Conclusions.* The network model described could be easily transferred to other similar settings.

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XL'EMOFILIA: A WEB-BASED CLINICAL RECORDS FOR OUTPATIENTS WITH HAEMOPHILIA AND ALLIED DISORDERS IN THE REGION OF EMILIA ROMAGNA

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Haemophilia treatment in developed countries is based on home self-infusions of coagulation concentrates: improving communication between Haemophilia Centres (HC) and patients is very important. The Emilia-Romagna Health Authority founded a project *Web connections of the region's HC* and the Hub Centre (Parma) designed new clinical records for outpatients *l'Emofilia*: a web application that does not require installation, is suited to the needs of HC, shares the databases of the region's HC, and can be accessed from anywhere. Data are managed with the *https* protocol, respecting Italian privacy legislation. Significant innovations are: - the *Problem List* - a summary of the patient's clinically significant data that can be consulted at a glance. It gives a concise, but full description of the patient's clinical profile and is updated automatically in real time when new data are entered; - easy compilation of therapeutic plans (necessary in Italy to obtain the drug from pharmacies); - *pathways* to help to enter data; - integration with the regional and national registries; - different login profiles (administrators, HC doctors, collaborating specialists, nurses, patients) enable access to different sections With a *web-identity* (a personal USB key for secure web access), patients can record bleeds and home infusions directly on-line, consult their own data and, if needed, selected data (in English or Italian) can be accessed by patients' general practitioners or Emergency departments in any part of the world. Since April 2007, 50 pilot patients in the region were trained and started to use *l'Emofilia* with success and training courses are on going in every Haemophilia Center of the Emilia Romagna Region.

P195

DISORDERS OF PRIMARY HAEMOSTASIS IN YOUNG WOMEN WITH MENORRHAGIA AND/OR IRON DEFICIENCY ANAEMIA

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Menorrhagia is the most common cause of iron deficiency anaemia in women in fertile age. In most cases gynaecological and endocrinological conditions may explain the abnormal menstrual loss, however more recently the possible contribution of undiagnosed disorders of haemostasis has been recognized. We investigated the presence of abnormalities of primary haemostasis in 38 consecutive young women (15-35 yrs) referred to our Centre with a history of menorrhagia and/or iron deficiency anaemia. Dysfunctional or organic gynaecologic abnormalities (pelvic ultrasonography and hormonal evaluation) or other clinically detectable causes of iron deficiency (including coeliac disease by testing anti-transglutaminase antibodies and other clear-cut gastrointestinal diseases) were excluded. The questionnaire for the collection of bleeding history and the computation of bleeding score, recently standardized in type 1 von Willebrand Disease (VWD), was used at patient enrolment. Primary haemostasis assessment, carried out in the first week of the menstrual cycle and in the absence of any hormonal treatment or signs of inflammatory states, included bleeding time (Ivy), PT, aPTT, FVIII:C, VWF:Ag and VWF:RCo and *in vitro* platelet aggregation (Born). Abnormal data were confirmed by repeated assessments. Two patients (5.2%) showed VWF abnormalities consistent with type 1 VWD (VWF:RCo <30%, RCo/Ag >0.7). In four patients (10.5%) abnormalities of *in vitro* platelet aggregation were detected and further studies led to diagnose storage pool disaese in two cases and agonist-receptor abnormalities in the other two patients. Both VWD patients and 2 out 4 patients with platelet defect showed responsiveness to desmopressin (normalization of bleeding time). On the whole, newly diagnosed abnormalities of primary haemostasis were found in 6/38 patients (16%) with idiopathic menorrhagia and/or iron deficiency anaemia, all with bleeding score ≥5. However, as expected, the specificity of such finding was 67% in this population. Despite the limited sample size and the lack of objective assessment of menorrhagia, our data support the role of underlying disorders of haemostasis as aetiologic/contributory factors in this setting. The standardized questionnaires and bleeding scores may be helpful to identify patients in which extensive haemostasis testing should be carried out. The diagnosis of such abnormalities may also provide specific therapeutic approaches (desmopressin, replacement factors).

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INHIBITOR DEVELOPMENT IN MILD HAEMOPHILIA A: COULD INFLUENZA VACCINATION HAVE BEEN THE TRIGGER?

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The major complication in haemophilia is the development of FVIII inhibitor. The frequency of inhibitors in severe haemophilia ranges from 20-30%, while it is less common (3-13%) in moderate/mild haemophilia. The physiopathology of inhibitor formation in mild haemophilia is only partially elucidated and it is mostly reported in relation to major bleedings, surgery and other reasons for frequent infusions. The role of causative mutation and family history of inhibitors is unclear. To date, no one claimed for a possible role of vaccination in inhibitor pathogenesis. GP is a 20 year old mild haemophiliac (FVIII:C ~7%), treated on demand with r-FVIII, who always presented only traumatic muscular and joint bleedings until adolescence. Screening for inhibitors was always negative, even after a long period of treatment (2000 U/die for 1 month, then 1000/die one month, and 1000 every other day, one further month) for reconstructive knee surgery at the age of 17. On December 2006 the patient was vaccinated against influenza virus with the trivalent adjuvated vaccine available in Italy for the season (FLUAD, Novartis Vaccine). On January 2007, GP presented macroscopic haematuria treated with rFVIII 25 U/Kg/day for 3 days, with complete remission and no inhibitor detection.

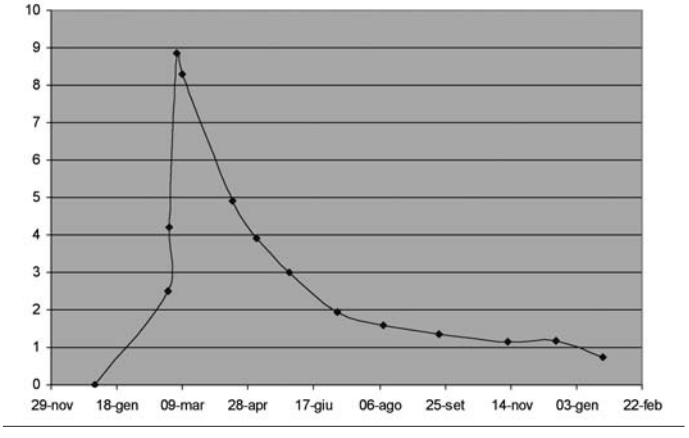

Figure 1.

One month later, the patient showed a severe spontaneous haematoma of his left calf and ankle. Inhibitor titre (IT) resulted in 2.5 Bethesda Units (BU). The patient was treated with DDAVP and rFVIIa, but IT arose to 8.85 BU, with slow reduction of clinical symptoms. Four days after, being IT unchanged, prednisone was unsuccessfully attempt-

ed. Later on IT spontaneously lowered to 3.9 BU in 8 weeks. Antibodies against influenza virus were titled in February (Feb) and April (Apr), showing the typical trend of a valid vaccination response (titre lowering along time): A/Wisconsin/67/105 (H3N2): 20-40 (Feb) 20(Apr); A/New Caledonia/20/99 (H1N1): 640-1280 (Feb) 640(Apr); B/Malaysia/2506/04: 40-80 (Feb) 40 (Apr). Thus a parallel increase and decline of antibodies titre was observed for FVIII and influenza viruses. The causative mutation of haemophilia was identified in Exon 26 G6977A-Arg2307Gln. This mutation is reported in 20 patients in HAMSTER and associated with inhibitors in another patient. We can conclude that 2/21 mild HA with Exon 26 G6977A-Arg2307Gln developed an anti-FVIII inhibitor and that an association between vaccination and inhibitor development may be hypothesized.

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TRANSCATHETER ARTERIAL EMBOLIZATION OF CONCURRENT SPONTAENEOUS HEMATOMAS OF THE RECTUS AND OBLIQUE LEFT ABDOMINAL MUSCLES IN A MODERATE HAEMOPHILIA B PATIENT

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Background. Evidence-based recommendations have recently been made with respect to many aspects of the acute management of the bleeding patients, which when implemented may lead to improved patients outcomes (Sphan DR *et al.* Crit Care.2007;11(1). With regard to the haemophilia population, cumulated evidence had proven that the substitution therapy according the prophylaxis regimens as well as the prompt infusion of concentrates had markedly reduced the occurrence of spontaneous muscle hematomas or their inevitable sequelae in terms of disability and/or signs of abdominal compartment syndrome or pseudotumor late development. A multidisciplinary approach is mandatory for the management of the massive muscle bleeding to create circumstances in which optimal care can be provided. *Aims.* However, the incidence of life-threatening spontaneous or post-traumatic muscle hematomas of the anterior and/or posterior abdominal walls remain to be feared even in moderate haemophilia which commonly are less infused. Generally, the treatment is conservative and intensive by replacement therapy because of the difficulty of revealing or controlling the bleeding arterial vessel(s) surgically. Furthermore, in these cases the haemorrhage is often multifocal and involves complex collateral pathways. *Methods.* We here report our recent observation of vaste concurrent spontaneous hematomas of the rectus and oblique left muscles of the anterior abdominal wall in a moderate haemophilia B patient 52 yrs old. By computed tomography (TC) an active and massive bleeding was documented from the epigastric artery with extravasation along the left retroperitoneum as far as the pelvic region. Despite the several infusions of FIX concentrates (AimaFIX® Kedrion), 19.000 IU/3 days, the epigastric artery haemorrhage lasted worrying. So, we considered to perform endovascular selective embolization by transcatheter arterial embolization (TAE) via transfemoral access to treat these muscle hematoma, as well as it has recently been done in non-haemophilia subjects, i.e. in patients undergoing anticoagulation (Basile A. *et al.* Cardiovascular and Interventional Radiology. 2004;27:1231-1234). Thus, because a part of the standard advantages (radiopacity, accuracy and safer development), coils and microcoils are able to pack the entire length of the major supplying vessel(s), preventing retrograde filling from collaterals with selective arterial embolization. *Results.* TAE was technically successful. No active bleeding was detected by angiography after the procedure. The patient recovered very well and he was discarged after 5 days. *Conclusion/Summary.* Uncontrolled bleeding contributes to 30% to 40% of deaths and is the leading cause of potentially preventable early in-hospital deaths (Holcomb JB *et al.* Crit Care.2004,8 [suppl 2]:557-60). No reports we found in the literature regarding TAE to treat muscle abdominis hematomas in haemophilia B patients. In our case the persistent and uncontrolled bleeding signs at CT as well as the unstable haemodynamic conditions of the patient indicated the use of angiography and the subsequent successful embolization to stop the severe haemorrhage and to prevent their inevitable sequelae. From this rare observation the embolization is effective and safe to control bleeding in these haemophiliac otherwise problematic cases where the conservative treatment seems to be insufficient and surgery can fail.

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LIVER TRANSPLANTATION IN A PATIENT WITH SEVERE HAEMOPHILIA A, HCV RELATED HEPATIC CIRRHOSIS AND HIV INFECTION: A CASE REPORT

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Background. In the '80s the use of contaminated biological coagulation factors led to a high number of haemophilia patients in serious viral infections (HIV, HCV, HBV). Orthotopic liver transplantation (OLT), where available, is the only therapeutic option for patients with end-stage liver disease. HIV infection itself is not, therefore, a contraindication for liver transplantation. The presence of serious haemorrhagic disease, associated with the need for continuous infusions of coagulation factors meat that only few OLTs could be carried out in haemophilic patients who were coinfected with HCV-HIV. In fact many of them died before they could be placed on transplant lists. *Case report.* A 39 year old male patient with severe haemophilia A (<1% FVIII), coinfected with HIV and HCV by previous transfusions of contaminated plasma derived FVIII concentrates, was affected with end stage liver disease and extremely short life expectancy. He was on prophylactic treatment with rFVIII due to the emarthrosis and frequent bleeding from oesophageal varices. In addition severe thrombocytopenia led to nose bleeding, successfully treated with rFVIIa. The severity of the disease (child index =15) and the absence of contraindications to transplantation due to HIV positivity allowed him to be placed on the waiting list for OLT. A recovery test with plasma-derived FVIII concentrates (Emoclot) was performed showing good response significantly increasing FVIII levels. The absence of anti-FVIII antibodies was also demonstrated. In June 2007 the patient underwent liver transplantation. Pre, intra and post-operative infusion of plasma-derived FVIII was carried out together with erythrocyte, platelet concentrates and fresh frozen plasma. Surgery was uneventful with no haemorrhagic complications. A gradual post-operative increase in endogenous FVIII levels was observed and therefore the infusion of plasma-derived FVIII was discontinued. During the 10 months of follow up FVIII levels remained between 30% and 40% without bleeding (Figure 1). No relapse of hepatitis occurred nor did any clinical events related to HIV). *Conclusions.* Our case demonstrates that, despite the high risk of haemorrhagic events, liver transplantation is possible in haemophilic patients with end stage liver disease and HIV infection, by appropriately infusing FVIII concentrates. Moreover liver transplantation frees the patient from the need for continuous infusion of FVIII concentrates.

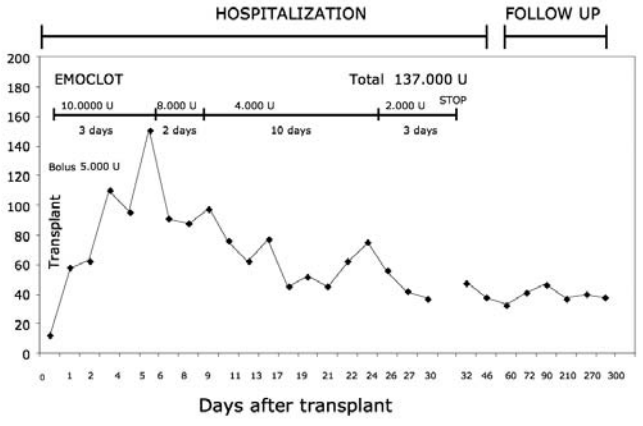

Figure 1. FVIII activity after liver transplantation a 10 months follow up.

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ACQUIRED HEMOPHILIA: A REVIEW OF EIGHT CASES

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Acquired hemophilia (AE) A is a rare but often severe bleeding disorder caused by the spontaneous development of autoantibodies against

coagulation factor VIII (FVIII). We have retrospectively evaluated 8 cases of AE diagnosed in our Hemophilia Center in a 15-year period. The patients were 5 females and 3 males, and the median age of FVIII inhibitor onset was 50 years (range 20-83). In 4 patients we found no underlying disorder, while 3 cases developed in post-partum period and one was associated with pemphigous. All the patients presented at our observation with different bleeding manifestations, such as uterine bleeding post-partum, hematuria, hemoperitoneum, muscle and skin bleeding. Only two cases required hemostatic therapy to control bleeding with recombinant activated factor VII and activated prothrombin complex concentrates. At the diagnosis, the median anti FVIII inhibitor titre was 16.6 Bethesda Units (BU) (range 2.6-48) and the median FVIII plasma levels were 3.8% (range 1-13). Seven patients were treated with the conventional immunosuppressive therapy (six with corticosteroids associated with intravenous immunoglobulins, and one adding also cyclophosphamide), while one patient, non responsive to the the traditional therapy, was also treated with two courses of rituximab. A complete remission was observed in 5 patients and FVIII inhibitor eradication with the normalization of FVIII plasma levels were attained after a median of 24 days (range 12-32); one patient achieved a remission within one month of immunosuppressive therapy, but after two months there was a relapse of FVIII inhibitor; the remaining two patients obtained only a partial remission, with FVIII plasma levels persistently below 50%. Among the patients without a complete remission, two died for concomitant disease not related to both FVIII inhibitor itself and immunosuppressive therapy. From our experience we conclude that a rapid diagnosis of AE and a prompt and effective immunosuppressive therapy has allowed to avoid further hemorragic episodes and to achieve a complete remission in 5/8 patients with FVIII inhibitor. However, even in the remaining patients we did not observe in the follow-up any bleeding complication.

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TWO UNUSUAL PRESENTATIONS OF ACQUIRED HEMOPHILIA

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We report on two unusual cases of acquired hemophilia. A 75 years old man with history of hypertension, myocardial infarction, type II diabetes and peripheral arterial disease, on treatment with ASA, was hospitalized for the occurrence of spontaneous hematomas initially involving the limbs and then the posterior thorax. As a result of prolonged APTT and INR the patient received FFP infusion. Three days later there was worsening of the hematoma with a fall in hemoglobin to 6.5 g/dL. The patient was transfused with 8 PRBC U and 6 FFP U. Clotting assays carried out in our laboratory revealed association of acquired hemophilia (FVIII:C 2%, 32 B.U.) with dysfibrinogenemia . With an electromechanical coagulometer (STA R, Roche) the APTTR was 2.43, the PTR 1.30, the TTR 1.53, the TCR 1.96 and fibrinogen levels were 60 mg/dL, but PT-derived fibrinogen levels (ACL 300, Instrumentation Laboratory, APTTR 2.57, PTR 1.10) were 662 mg/dL. The patient received PCC (75 U/Kg), with cessation of bleeding, and methylprednisolone, insulin and cyclophosphamide. FVIII:C was 13% (8 BU) 20 days and 132% 54 days later, with persisting dysfibrinogenemia. A 71 years old man with diagnosis of generalized myasthenia gravis and autoimmune primitive hypothyroidism in 2005, was treated with prednisone, levothyroxine, cholinesterase inhibitors and azathioprine (interrupted because of liver toxicity) with only mild improvement of symptoms and then with i.v. IgG for 5 days and monthly boluses of cyclophosphamide for 8 months with no benefit.. In 2007, 20 days after IgG infusion for 5 days, the patient presented with a large hematoma involving thorax, gluteus and thigh in association with a rapid fall in hemoglobin (from 15 to 8 g/dL). Acquired hemophilia was promptly diagnosed (APTT R 2.25, FVIII:C <1%, 64 BU). The patient was first treated with prothrombin complex concentrate (75 U/Kg) and then twice with FEIBA (75 U/Kg and 100 U/Kg) with poor control of bleeding and requirement for repeated transfusions of PRBC (10 U over 15 days). Methylprednisolone (1 g daily boluses for 3 days) and cyclophosphamide (150 mg daily), immediately administered after diagnosis, prompted a rapid fall in FVIII inhibitor titers (16 BU after 2 weeks) resulting in measurable FVIII:C levels (5%), which were associated with cessation of bleeding and increased to 20% one month after diagnosis. Normalization of APTT occurred 42 days after diagnosis (R=1.11) and FVIII:C levels were 97% 15 days later.

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THE MODERN THROMBOELASTOGRAPHY AS A PROMISING TOOL TO EVALUATE HAEMOSTASIS CAPACITY IN HAEMOPHILIA PATIENTS TO OPTIMIZATE THE SUBSTITUTION THERAPY

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Background. To date, in haemophiliac patients, correlation is usually observed between the clinical expression of the disease and plasmatic factor VIII/factor IX (FVIII/FIX) activity. However, the clinicians observe that some haemophiliacs, despite similar FVIII/FIX plasma levels and/or with identical mutations, could exhibit different bleeding phenotypes. So, FVIII/FIX clotting activity assays have a poor predictive value of individual bleeding risk. Therefore, a big concern remains when substitution therapy with the several commercial products must be carried on demand, prophylaxis or immune tolerance programmes as well as during surgery. Indipendently from FVIII:C/FIX:C plasma concentrations, the individually tailoring regimens as well as the adapting clotting factors infusions remain until now uncertain or even empiric. *Aim.* From years clinical haemostasis laboratories are ideally searching precious tests for physicians in management of haemophilia A and B, but these assays assess circumscribed elements of the haemostatic and coagulation mechanisms and often they do not reflect physiological state. Then, it emerges that the quality of the results are critically dependent upon preanalytical conditions. In this scenario, recently there has been considerable interest in the potential value of more global assays in the assessment of bleeding and for monitoring procoagulant therapies. Present cumulated evidences suggest that thromboelastograph/thromboelastomer technique could be of interest in the follow-up of haemophiliacs under the different therapeutical protocols. *Methods.* Since the minimum level of FVIII/FIX that will prevent bleeding physiologically is not known, we here propose that ROTEM® thromboelastogram (Sysmex, Dasit), a commercial available device for the application of the technique at the point care, may have important implication for optimization of therapies. *Results.* The ROTEM® system in whole blood samples serves as an informative global test of coagulation. The principal approaches measure the area under thrombin generation curve (AUC), the endogenous thrombin potential (ETP) together with lag time and the time to peak. *Conclusion*s. Even if applying standard pharmacokinetics to coagulant activity values is questionable in principle it was very convenient in practice (Berntorp SB *et al.* Clin Pharmacokinet. 2001:40(11):815-832), but a number of methodological problems may yeld rather discrepant values even in the same person. These issues may have strong importance for factor consumption and treatment cost. Thrombin generation associated with plasma FVIII/FIX measurements might represent a promising tool to evaluate more precisely the efficacy of a prophylactic regimen and thus to improve the overall cost-effectiveness of anti-haemophilic treatments. Furthermore, in order to approach *in vivo* conditions more closely, the measurements in whole blood might be more informative. By these approaches we can firmly establish the correlation between clinical expression of the coagulopathy, function tests (i.e. thromboelastogram and FVIII/FIX plasma activities) and immune tolerance programmes.

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ACQUIRED HAEMOPHILIA A: A CASE REPORT

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Acquired haemophilia A (AH) is a rare bleeding disorder caused by specific antibodies, which are directed against coagulation factor VIII (FVIII). These antibodies are able to inhibit FVIII clotting activity and can cause an unexpected bleeding episode in individual with no haemorrhagic history. Up to 50% cases are idiopathic, but AH can be associated with autoimmune diseases , postpartum period, malignancies, drug reactions. Laboratory tests reveal a prolonged aPTT, low level of FVIII and presence of inhibitor of FVIII (BU). The aim of treatment is control of bleeding and eradication of inhibitor. We report the case of a 66-year-old man, who was admitted to hospital because of spontaneous bleeding into right gluteus muscle and severe anaemia. His medical history included Hyperthyroidism, Hypertension and an Aortic Abdominal Aneurysm wide nearly 5 cm. Laboratory tests showed: aPTT 88", FVI-II 5%, presence of inhibitor FVIII (4BU), Hb 7.45g/dL. We started therapy with Prednisolone 80 mg.i.v., recombinant factorVIIa 3.6 mg, blood

transfusions (3units). After 8 days, he was discharged and came to hospital as outpatient: Hb was 10 gr/dL, FVIII 23%, inhibitor 2BU, aPTT 66". The ultrasonography showed enlargement of right thigh's haematoma and a new haematoma in left psoas; few days later he returned to hospital as inpatient. C.T.revealed the presence of haematomas in femoral quadriceps from inguine to knee, in gluteus and sural triceps. Laboratory tests showed severe anaemia, aPTT 72", low level of FVIII without inhibitor. We treated the patient with Prednisolone 80 mg/day , 5 units of blood transfusion , fresh frozen plasma, recombinant FVIIa 1.2 mg/day 5 days a week for 3 weeks. A septicaemia from Staphylococcus aureus was treated with antibiotics. When bleeding was stopped, the right leg septic haematoma was drained surgically. No malignancies or autoimmunity diseases was detected. After 3 months we obtained a clinical and haematological remission and the patient was transferred to a rehabilitation clinic; few days later the sudden rupture of abdominal aortic aneurysm compelled a surgical procedure performed without bleeding. After 24 months, the patient is well, aPTT and FVIII are normal. *Conclusions.* This report presents a patient successfully treated with corticosteroids and with a unusual dosage of recombinant FVIIa as bypassing agent. We think that this kind of therapy led to success and could be a different approach for these patients.

P203

TTV INFECTION AND ITS RELATION TO LIVER FUNCTION IN SICILIAN HAEMOPHILIA POPULATION

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Introduction. It is well known that at last the careful screening of blood-transmitted viruses in healthy blood donors and after the introduction of HBV vaccination as well as the inactivation of viral agents by physical and chemical procedures in normal pooled plasma have pratically removed the risk of possible infections in multitransfused patients with congenital clotting disorders. Nevertheless, haemophiliacs remain at putative risk of infections with blood-transmitted agents. In the past decade a novel DNA non-enveloped virus of 30-50 nm and a single straned genome of 3582 bases has been described in a patient with non A-E post-transfusion hepatitis of unknown etiology, so called TTV a member of new virus family named Circinoviridae (Nishizawa I *et al.* Biochem Biophys Res Commun. 1997; 241: 92-97. Mushahwar IK *et al.* Proc Natl Acad Sci U.S.A. 1999; 96: 3177-3182). *Aim.* By the present study we propose to determine the prevalence of TTV infection in Sicilian healthy controls and in multi-treated haemophiliacs of our Centre. Also, in haemophiliacs we searched for the liver disease or contribution to the severity of liver damage by a possible addition TTV infection. Parallely, RBC multitransfused homozygous β-thalassemia (β-Th) and sickle cell disease (SCD) Sicilian patients were considered. *Methods.* Adult/older Sicilian haemophiliacs A (n=24), B (n=6) and C (n=5, 4 females and 1 male), HBV+ and HCV+ (n=35), HIV- (n=32), age ranging 28-79 yrs were studied. All of them were hystorically multitreated with pooled-plasma concentrates during the period 1976-1992. Homozygous β-Th (n=16, 9 females and 7 males) and SCD (n=13, 8 females and 5 males), age ranging 25-66 yrs, all of them HBV+ and HCV+, HIV- were comparatively investigated.DNA TTV was determined by primers PCR (TIB-MOLBIOL) NG059 (sense) and NG063 (antisense), followed by eminested procedure with NG061 (sense) and NG063 (antisense) primers. Both PCR were amplified by 30 pmol of each primer and 2.5 U of ampli Taq Gold (Perkin Elmer) as currently done in our laboratory. Agarose gel electrophoresis (2%) and ethidium-bromide staining procedures were carried out. All serum samples of haemophiliacs and anaemic haemolytic patients were collected between January and November 1992. At that time the haemophiliacs were being treated with clotting concentrates that had been exposed to solvent-detergent and heat temperature for viral inactivation. It is known that solvent-detergent does not destroy non-enveloped viruses. Furthermore, in 1992 Italian National Health Service had stated that all haemophiliacs who reported hepatitis infections have been included on the National Registry to receive biological damages. Of the 35 haemophiliacs, 29 were HCV-RNA positive and 4 were HBV-DNA positive with elevated serum alanine aminotransferase (ALT) levels (n=31). Of the hereditary haemolytic patients (n=29), 25 were HCV-RNA positive and 3 were HBV-DNA positive, with increased serum ALT amounts (n=27). Finally, 19 healthy blood donors (9 females and 10 males), aging 21-51 yrs, of our hospital Transfusion Medical Service were choosen. Results 1. Out of 19 healthy blood donors, 4 (15.8%) were positive for TTV while 15 (84.2%) were negative, all of them with normal serum ALT values. Of 35 patients with congenital clotting disorders who had received frequently blood or plasma concentrates before 1990, 19 (54.4%) were positive for TTV DNA in serum; sixteen of them (45.6%) were negative. Elevated serum ALT levels were observed in 15 (78.8%) of the TTV DNA positive patients.The number of patients with elevated ALT was significantly higher in the TTV DNA positive group, but the mean ALT levels of 19 TTV DNA positive patients was 61.4±68.9 U/L and did not differ from the 59.7±61.6 U/L of the 16 TTV DNA negative cases. The mean age of TTV DNA negative patients was slight higher than that of TTV DNA positive patients (51.9±13.4 *vs.* 49.8±14.4 yrs, $p<0.4$) (Table 1). Results 2. With regard to the group of β-Th (n=16) and SCD patients (n=13), 4 (13.1%) were positive for TTV while 25 (86.9%) were negative. Elevated serum ALT values (73.4±39.8 U/L) were observed in all of them without any difference between TTV DNA positive and TTV DNA negative subsets (Table 1).

Table 1. Characteristics of TTV DNA Positive and Negative Patients with Congenital Clotting Disorders (n=35), Hereditary Haemolytic Anaemia (n=29) in Comparison with Healthly Blood Donors (n=19).

	Haemophilia (n=35)			Homozygous ± TH (n=16)	SCD (n=13)	Healthy Blood Donors (n=19)
	A (n=24)	B (n=6)	C (n=5)			
Sex						
Male	24	6	1	9	8	10
Female			4	7	5	9
Age (yrs)	29-73	28-37	31-59	26-66	25-63	21-51
Anti-HBc						
Positive	20	6	4	14	12	---
Negative		---	---	---	---	---
HBV-DNA						
Positive	4	---	1	2	1	---
HCV						
Positive	24	6	5	16	13	---
Negative	---	---	---	---	---	---
HCV-RNA						
Positive	22	4	3	11	9	---
Negative	3	2	1	5	4	---
TTV negative	10	2	4	13	12	15
TTV positive	14	4	1	3	1	4
Elevated ALT	22/2	5/1	4/1	16/0	13/0	0/19
Mean ALT (U/L) in TTV positive		61.4±68.9		73.4±39.8		19.3 ± 11.5
Mean ALT (U/L) In TTV negative		59.7±69.6		79.1±16.6		20.8 ± 9.1

Summary and Conclusions. The prevalence of TTV infection in blood donors as reported in the literature varies from country to country (Okamoto *et al.* J Med Virol 2000; 74: 1132-1139. Desai SM J Infect Dis. 1999; 179: 1242-1244. Ali S *et al.* J Med Virol 2002; 66: 561-566). In the present study, a prevalence of TTV infection of 15.8% was found in apparently healthy Sicilian blood donors, with a lesser degree respect to other countries as well as Belgium, Scotland, U.S.A. and Japan. Notoriously, patients with congenital clotting disorders are at high risk for infection with blood transmitted agents. On this basis, we parallely investigated subjects with hereditary haemolytic anaemias who are frequently transfused with packed RBC units, with high rate of hepatitis infections in the past and iron overload. In our haemophiliac population we found a higher prevalence of TTV infection than that of healthy blood donors. The high rate of co-infection of TTV and HBV+/HCV+ in the patients is most probably due to the fact that TTV and HCV can be present in the past in pooled plasma concentrates. There were more patients with an elevated ALT in the TTV positive group than in TTV negative patients, but the mean ALT value was similar in both groups. The β-Th and SCD patients had prevalence of TTV infection comparable to that of healthy blood donors with normal ALT values, thus demonstrating a lack of impact of TTV infection alone on ALT levels in haemolytic patients. We conclude that a high prevalence of TTV infection is found in apparently healthy blood donors at same degree of that present in hereditary haemolytic subjects. The higher prevalence in patients with congenital clotting disorders is dependent on the frequent infusions of pooled haemoderivates that were contaminated with TTV

obtained from the infected healthy blood donors. In this regard, it is interesting to note that not all patients were TTV positive despite multiple infusions of infected plasma sources before the virucidal inactivation combined procedures started from 1986. Interestingly, the majority of our cohort of haemophiliac and haemolytic patients were also co-infected with HCV. In both groups TTV positivity seems to bear no correlation with serum ALT amounts. Therefore, we suggest that TTV alone does not cause liver disease or contribute to the severity of liver disease.

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IN GOOD CLINICAL PRACTICE PROGRAMMES THE OPTIMIZATION OF DOSES WITH FVIII RECOMBINANT PRODUCTS IS MANDATORY TO TREAT HAEMOPHILIAC PATIENTS

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Background. To date the optimal doses and individual responses to replacement therapy in haemophilia remain controversial even not defined. At present, the clinical practice is largely based on data from observational non-controlled studies. Recently, it has been postulated that lower doses of FVIII commercial products could achieve the same results as more intensive treatments with better cost-benefit outcome. In addition, several variations persist until now in specifications and *in vivo* recovery between the FVIII concentrates. Apart from that, it is well known that laboratory tests for measuring FVIII and FIX activities vary much in haemophiliac population. From a clinical point of the view all these variations have practical implications when attempts are made to correlate therapeutic responses to the doses infused on the one hand and to plasma levels achieved on the other hand. Now then, even if clinical studies have abundantly demonstrated that FVIII recombinant products are efficacious and safe, the amounts to be infused persist often imprecise in the haemophiliacs. *Aim.* Regarding to *in vivo* functional expression of the FVIII concentrate formulations on the market, two main factors can be taken into consideration: - the resistance of activated FVIII (FVIIa) to *in vivo* inactivation by activated Protein C(APC); - FVIII half life mediated by low density lipoprotein receptor-related protein(LRP). At present, the exact role of APC remains unknown. It would therefore be interesting to evaluate APC resistance ratios and circulating FVIII plasma amounts before and after infusion in individual haemophiliac. Two major binding sites between FVIII and LRP, located on domains A2 (heavy chain) and C2 (light chain), have been identified. The C2 domain site is related to von Willebrand factor (vWF) and various interactions have been noted between FVIII,LRP and vWF. Therefore, it is useful to evaluate LRP, vWF plasma levels before and after FVIII recombinant infusion. *Methods.* We here propose a study intended to collect data on the employment of FVIII recombinant products based on evidence including individual features like the following laboratory parameters: FVIII:C (one stage method); vWF (ELISA technique); APC resistance (automated coagulometer); FXa (ELISA testing); D-dimer (automated coagulometer) as thrombin formation and fibrin deposition marker; Interleukin-6 (ELISA method) as major cytokine involved in the vascular endothelium responses; Interleukin-10 (ELISA method) as relevant cytokine implicated in immune response; P-selectin (ELISA method) as cytokine derived from activated endothelial cells. Thromboelastography as global kinetic indicator of coagulation with reference to measurements of the time of the peak, area under curve and endogenous thrombin potential before and after FVIII infusion at the times: 0, 10 min, 30 min, 1 hr, 12 hrs and 24 hrs. *Results.* By this promising approach we can monitor and optimizate the necessary therapeutical levels in various clinical situations of haemophiliacs. Individual guideline also reviews appropriate physiological targets and suggests use and dosing of the coagulation factor replacement. *Conclusions*. In our opinion, this promising approach is mandatory for optimizing doses for treatment regimens on demand, prophylaxis, immune tolerance programmes or during surgery as well as to assess clinical efficacy and to promote cost-benefit large programmes in haemophilia population.

Pathophysiology of Coagulation

P205

IMMUNOLOGICAL CHARACTERIZATION AND SEQUENCE ANALYSIS OF THE COAGULATION PROTEINS IN PRIMATES AND PIGS

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Background. The shortage of human donors for organ transplantation has led to an increasing interest in the possible use of pig organs for transplantation. However, microvascular thrombosis, following the activation of the clotting cascade, are frequently observed in the rejection process when pig organs are transplanted into primates. This coagulation disorder is becoming a severe barrier to the long-term survival of pig organs transplanted into primates. Compatibility between human and porcine coagulation system is determinant in xenotransplantation. *Aim of the study.* Immunoblot technique and bioinformatic approach were used to evaluate the potential molecular incompatibility between porcine and primate coagulation system. *Materials and methods.* Twenty healthy human donors, fifteen cynomolgus monkeys and fifteen pigs were enrolled. The vitamin K-dependent proteins were separated from plasma by barium citrate absorption. FV was purified from plasma by immunoaffinity chromatography. Plasma and platelet proteins were analyzed by SDS/PAGE and Western blot. The amino acid sequences of human, monkey and pig coagulation factors were aligned using MOE 2006.08 suite with blosum 62 substitution matrix. The sequences were extracted from ExPASy database. *Results.* Immunoblot analysis revealed that porcine PC had a similar phenotype to human, however with a lower MW. FX and PS presented as a band of equal MW in human and monkey. However, FX and PS in pig were of a lower MW. FII and FIX showed a similar MW between human and pig. However, in monkey FII and FIX were of a higher MW. FV immunophenotype was similar in the three species. Human FVII showed a lower MW respect to monkey and pig. The percentages of amino acid identity between human and porcine coagulation factors were lower than those between human and monkey. The sequence alignments showed that the porcine coagulation factors, compared to the human ones, contained highly conserved sites, but also insertions, deletions, important single amino acid substitutions and small regions completely changed. *Conclusions.* Coagulation factors of the three species present immunophenotypic differences. The sequence variations might induce differences in substrate specificity, reaction velocity and binding affinity to calcium ions and phospholipids when porcine coagulation factors interact with plasma from primate. These differences might explain the abnormal coagulation responses observed in pig to primate xenotransplantation.

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WHOLE BLOOD THROMBOELASTOMETRY PROFILE IN FACTOR V LEIDEN SUBJECTS AFTER *IN VITRO* ADDITION OF HUMAN ACTIVATED PROTEIN C

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Introduction. The Rotation ThromboElastoMetry ROTEM® (Pentapharm, Munich, Germany) is useful for studying the dynamics of whole blood (WB) coagulation and lysis, evaluating, in contrast to traditional coagulation assays, the interaction between plasma, platelets, leukocytes, and red blood cells. ROTEM has been reported to detect a variety of haemostatic disorders ranging from hypo- to hypercoagulative conditions. Recently, ROTEM® failed to identify a hypercoagulable profile in approximately 50% of carriers of standards thrombophilic traits. Aim of our study was to perform ROTEM® in 6 carriers (2 homozygous and 4 heterozygous) of factor V (FV) Leiden mutation and in 10 healthy subjects before and after the, *in vitro*, addition of human activated protein C (hAPC). *Patients and Methods.* After informed consent, 4.5 mL of WB were collected in a syringe prefilled with 0.5 ml of Na-Citrate 105 mMol from 6 carriers (2 homozygous and 4 heterozygous) of FV Leiden mutation and 10 healthy subjects, age and sex matched. INTEM test, in which the intrinsic coagulation pathway is

triggered, was performed before and after the, *in vitro*, addition of hAPC (Enzyme Research, Cabru, Italy) to reach a final concentration of 1 microg/mL. The following ROTEM® parameters were considered: Clotting Time (CT, sec), time from the beginning of the coagulation until an increase in amplitude of 2 mm; Clot Formation Time (CFT, sec), time between an increase in amplitude from 2 to 20 mm; Alfa-angle, tangent to the clotting curve at 2 mm point; Maximum Clot Firmness (MCF, mm), maximum amplitude of thromboelastogram; Area Under Curve (AUC, mmx100), area under the velocity curve. *Results.* No statistically significant differences were found between carriers of FV Leiden mutation and controls, at baseline. In controls, CT (mean±SD) was longer (468 ±100 sec), after hAPC addition than at baseline (176±32 sec). In carriers of heterozygous and homozygous FV Leiden mutation, CT (mean ±SD) was milder prolonged (246±18 and 220±36 sec, respectively) after hAPC addition either in respect to baseline and in respect to controls. Interestingly enough, CT ratio (baseline/after hAPC addition) was 2.7 in controls, 1.5 in heterozygous and 1.4 in homozygous carriers. *Conclusions.* WB thromboelastometry, performed by ROTEM® according to the standard protocols, failed to identify a hypercoagulable profile in carriers of FV Leiden mutation. This modification of INTEM allows to monitor with thromboelastometry the effect of the addition of APC to whole blood and to study the modification of clotting profile related to the presence of APC resistance associated to FV Leiden mutation.

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WHOLE BLOOD ROTATION THROMBOELASTOMETRY (ROTEM®) PROFILE IN 40 CYNOMOLGUS MONKEYS

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Introduction. Microvascular thrombosis is a hallmark of the rejection of porcine solid organ xenografts. Little is known about dynamics of whole blood (WB) coagulation and lysis in primates. WB rotation thromboelastometry, performed by ROTEM® (Pentapharm, Munich, Germany), evaluates the interaction between plasma, platelets, leukocytes, and red blood cells in colt formation. Aim of our study was to record WB thromboelastometry profile in 40 naïve cynomolgus monkeys versus 52 humans. *Materials and Methods.* Forty naïve cynomolgus monkeys were enrolled in the study; a group of 52 healthy human volunteers acted as control. Four ROTEM assays (INTEM, EXTEM, FIBTEM, NATEM) were performed according to protocols supplied by the manufacturer. The following ROTEM® parameters were considered: Clotting Time (CT, sec), time from the beginning of the coagulation until an increase in amplitude of 2 mm; Clot Formation Time (CFT, sec), time between an increase in amplitude from 2 to 20 mm; Alfa-angle, tangent to the clotting curve at 2 mm point; Maximum Clot Firmness (MCF, mm), maximum amplitude of thromboelastogram. *Results.* In all four tests considered, CT and CFT were statistically significant shorter in monkeys than in humans ($p<0.00001$). MCF, α angle and AUC were statistically significant higher in primates than in control group ($p<0.00001$). No differences were seen in platelet count among monkeys and humans, on the contrary fibrinogen levels were statistically significant higher in primates than humans ($p<0.0001$). *Conclusions.* WB thromboelastometry showed a hypercoagulable profile in primates as compared to humans. Prospective studies are needed to define the potential applications of ROTEM® in xenotransplantation setting.

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TWO NOVEL FACTOR V GENE MUTATIONS IN TWO PARAHAEMOPHILIC SUBJECTS

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Background. Coagulation factor V (FV) deficiency is a rare autosomal recessive bleeding disorder also called *Parahaemophilia*. The molecular bases of the disease are poorly characterized. We analysed the molecular genetic defects of two unrelated Italian patients with hereditary FV deficiency with recurrent bleeding events, and their relatives. Aim of the study. To screen FV gene (F5) for mutations involved in FV deficiency. To characterise with bioinformatics tools the novel mutations and their effect in the three-dimensional structure of the protein. *Methods.* In the two patients we determined plasma FV antigen levels (FVag) and FV activity. Genomic DNA was isolated from peripheral blood cells. The entire length of the 25 exons with flanking regions of F5 was amplified by polymerase chain reaction (PCR) and the amplified fragments were subjected to direct DNA sequence analysis. We performed sequence analysis comparing human FV with his hortologues in other species using CLUSTAL W program. A preliminary three-dimensional analysis was performed using a freeware program (GENO 3D). *Results*. The two patients were homozygous for FV deficiency and had very low FV antigen (<3%) and activity (<3%). DNA sequence analysis revealed the presence of double heterozygous mutations in both patients. Patient one was compound heterozygous for a previously reported mutation and a novel insertion at position 828 in exon 6 of F5 that introduce a stop codon at position 223 in FV protein. Patient two was compound heterozygous for a previously reported mutation and a novel mutation T4957G in exon 14 of F5 that determine the substitution Tyr1595Asp in the A3 domain of FV. This last mutation lies in a highly conserved region of the protein. *Conclusions.* In our study we identify two new mutations in F5. The preliminary studies of Tyr1595Asp three-dimensional FV structure reveal a modification of the structure. To confirm a correlation between the Tyr1595Asp mutation and FV deficiency we're going to analyse the expression of the mutated protein in a cell culture model.

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SIMULTANEOUS ASSAY OF THE METABOLITES 6-KETO-PGF1α AND TXB2 IN PLASMA SAMPLES USING TRIPLE QUADRUPOLE TANDEM MASS SPECTROMETRY COUPLED TO A TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY (2D-LC/MS/MS)

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Prostanoids, which include prostanglandine (PG) and thromboxane (TX), are synthesized from arachidonic acid by cyclooxigenase. Quick metabolism of PGI2 and TXA2 lead to the 6-keto-PGF1 and TXB2 metabolites in plasma, respectively. These prostanoids have pathological effects and the abnormalities they produce have been implicated in a wide range of disease, including haemostatic disease. TXA2 has aggregatory, vasocostrictory and bronchoconstrictory effecty, while PGI2 has antiaggregatory and vasodilatory effects. Therefore, the relative levels of these mediators have physiological significance and may be helpful in the diagnosis of various pathophysiological conditions. Some methods have been published for the analysis of the arachidonic-acid derived compounds and some are dealing with mass spectrometry but none are specifically centered on these specific compounds with a fast and cheap protocol. Here we describe an analytical strategy that incorporates a two-dimensional chromatography running coupled to tandem mass spectrometry that minimizes the sample preparation and addresses the presence of the 6-keto-PGF1 and TXB2 for a robust quantitation measurement. Sample preparation is limited to a protein precipitation step of the indometacine-stabilized plasma samples. The 2D-chromatography is coupled to a tandem mass spectrometer operating in negative mode for the quantitative measurement. Results are generated by using as internal standards their deuterium-labeled analogues. With the proposed configuration, total analysis time per sample results in 10 with a LOQ of 100 pg/mL. Linearity has been tested from LOD (estimated as 40 pg/mL for 6-keto-PGF1 and 60 pg/mL for TXB2) up to over 4 ng/mL. The range of the total assay precision is 5-10%. (n=6) for concentrations over 200 pg/mL and reaches values close to 20% for concentrations approaching the LOQ. The proposed methodology enables reliable quantitation of 6-keto-PGF1α and TXB2 metabolites in plasma samples after performing just a simple protein precipitation. Chromatography takes care of the presence of the two TXB2 anomers which, if not conveniently addressed, should affect the quantitative measurement results of TXB2. It has been demonstrated as stabilization with indometacine is mandatory for avoiding any further TBX2 production generated by the erithrocytes lysis after specimen collection. The reduced sample preparation makes the entire protocol suitable for large-scale studies.

P210

A HYPOFIBRINOLYTIC STATE IS DETECTABLE IN PATIENTS WITH RETINAL VEIN OCCLUSION

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Retinal vein occlusion (RVO) is an important cause of permanent visual loss but its pathogenesis has not been well understood. RVO has been associated with different systemic diseases, but also with a thrombophilic state. Previous studies have suggested a possible role for hypercoagulability and hypofibrinolysis in these patients. Recently, new global tests have become available, so possibly improving the capability of studying the pathophysiology of this disease. The aim of our study was to search for coagulation and fibrinolytic alterations in OVR patients by two global tests: Endogenous Thrombin Potential (ETP) and Clot Lysis Time (CLT). We studied 102 RVO patients (52 males and 50 females; median age 65 years; range 18-92 years;) and a control group of healthy subjects matched for age and sex. The ETP was measured by using a functional chromogenic assay (Dade Behring ETP) and the results were expressed as a percentage relative to the ETP value of the standard (ETP %). CLT (min) was determined by tissue factor-induced clot lysis assay, in which a fibrin clot is lysed by exogenously added t-PA. TAFI ag (micrograms/mL) and PAI ag (ng/mL) were determined by ELISA by using commercial kits. CLT, ETP and TAFI values were significantly higher in patients than in control group (CLT: 78±15 *vs.* 69±12 min; ETP: 111±18 *vs.* 98±9 %; TAFI: 13±3 *vs.* 10±2 micrograms/mL, $p<0.0001$ for all). TAFI and ETP were also higher in patients >50 yrs than in patients <50 yrs ($p<0.05$ and 0.005, respectively). Our results suggest that in RVO patients an increased thrombin generation exists which may lead to an impaired fibrinolysis possibly via an increased TAFI production and activation.

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ASSESSMENT OF FIBRINOLYTIC ACTIVITY BY MEASURING THE LYSIS TIME OF A TISSUE FACTOR- INDUCED CLOT: A FEASIBILITY EVALUATION

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A Clot Lysis Time (CLT) assay in which a tissue factor-induced fibrin clot is lysed by exogenously added t-PA has been recently reported. However, no consensus exists on assay conditions and the assay is not standardised. We evaluated the feasibility of CLT, its correlation with TAFI and PAI-1 levels and its standardization. In 185 healthy subjects CLT, TAFI antigen and activity, PAI-1 antigen plasma levels were evaluated. CLT was studied by monitoring changes in turbidity during clot formation and subsequent lysis using a computerized kinetic spectrophotometric microtiter plate. After preliminary experiments, 100 and 160 ng/mL t-PA concentrations were chosen for the study. CLT was calculated by a new mathematical analysis of the lysis curve based on discrete derivative. CLT, TAFI, PAI-1 plasma levels showed a normal distribution. For both concentrations of t-PA, CLT progressively increased with age ($p<0.0001$) and was significantly correlated with TAFI antigen and activity, PAI-1 antigen (at least $p<0.01$). At linear regression analysis TAFI and PAI-1 antigen were found to significantly influence CLT (at least $p<0.01$). CLT determination has a good laboratory performance. Our new method of calculation is independent of the time of reading and allows a more accurate and consistent detection of both short and prolonged lysis times. Our data suggest the feasibility of the use of this test in the routine work of specialised haemostasis laboratory.

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CHANGES IN CLOTTING AND IN INFLAMMATORY PARAMETERS FOLLOWING THE INFUSION OF XIGRIS OR CEPROTIN ARE SIMILAR IN SPITE OF A 20-FOLD DIFFERENCE IN CIRCULATING APC LEVELS

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After the introduction of Xigris® (APC), a series of studies revealed that APC administration was associated with an increased risk of bleeding, and these results have had a negative impact on the use of Xigris in severe sepsis patients. Two pivotal endothelial receptors are required for the anti-apoptotic and barrier-protective effects of APC: the protein C (PC) receptor (EPCR) and protease-activated receptor 1 (PAR1). At variance with PAR1 cleavage by thrombin, PAR1 cleavage by EPCR-bound APC leads to an antinflammatory endothelial response. A number of reports suggest a beneficial effect of the infusion of PC zymogen (Ceprotin®) on the survival of pediatric and adult patients with severe sepsis.We have compared changes in APC, F1.2, protein C, D-dimer, T-AT complex, IL-6, IL-8, IL-10 and e-selectin levels in 4 severe sepsis patients treated with Xigris and 7 severe sepsis patients treated with Ceprotin. Average baseline protein C (%), F1.2 (pmol) and APC (pmol) levels were 40±17, 572±512 and 2.9±4.0 in patients receiving Xigris and 40±18, 330±161 and 0.8±0.7 in patients receiving Ceprotin. The average values of the same parameters during treatment were 60±27, 403±333 and 156±9 with Xigris and 71±25, 359±215 and 8.5±7.0 with Ceprotin. In spite of 20-fold higher APC levels in patients with Xigris, coagulation and inflammatory markers decreased rapidly and to a similar extent in both groups of patients. In patients treated with Xigris, there was an average 595-fold excess of circulating protein C zymogen over APC levels and an average 247 pmol excess of F1.2 over APC levels. Given the identical affinity of protein C and APC for EPCR, the 600-fold excess of protein C over APC levels argues against the possibility of exogenous APC binding effectively to EPCR and exerting anti-inflammatory activity. In addition, the 1000-fold higher affinity of thrombin than of APC for PAR1 together with excess of thrombin formation over circulating exogenous APC levels challenge the concept of effective APC competition with thrombin for PAR1. Our data support the switching of the PAR1-dependent signaling specificity of thrombin from a permeability-enhancing to a barrier-protective response by the ligand occupancy of EPCR. A non-inferiority study comparing enzyme and zymogen for the survival of patients with severe sepsis is urgently needed. However, a rationale exists for the administration of the zymogen in patients presenting with contraindications to Xigris.

P213

THE USE OF PROTEINE C CONCENTRATE (PC) IN THE TREATMENT OF SEVERE SEPSIS IN IMMUNOSUPPRESSED PATIENTS IN THE COURSE OF HAEMATOLOGICAL DISEASE

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Severe sepsis is a complication of treatment of haematological disease. Chemotherapy of these diseases results, among others in impairment of both humoral and cellular immunity and consequent high susceptibility to infections, expecially those caused by bacteria residing in the oral cavity, nasopharynx, gastrointestinal tract. Such infections in patients under immunosuppression, additionally enhanced by chemotheraphy, often lead to the development of severe sepsis, associated with high mortality, ranging from 30% to 50%. Severe sepsis is currently defined as the present of SIRS (systemic infiammatory response syndrome), a source of infection, and dysfunction of at least one vital organ. Appropriate treatment includes detection and elimination of the source of infection as well as supporting organ function by maintaining tissue perfusion. In severe sepsis microcirculation is impaired as a result of activated SIRS, disseminated intravascular clotting (DIC), and vascular endothelial damage. This cascade of events lead to eventually to multiorgan dysfunction syndrome, which considerably deteriorates the prognosis. We have treated two cases of severe sepsis utilizing proteine C concentrates in patients with Acute Myeloid Leukemia (AML) and Non Hodgkin Lynphoma (NHL). Besides intensive, multidirectional treatment in these patients was utilized protein C concentrate.The recombinant human activated protein C (rhAPC) was controindicated in consid-

eration of bleeding risk (platelets count was <20.000 in both patients). In these patients the protein C plasma level was lower than 50%. Protein C concentrate was administrated as a bolus of 100 IU/kg initially , followed by continuos infusion for 72 hours. The protein C plasma level were brought again to the physiological values (activity 80-120%). Coagulation system, protein C level, platelets counts and biochemical parameters were monitored daily. In either patients starting from the second day from the beginning of the treatment a gradual normalization of laboratory parameters and improvement of the patients's clinical condition was observed. No adverse reaction or bleeding complication were seen. In our experience protein C concentrates are proven to be safe and particularly useful in the control of the coagulopathy triggered and sustained by sepsis in patients with clinical contraindication to the activated protein C (APC).

P214

A CASE OF DYSFIBRINOGENEMIA ASSOCIATED TO FV LEIDEN POLYMORPHISM AND MILD HYPERHOMOCYSTEINEMIA

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A 48yrs old woman came to our observation for suspected FXIII deficiency and dysfibrinogenemia, diagnosed when she was 8; she could not produce any documentation about her problem and family history. At 12 yrs she underwent appendicectomy surgery without any complications. She reports large mestrual losses, prolonged bleeding with a slow granulation and a tendency to form chieloidal recovery of worm. She reports in 1980 a miscarriage at the 10th week of pregnancy, in 1981 a deep vein thrombosis during the 5th month of pregnancy and disseminated intravascular coagulation during the 8th month with an emergency caesarean section and chronic renal failure (CRF) so she began haemodialytic treatment. She did not smoke, nor had arterial hypertension or diabetes; cholesterol, triglycerides, HDL and LDL levels were normal ANA and ENA were absent. In 2006 coagulation tests were: PT 96%, APTT 31 sec, fibrinogen concentration (Clauss) 50 mg/dL. On the basis of our evaluation, the patient is affected by dysfibrinogenemia, FV Leiden, mild hyperhomocysteinemia, probably in part related to CRF; MTHFR 677TT genotype was found. No FXIII defect (cromogenic method, 98%, 76%) was detected. Laboratory values of clinical interest were: PT 61%, TT 2.4 (0.9-1.2), fibrinogen (Clauss) 61 mg/dL (300-450), immunological fibrinogen level 306 mg/dL (200-450), homocysteine 21.3 umol/L (<13), APCR 1.9 (>2.2). Normal values were found for other thrombophilic tests. No genomic sequencing and polymerization of fibrinogen were carried out. Now the CRF is under control and she is undergoing since 1992 erithropoietin therapy (10 units/ten days). After 3 months with combined therapy of vitamin B12, B6, folates and betaine, the methionine loading test was normal. At follow up after 6 months the patient is in good health. Only a few cases of co-inheritance of dysfibrinogenemia and the FV Leiden polymorphism associated with severe thrombotic diathesis have been reported. The dysfibrinogenemia is Cedar Rapids type (gamma R275C substitution), in which a delayed plasmin lysis and abnormal platelet aggregation are reported. Individuals affected only by Cedar Rapids usually do not develop any thrombosis, suggesting that the combination of FV Leiden and dysfibrinogenemia makes the patient prone to thrombotic disease. No cases with associated hyperhomocysteinemia had so far been reported.

P214bis

PROTEIN Z DEPENDENT PROTEASE INHIBITOR AND PROTEIN Z IN PERIPHERAL ARTERIAL DISEASE PATIENTS

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Introduction. Protein Z is a vitamin K-dependent protein that serves as a cofactor for the inhibition of factor Xa through the serpin protein Z dependent protease inhibitor (ZPI). Recent studies have suggested a role for protein Z in atherosclerosis. We have previously determined protein Z plasma levels in patients with peripheral arterial disease (PAD), but ZPI levels have not yet been reported. The aim of this study is to more fully assess the protein Z/ZPI system in PAD patients in order to clarify its role in this particular model of atherosclerotic disease. *Material and methods*. We measured protein Z and ZPI in 60 PAD patients (48 M; 12 F) [median age: 73 (50-86) years] and in 60 healthy subjects comparable for age and gender. Protein Z and ZPI plasma levels were measured using home-made protein Z and ZPI immunoassays and a ZPI functional assay developed at the Division of Hematology of the Washington University, St. Louis, USA. *Results*. Protein Z antigen and ZPI function were found to be significantly lower in PAD patients with respect to healthy controls [Protein Z: median 73.56 (range: 3.37-123.66)% *vs*. 98.5 (42.34-203.16)%, p=0.0001; ZPI function: 83.30 (21.06-135.17)% *vs.* 96.91 (54.11-175.48)%, p=0.001]. On the other hand, only a trend of significance for ZPI antigen was reported between patients and healthy controls [(ZPI antigen: median 90.82 (27.17-149.54)% *vs.* 93.14 (range: 48.98-171.29)%]. In order to evaluate the possible involvement of protein Z/ZPI system on the pathogenesis of PAD we analysed the relationship between these proteins and the clinical severity of the disease. A general linear model, after adjustment for age, gender, and traditional cardiovascular risk factors showed significant lower levels of ZPI function according to the Fontaine's stages [ZPI function, stage IIb: 90.16 (82.89-97.43)%; stage III: 57.69 (40.22-75.17)%; stage IV: 37.64 (10.36-64.92)%]. Finally, the whole population was grouped on the basis of the number of traditional risk factors, and a trend of decrease for Protein Z and ZPI antigen according to the number of risk factors was observed. *Conclusions*. Our data show, for the first time, an association between protein Z/ZPI system and PAD, and suggest a potential role for this coagulation regulatory system in the occurrence and/or progression of the atherosclerotic disease.

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TISSUE FACTOR INHIBITION IN HUMAN CANCER AND TUMOR-ASSOCIATED HOST CELLS BY EPOPROSTENOL

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Background. It is now recognized that the interaction between tumor cells and the hemostatic system of the host plays an important role in cancer growth and dissemination; prostacyclin and its stable analogues have been shown to interfere particularly with metastatic process. Tissue factor (TF), the trigger of blood coagulation, is a mediator of intracellular signaling events of possible relevance for tumor growth, metastasis, angiogenesis and inflammation. We decided to investigate whether epoprostenol (prostacyclin), could modulate the procoagulant potential at tumor/host interface, by studying MCF7, a metastatic human breast carcinoma cell line, and host cells, such as human monocytes and endothelial cells. *Methods.* MCF7 cells were grown in 10% FCS DMEM until confluency. Human mononuclear cells (MNC) were obtained from whole blood collected from healthy donors by Lymphoprep sedimentation. Human umbilical vein endothelial cells (HUVEC) were isolated from human umbilical cord vein and grown in 15% FCS 199/DMEM until confluency. Cells were then incubated with epoprostenol and the different reagents in various combinations at 37°C. At the end of incubation, cells were disrupted and procoagulant activity was assessed by a one-stage clotting assay and expressed in arbitrary units (U) by comparison with a standard preparation of human brain thromboplastin. TF antigen cellular expression was determined by flow-cytometry. *Results.* MCF7 incubated for 16 hours at 37°C expressed strong TF activity, while exposure to epoprostenol decreased TF expression in a dose-dependent way. The decrease in TF activity was accompanied by a decrease in TF antigen measured by flow cytometry. Similar results could be observed in TF activity of endotoxin-stimulated MNC and HUVEC, cultured in the presence of epoprostenol. When MNC were exposed to TNF-α and IL-1 β, the presence of epoprostenol could once more be effective in TF activity inhibition. Surprisingly, no TF activity modulation was observed in cytokine-stimulated HUVEC. Inhibitory antibodies demonstrated that the activity was solely attributable to Tissue Factor, which was expressed by the different cells at various degree. *Conclusions.* These data support the hypothesis that epoprostenol, by its downregulation of TF, could exert a beneficial effect in thrombotic disorders where enhanced MNC/HUVEC and or tumor cell procoagulant activity may play a role. *Supported by unrestricted grant from GSK (Verona, Italy)*

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MULTIPLE MYELOMA PATIENTS UNDER THALIDOMIDE TREATMENT: PREDICTIVE VALUES FOR VENOUS THROMBOEMBOLISM OF HEMOSTATIC MARKERS

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An increase in the venous thromboembolic (VTE) rate has been observed in multiple myeloma (MM) patients receiving thalidomide in combination with oral steroid and chemotherapy. Aim of this study was to investigate whether the pre-thalidomide (Thal) plasma levels of hemostatic markers may predict for Thal-associated thrombosis in MM patients. We enrolled 50 consecutive MM patients (22 F/28 M; age range: 44–81 years), 21 newly diagnosed MM, before starting 1st line therapy (Thal ± dexamethasone or MP), and 29 with relapsed MM, before starting 2nd line therapy (Thal ± dexamethasone ± MP). At enrolment, patients were screened for VTE by doppler ultrasonography. Patients with newly diagnosed MM received thrombo-prophylaxis with LMWH enoxaparin 4 mg/d (n=20) or with acenocumarol (n=1). The group with relapsed MM did not receive any thromboprophylaxis, except for 6 subjects, who were on antithrombotic therapies (4 LMWH and 2 aspirin) for chronic cardiovascular diseases. Plasma levels of hypercoagulation (TAT, F1+2, D-Dimer), endothelial (vWF:Ag, t-PA, PAI-1) and leukocyte (elastase) activation markers were measured by ELISA. Ten of 50 patients (20%) developed VTE during therapy: 3/21 with newly diagnosed MM (14%) and 7/29 (24%) with relapsed MM. At baseline (i.e. pre-Thal), hypercoagulation TAT, F1+2 and D-Dimer, and endothelial vWF:Ag and t-PA marker levels were significantly higher in patients compared to controls ($p<0.05$), whereas PAI-1 and elastase levels were not statistically different from controls. Pre-Thal levels of TAT, F1+2, D-Dimer, t-PA and PAI-1 were significantly ($p<0.05$) greater in the group of patients who developed VTE compared to the VTE-free group. The relative risk (RR) for VTE of these laboratory markers was calculated by Fisher's exact test. A significant predictive value was found for pre-Thal levels of TAT ≥ 3 ng/mL (RR = 9.5; 95% CI = 8.98-23.66, $p<0.003$) and F1+2 ≥ 0.25 nmol/l (RR = 6.6; 95% CI = 0.06-11.26, $p<0.05$). The data suggest that pre-Thal plasma levels of TAT and F1+2 may be useful for predicting the development of a subsequent VTE during Thal treatment for MM. However, large prospective studies to evaluate the possible role of these markers in the management of VTE in MM patients are required

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ACQUIRED AND INHERITED RISK FACTORS FOR THE DEVELOPMENT OF VENOUS THROMBOEMBOLISM (VTE) IN AMBULATORY CANCER PATIENTS RECEIVING ADJUVANT CHEMOTHERAPY FOR BREAST OR GASTROINTESTINAL (GI) CANCER: A PROSPECTIVE TRIAL.

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To assess the thrombogenic role of adjuvant chemotherapy, following radical surgical resection, in ambulatory cancer patients, is of particular interest, because of the little or no tumor burden. The relation between adjuvant chemotherapy and thrombosis has been investigated in breast cancer. Limited information is available in GI cancers. Aim of this study was to investigate the acquired and inherited risk factors for VTE and the incidence of symptomatic VTE in patients on adjuvant chemotherapy for breast or GI cancer. In a prospective observational study (January 2003 and February 2006), 199 GI (82F/117M; age range, 26-84 years) and 182 breast (180F/2M; age range, 29–85 years) cancer patients were enrolled and followed-up for symptomatic VTE during adjuvant chemotherapy. We prospectively evaluated the effect of acquired (i.e. age, chemotherapy, tumor hystotype, history of thrombosis, body mass index and smoke) and inherited risk factors (i.e. antithrombin, protein C, protein S, homocysteine, activated protein C [APC] resistance, factor V Leiden [FVL] and Prothrombin [PT] mutations). Overall 30 VTE events (7.8%) were recorded: 28 (7.34%) during treatment and 2 (0.55%) during the subsequent follow-up. Among all the 381 cancer patients, FVL was detected in 14 cases (3.6%) and PT mutation in 10 cases (2.5%). None of the other inherited factors was found. One patient with GI cancer was double heterozygous carrier for both mutations. There was no association between the FVL or PT mutations and the risk of VTE. Among the 30 patients with VTE, PT mutation was present in 1 patient (3.3%), and FVL in none. By multivariate analysis, only chemotherapy was strongly associated with VTE development ($p<0.001$). In conclusion, our data demonstrate that, in the adjuvant setting, VTE is frequent and chemotherapy is the major risk factor.

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CANCER-RELATED VENOUS THROMBOSIS: RESIDUAL VEIN THROMBOSIS IMPROVES SCREENING FOR OCCULT CANCER

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Background. Clinical advantages of extensive screening for occult cancer in patients with idiopathic Deep Vein Thrombosis (DVT) is still debated since this approach improves early detection of cancer but not cancer-related mortality. Recently, we have demonstrated that patients with Residual Vein Thrombosis (RVT), 3 months after DVT, have a high risk for cancer in the subsequent 2 years (Siragusa S *et al.* Blood 2005;106(11):OC262). At the present it is unknown whether RVT assessment may be used to select patients, with idiopathic DVT, who require screening for occult cancer. Objective of the study. We conducted a prospective study evaluating whether a RVT-based screening for cancer

is sensitive and influences cancer-related mortality. *Study design.* Prospective with two cohorts of DVT patients: the first cohort was monitored for clinical overt cancer only (Group A), while the second (Group B) received complete screening for occult neoplasm and subsequent surveillance. *Materials and methods.* Consecutive patients with a first episode of DVT who presented RVT after 3 month of anticoagulation and without signs and/or symptoms for overt cancer. Screening for occult cancer was based on: ultrasound and/or CT scan of the abdomen and pelvis, gastroscopy, colonoscopy or sigmoidoscopy, hemoccult, sputum cytology and tumor markers. These tests were extended with mammography and Pap smear for women and ultrasound of the prostate and total specific prostatic antigen (PSA) for men. All investigations had to be completed within four-weeks from the assessment of RVT. All patients were followed-up for at least 2 years. Incidence and cancer-related mortality was compared between the two groups by survival curves (Kaplan-Mayer) and related Breslow test for statistics. *Results.* Over a period of 6 years, 345 patients were included in the analysis: first cohort included 213 patients (Group A), second cohort 132 (Group B). Clinical characteristics between groups were homogenous. During the follow-up, 8.4% of patients developed overt cancer in group A; in group B, 8.3% of patients had diagnosed cancer at the moment of extensive screening while one new case (0.7%) occurred during the follow-up (Table 1). The sensitivity of this approach was 91.6% (95% confidence intervals 74.7-108.5). Cancer-related mortality was 6.5% in group A and 3.0% in group B (p<0.001). *Conclusions.* Our study demonstrates that RVT-based screening for occult cancer improves early detection as well as cancer-related mortality.

Table 1. Cancer events during surveillance

Characteristic	*Group A (n= 213)*	*Group B (N= 132)*
Cancer, No (%) at screening time*	- -	**10 (8.3)
Cancer, No (%) during clinical surveillance**	18 (8.4)	1 (0.7)
Density incidence (cases x 1000 p/y)	41.9	40.3
Mean time cancer diagnosis (months±SD)	6.9 (1.3)	3.6 (0.7)
Cancer-related mortality, No (%)	14 (6.5)	4 (3.0)
Cancer-related mortality, mean time (months SD)	19 (3.4)	18 (2.8)

*4+0.5 months; **At least 24 months.

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THE IMPACT OF ANTI-THROMBOTIC PROPHYLAXIS ON INFECTIOUS COMPLICATIONS IN CANCER PATIENTS WITH CENTRAL VENOUS CATHETERS: AN OBSERVATIONAL STUDY

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Background. Potentially serious infections occur in 2-43% of cancer patients after implantation of a central venous device (CVD).[1] The possibility of limiting this complication with anti-thrombotic drugs is still debated. *Methods.* For this observational study we recorded the routine management of CVD in cancer patients at 18 oncology centers in Lombardy (N. Italy) and assessed the effect of anti-thrombotic prophylaxis (AP) on catheter-related infections. *Results.* Out of 1410 patients enrolled, 451 received AP continuously after implantation of the central line. The most frequent AP was very-low-dose warfarin, with 180 patients receiving this treatment according to Levine[2]; another 163 received very-low-dose warfarin according to other regimens. A group of 108 patients received continuous low-dose subcutaneous low-molecular-weight heparin (LMWH). During a mean follow-up period of 30 months, 57 catheter-related infections were reported in the 1390 pts seen at least once at follow-up examinations (4.1% of the whole group), giving an overall incidence of 0.10 infections per 1000 catheter days. This complication was significantly more frequent among patients with an indwelling central venous catheter (CVC) or peripherally inserted catheter than among those with a port device, and the group not given AP had 0.14 infective complications/1000 CVD days compared to 0.05/1000 CVD days (OR 2.4; 95% CI 1.7-5.0) for those treated. AP protected against infections at the catheter exit site and track but not against systemic infections. *Conclusions.* The reduction in infectious complications and in the systemic venous thrombosis burden when cancer patients with a CVD are given AP may be one of the mechanisms explaining why they survive longer than patients not given anticoagulants.

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CORRELATION BETWEEN LEUKOCYTOSIS AND THROMBOSIS IN PHILADELPHIA NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES

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Introduction. Recent investigations suggest that leukocytosis may cause thrombosis in Philadelphia negative (Ph-neg) MyeloProliferative Diseases (MPD). We investigated the relationship between leukocytosis and the occurrence of arterial and venous thromboembolic events in Ph-neg MPD patients over a period of two years. *Material and methods.* Seventy-five patients [46 females and 29 males, median age at diagnosis: 54 years; 42 with Essential Thrombocytemia (ET), 25 with Polycythaemia Vera (PV) and 9 with Idiopathic Myelofibrosis (IM)] were evaluated during the period 2000-2005; all of them received at least 2-years of follow-up. Patients were treated with cytoreductive therapy, anagrelide or α-IFN accordingly to age and type of MPD. Twenty-one patients had at least one episode of objectively confirmed thrombosis (arterial or venous) at the moment of diagnosis, or six months prior to diagnosis or during the follow-up. *Results.* A total of 28 vascular events were observed: 12 (42.8%) occurred in patients with PV (3 in the follow-up), 13 (46.4%) in patients with TE (3 in the follow up) and 2 (7.1%) in patients with IM(1 in the follow up). A leukocytes count above 8.5×10^9/L (median value) was statistically associated with an increased risk of thrombosis (p=0.03). The multivariate analysis, evaluating the interaction between conventional risk factors for thrombosis and leukocytosis, showed that the increased leukocytes counts was the most important risk factor for thromboembolic events. The relation between standard risk factors and leukocytosis is reported in the Table 1. *Conclusions.* The presence of a median leukocytes count >8.5×10^9L confers a high risk of thromboembolic events in Ph-negative MPD. These data may suggest the choice of cytoreductive therapy, but this approach must be confirmed in properly designed clinical trials, specially in young patients.

Table 1. Interaction between standard risk factors for thrombosis and leukocytes count.

*Risk factors**	*Hazard ratio (95% CI)*
Low risk with low WBC, n°1/11 (9%)	1 (Reference)
Low risk with high WBC**, n°4/7, (57%)	6.3 (4.1-8.4)
High risk and low WBC, n°6/28, (21%)	2.3 (1.2-3.4)
High risk with high WBC, n°18/29, (62%)	6.8 (2.9-10.7)

** Patients >60 years and/or previous thrombosis; ** Luekocytes >$8.5x10^9$/L (median)*

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THE RISK OF CANCER PROGRESSION IN WOMEN WITH GYNAECOLOGICAL MALIGNANCIES AND THROMBOPHILIC POLYMORPHISMS. A PILOT CASE-CONTROL STUDY

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Background. Cancer produce a hypercoagulable state which might lead to thrombosis and, on the other hand, unprovoked venous thromboembolism(VTE) might be the manifestation of an occult cancer. *Objective.* In this pilot case-control study we assessed the risk of gynaecological malignant diseases related to the presence of the factor V Leiden (FVL)

and prothrombin G20210A (PT-G20210A) polymorphisms. *Methods.* Fifty-two women underwent an operation for gynaecological malignancy and were enrolled in the study. Women that underwent an operation for gynaecological non malignant disease in the same days of cases were considered as controls. The presence of FVL and PT-G20210A was assessed in case and control groups. *Results.* Seven out of 52 cases were carriers of the two polymorphisms as compared to 20 out of 198 controls (OR 1.3; 95% CI 0.6 to 3.0). The results were similar also when the risk was considered separately for the site of cancer. As for advanced and metastatic malignancies the ORs were 2.3 (95% CI, 0.9 to 6.0) and 3.3 (95% CI, 1.0 to 11), respectively, as compared to non-cancer patients. When these two groups were compared to non-advanced cancer group the ORs for carriers of polymorphisms were 2.7 (95%CI, 0.7 to 11.0) and 3.9 (95%CI, 0.8 to 18.6) for advanced cancer and metastatic malignancies, respectively. *Conclusions.* Women with FVL or PT-G20210A polymorphisms, who developed gynaecological malignancy, might present with a higher stage of cancer at the time of surgery. Larger studied in similar cohort of patients are needed to confirm these findings.

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ORAL ANTICOAGULATION THERAPY IN SOLID AND HAEMATOLOGICAL CANCER PATIENTS: MANAGEMENT AND COMPLICATIONS

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According to most studies, patients with cancer, compared to other patients, have a higher risk of recurrence of venous thromboembolism and of bleeding while receiving oral anticoagulant therapy (OAT). It remains still unclear whether these results could be entirely extrapolated to patients with haematological malignancies. Our goal was to compare the anticoagulation control (i.e. % time in target INR range) and the clinical outcomes between patients with and without cancer in OAT; moreover, we focused on haematological malignancies. A total of 500 patients were recruited in 6 years, regardless of the indication for OAT; 146 (29%) of them were affected by cancer (47 haematological and 99 solid neoplasms); those being treated with warfarin for less than 1 month were excluded from this analysis. Cancer patients had a worse anticoagulation control, compared to those without cancer (55% *vs.* 60%; $p<0.001$). The rate of thrombosis was similar (6/146=4.1% vs 14/354=3.9%; OR=1.1); moreover the former had a tendency toward a higher rate of major bleeding complications (3/146=2.1% and 5/354=1.4% respectively; OR 1.5). The quality of anticoagulation control did not differ between haematological and solid malignancies (54% vs 55%, *p*=ns); no significant differences were found in the major bleeding (1/47=2.1% and 2/99=2.0% respectively; OR=1.05) and thrombotic complications (2/47=4.3% and 4/99=4.1% respectively; OR=1.05). The cancer patients discontinued warfarin primarily for surgical, endoscopic diagnostic or therapeutic procedures (32% vs 19%; $p<0.001$). The 47 haematological patients showed a younger median age compared to those with solid neoplasms (67 *vs.* 72 years, $p<0.001$), and discontinued OAT often because of invasive procedures (45% vs 26% of cancer patients, $p<0.02$). In the patients with solid neoplasms the surgical procedures more frequently were the reason for discontinuation (27% *vs.* 4% of the haematological, $p<0.001$). In our experience the haematological patients are not at increased risk of thrombosis recurrence and bleeding during OAT. Such a result probably reflects a good management of OAT even in presence of risk factors like central vein catheter or thrombocytopenia. Moreover, an appropriate initial decision of employing LMWH rather than OAT in particular subsets of haematological patients (for example those with life-expectancy less than 2-3 months or undergoing high-dose chemotherapy for stem cell transplantation) can reduce the hemorragic risk.

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THROMBOTIC EVENTS IN A COHORT OF 98 THROMBOCYTHEMIC PATIENTS

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The clinical corse of Essential Thrombocythemia (ET) may be complicated by thrombohaemorragic episodes. We have retrospectively evaluated 98 patients with diagnosis of ET with regard to thrombotic episodes. Overall 14/98 patients (14.3%) experienced a thrombotic event. The median age and the median platelet count at the diagnosis of thrombosis were respectively 55 years (range 30-85) and 529×10^3/mmc (range 250-915). An arterial thrombosis was observed in 59% and a venous thrombosis in 41% of these patients (10 and 7 episodes respectively). In 10/14 patients the thrombotic event coincided with the diagnosis of ET. The arterial thrombotic events were frequently cerebrovascular accidents (3 Strokes and 4 Transient Ischaemic Attacks) and Myocardial Infarctions in 3 cases. Among the patients with vein thrombosis we observed 5 episodes of intra-abdominal thrombosis (portal and hepatic vein thrombosis in 4 and 1 patient respectively), 1 cerebral vein thrombosis and 1 lower extremity deep venous thrombosis. Advanced age (>60 years), smoking and arterial Hypertension were more common acquired risk factors (5/8) in patients with arterial thrombosis, oral contraceptives among women with history od vein thrombosis (2/6 patients). A prothrombotic genetic defect (the G20210A prothrombin gene mutation) was demonstrated in 2 patients with portal thrombosis and cerebral vein thrombosis. In our experience cerebrovascular accidents are predominant arterial thrombotic events. In patients with venous thrombosis we observed a high rate of episodes in unusual sites and particularly of intra-abdominal thrombosis. The presence in two of these patients of a thrombophilic defect raises the issue of the potentilal role of these defects in patients with Myeloproliferative disorders and venous thrombosis.

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IRINOTECAN AND INCREASED TRIGLYCERIDES LEVEL IN PARANEOPLASTIC DEEP VENOUS THROMBOSIS: A STRANGE LINK IN AN OLD PROBLEM

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Background. Deep venous thrombosis (DVT) is present in about 3-15% of cancer patient. Paraneoplastic thrombosis pathogenesis is multifactorial. Chemotherapy is frequently involved in DVT genesis as proinflammatory/prothrombothic factor. Ther are few data about incidence of DVT in patients treated with Irinotecan. *Aims.* Aim of our study is to define if thrombotic risk in patient treated with Irinotecan is increased and eventually through wich mechanism probably Irinotecan acts in DVT genesis. With this purpose we considered in our patient complement fraction C3 and C4 and immune circulating complex (ICC) because they activate macrophage and platelets and increase tissue factor level. Moreover we recognize also total cholesterol and triglycerides level, because they are linked with factor VII activation. *Methods.* We considered C3, C4, ICC, cholesterol and triglycerides level, dose density and chemotherapeutic agents used in 116 patients with solid neoplasm (62colon, 26lung, 18gastric,) and without anticoagulant prophilaxis. Median age was 68.5 years (R 57-83). M/F ratio was 66/40. The threshold value of third quartile was chosen as risk cut-off (C3: 130 mg/dL; C4: 32 mg/dL; ICC: 2.9 mcg/mL; total cholesterol: 205mg/dl; triglycerides 120 mg/dL). The statistical analysis was conducted with Yates corrected chi square test, Fisher's exat test, Odds Ratio (OR), realtive risk (RR). *Results.* 24 patients (20%) showed DVT. Patients treated with Irinotecan were 42. DVT occurred in 14 of these patients (32%) and in 10 of 74 patients treated without Irinotecan (14%) with a Yates corrected chi square test of 4.6 (p 0.03), a Fisher's exact test with p0.03, an OR of 2.98 (95%CI 1.2-7.3) and a RR of 2.35 (95%CI 1.1-4.8). CRP and ESR at Pearson's test were not related with C3, C4, ICC, total cholesterol and triglycerides levels. All patients treated with Irinotecan had advanced stage colon cancer. High levels of triglycerides (>120 mg/dL) were present in 17 out of 42 patients treated with Irinotecan (40%) and in 17 out of 74 patients treated without Irinotecan (23%) with a Yates corrected chi square test of 3.1 (p 0.07), a Fisher's exact test with p 0.05, an OR of 2.2 (95%CI 1-5) and a RR of 1.7 (95%CI 1-3). *Summary/Conclusions.* Althoug patients receiveing Irinotecan and developing DVT were those with advanced stage colon cancer, influence of chemotherapy in DVT genesis in our study cannot be excluded. Probably Irinotecan contributes to DVT increasing triglycerides levels. In fact increased levels of triglycerides are linked *in vivo* with factor VII activation. This relief suggest also that other risk factors and other drugs (i.e. hypolypemizant drugs) might be used in paraneoplastic DVT prevention. Nevertheless these data need confirmation on a larger cohort of patients.

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ARSENIC TRIOXIDE MODULATES THE PROCOAGULANT ACTIVITY OF ACUTE PROMYELOCYTIC LEUKEMIA NB4 CELLS

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Arsenic trioxide (ATO), as a single agent, has proven effective in inducing molecular remission in APL patients. Recent studies suggest that the combination therapy of all-trans retinoic acid (ATRA) + ATO is effective in inducing APL remission in newly diagnosed patients, and may provide an alternative to ATRA+chemotherapy in this disease. Both ATRA and ATO determine a rapid resolution of the APL-associated coagulopathy. One mechanism of ATRA effect on the coagulopathy consists in ATRA capacity to reduce APL cell procoagulant activities (PCA) [i.e. tissue factor (TF) and cancer procoagulant (CP)]. However, little information is available on the effects of ATO or the combination ATO+ATRA on the PCA of APL cells. In this study we evaluated the capability of ATO and ATO+ATRA, as compared to ATRA alone, to modulate TF and CP of the NB4 APL cell line. The association between the PCA variations and the occurrence of cell differentiation, proliferation, and apoptotic/necrotic phenomena was also explored. NB4 cells were incubated with either 0.1 μM ATO, 1 μM ATRA, 0.1 μM ATO/1 μM ATRA or the vehicle (control cells). At different incubation times (i.e. 24, 48, 72 and 96 h) cells were tested for TF and CP expression, cell differentiation (as an increase in CD11b expression), proliferation and apoptosis/necrosis (by annexin V/propidium iodide staining). The results show that, starting from 24 h incubation, TF activity was 41% reduced by ATO ($p<0.05$) and 89% by ATRA ($p<0.01$). The reduction in TF activity was associated with a parallel and similar decrease in TF mRNA and TF antigen levels. CP activity was 18% reduced by ATO ($p<0.05$) and 61% by ATRA ($p<0.01$). The ATO+ATRA combination was more effective in modulating the two procoagulants than ATO, with no significant difference from ATRA alone. Simultaneously to the PCA downregulation, cell proliferation was inhibited by ATO by inducing cell necrosis, and by ATRA or ATO+ATRA by inducing apoptosis and cell differentiation, without necrosis. These data indicate that ATO can modulate both TF and CP activities of NB4 cells, although less than ATRA, and, in combination with ATRA, its effect on PCA is augmented. It is important that, differently from ATO alone, the ATRA+ATO combination is not associated to the induction of necrotic cell death which often causes extensive tissue damage and intense inflammatory response. A role for ATO in the molecular-targeted anticoagulant control of APL-associated coagulopathy is suggested.

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CHARACTERIZATION OF PRO-COAGULANT AND PRO-ANGIOGENIC ACTIVITIES OF SMALL CELL LUNG CANCER H69 CELLS

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The pathogenesis of thrombophilia in cancer patients partly relies on the capacity of tumor cells to produce procoagulant factors -i.e. Tissue Factor (TF) and Cancer Procoagulant (CP)-, and to activate the endothelium to express procoagulant proteins. Furthermore, cancer cells induce tumor neoangiogenesis by coagulation-dependent and -independent mechanisms. Clinical studies with anticoagulant drugs indicate a favorable effect of low molecular weight heparins (LMWH) on survival of patients with SCLC. In this study we wanted to: 1. characterize the procoagulant and pro-angiogenic activities of the SCLC H69 cell line, and, 2. evaluate the effect of LMWH on *in vitro* angiogenesis induced by these cells. H69 were grown in RPMI1640+10% FBS and different cell lysates or extracts were prepared and assayed for: TF activity by specific chromogenic assay; TF antigen by ELISA; TF mRNA by RT-PCR; and CP activity by specific chromogenic assay. Angiogenesis was evaluated by the Matrigel-based capillary-like tube formation employing human microvascular endothelial HMEC-1 cells. For inhibition studies, HMEC-1 were incubated for 24 h with H69 tumor cell conditioned medium (TCM) ± increasing concentrations (i.e. from 0.01 to 1 IU/mL) of LMWH dalteparin (DLT) or enoxaparin (ENX), or control medium. TCM content of pro-angiogenic factors (i.e. VEGF and bFGF) and TF was quantified by ELISA. The results show that H69 cells express very low TF, as shown by assays of either activity (2.2±0.8 pmol/10^6 FXa), antigen (18.8±6.2 pg/10^6 cells), and TF mRNA, and moderate CP activity (21.4±3.4 mU/mg protein). In the angiogenesis assay, H69 TCM significantly increased (36% mean increase) the capillary tube formation compared to control medium (TCM: 1,210±162 mm/cm^2; Control: 887±95 mm/cm^2; $p<0.05$). TCM-induced tube formation was dose-dependently inhibited by both DLT and ENX, reaching a 100% mean inhibition at 1 U/ml heparin ($p<0.05$ for both LMWH vs control). Cytokine content of H69 TCM showed a high level of VEGF (112±22 pg/mL), with low quantities of bFGF and TF. In summary, SCLC H69 cells express moderate CP and low TF procoagulant activities, while are capable to significantly induce endothelium capillary tube formation *in vitro*, which is sensitive to inhibition by LMWH. The data suggest that H69 may represent a suitable model for studying non-coagulation-dependent mechanisms of LMWH anti-tumor properties.

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HUMAN BREAST CANCER CELLS AND BREAST CANCER CELL LINES EXPRESS ENDOTHELIAL PROTEIN C RECEPTOR AND THROMBOMODULIN

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Background. Endothelial protein C receptor (EPCR) aids the thrombomodulin (TM)-thrombin complex in protein C activation, thereby providing anticoagulant and anti-inflammatory protection to the endothelium. The breast cancer cell line MDA-MB-231 also expresses EPCR. *Objectives.* To test whether or not: 1) human breast cancer cells and breast cancer cell lines express EPCR and TM; 2) protein C can be activated by breast cancer cell lines; 3) EPCR and TM expression is correlated with invasiveness and the expression of other established signatures of breast cancer. *Methods.* 1) Specimens of breast cancers that had been excised from 96 women were fixed in 10% buffered formalin and processed for conventional histopathological examination of EPCR and TM. 2). Five breast cancer cell lines were analyzed by flow cytometry, Western blot and mRNA for the expression of EPCR and TM and the activation of protein C. *Results.* Normal breast tissue was negative for EPCR and TM expression, whereas 49 (51%) breast cancers expressed EPCR, and 17 (18%) expressed TM, localized to the epithelial cancer cells. EPCR expression was prevalently detected in lower stage cancers and was inversely related to c-erb-B2 (r=-0.242, $p=0.018$). Results of the breast cancer cell lines study are shown in the Table 1. *Conclusions.* Half breast cancers in our series expressed EPCR, and a fifth TM. These are prevalently small c-erb-B2 negative cancers. *In vitro* expression of EPCR associated with protein C activation is also associated with invasiveness and absence of hormone receptors.

Table 1.

Breast cancer cells	*EPCR (%)#*	*TM (%)#*	*Protein C activation (nmoles/ 20 min)*	*Invasiveness*	*Estrogen (E), Progesterone (P) and c-erb-B2 (H) expression*
BT 474	0.1	1.14	0	++	E, P, H
Hs 578t	74	1	0.1	+++	H (low)
MDA-MB-231	97	0.1	2	+++	H (low)
MDA-MB-361	98	0.03	0.3	++	E(high), P, H(high)
T47D	95	0.32	0	+	E (low), P, H(low)

#expression detected by flow cytometry.

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EVIDENCE FOR THE OFF-LABEL USEFULNESS OF FEIBA® TO MANAGE THE CRITICAL GASTRO-INTESTINAL BLEEDING IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES

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Background. During the clinical course of haematological malignancies in patients with acute leukemias at onset of disease as well as in or after the intensive chemotherapies, the massive gastro-intestinal (GI) haemorrhages constitute life-threatening complications. In this scenario, several coagulation abnormalities and severe thrombocytopenia are considered causative factors of GI bleedings. The complex treatments include administration of platelet concentrates (PC), red blood cell (RBC) units, antifibrinolytic drugs, plasma infusions, somatostatin acetate and H2-antagonist intravenously, even recombinant activated Factor VII (rFVIIa) boluses with unsatisfactory results. *Aims.* In the past decade we experienced that FEIBA® (Baxter Bio Science, Pisa) at 90 IU/Kg infusions were capable to stop dramatic GI bleedings in patients with haematological malignancies not respondent to various therapeutic attempts. *Methods.* 12 consecutive patients (7 females and 5 males), age ranging 19-79 yrs, with acute myeloid leukaemia (n=5), acute lymphoblastic leukaemia (n=4), non-Hodgkin lymphoma (n=3) at onset of disease (n=4) and during intensive combined chemotherapy (n=8) were considered during 14 episodes of massive GI bleedings. In all patients the haemostatic profile showed as expected several coagulation changes as PT and aPTT slight prolongation, D-dimer elevation and fibrinogen is normal range; sometimes Protein C and Protein S together with Antithrombin III variable reduction. No sepsis findings were documented. Severe thrombocytopenia (<20.000/μL) and marked anaemia (Hb <7gr/dl, HCT <21%) were also present. *Results.* By FEIBA® administration (90 IU/Kg) at first infusion (n=3) ,at second infusion (n=5) after 6 hrs, at third infusion (n=2) after 12-18 hrs, at fourth infusion (n=4) after 24 hrs we achieved GI haemorrhages complete control with safe and efficacious outcomes, even without PC in 6 patients. Interestingly, in 5 patients we carried out during their critical haemorrhagic occurrences a scintigraphy with homologous RBC Cr-51 labelled to explore the site of GI tract lesion. We noted small bowel origin of bleeding (n=3) and in caecum/ascendent colon (n=4). Obviously, the surgeon excluded the operation as well as the digital subtraction angiography (DSA) to perform transcatheter embolization even in emergency owing to massive bleeds and/or critical conditions of the patients. It is noteworthy that after FEIBA® in 4 of these same patients we demonstrated by RBC labelled Cr-51 findings no GI haemorrhage, thus providing strong evidence that bleeds has been resolved. *Summary/Conclusions.* Despite the mechanisms by which FEIBA® determines bleedings' stopping in life-threatening haemorrhages of patients with haematological malignancies are to be defined, from our observation and for critical practice we propose FEIBA® employment safely in GI dramatic and massive bleeds, even if this indication is off-label, but efficacious based on clinical endpoints.