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**POSTER**

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**Editorial Office**
SIMTI Servizi Srl
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Tel.: +39 02 23951119
Fax: +39 02 23951621
francesca.fermi@simtiservizi.com

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E.g.:

*Momi S, Cecchetti L, Gresele P. Platelet-derived matrix metallo-proteinase (MMP)-2 contributes to atherosclerosis in strongly hypercholesterolemic mice. Blood Transfus 2010; 8 (Suppl 4): OC035.*

To the associates of the Italian Society for Thrombosis and Haemostasis

To the readers of Blood Transfusion

To all those interested in Thrombosis and Haemostasis

As the President of the Italian Society for Thrombosis and Haemostasis (SISET), and on behalf of the whole Executive Committee of the society, I am glad to present to you this Journal Supplement reporting all the abstracts of the scientific papers accepted for communication at the XXI National Congress of our Society.

These abstracts were peer reviewed by three to five blinded reviewers each; the reviewers were selected among the senior members of the society with a valuable record of publications; the scores assigned by the referees were mediated and used to rank the abstracts, and select them for oral communication or poster display.

We opted to maintain this organisation in this Supplement, presenting the abstract aggregated in oral communication session and the posters by broader discipline areas.

As the reader will easily understand going through this collection, the biennial National Congress of the SISET is a showcase for all the scientific activity that the members developed in the preceding two years, so that some results presented here might have been recently published, overlap, in part or at all, with those presented at the last Congress of the International Society for Thrombosis and Haemostasis, held in Boston on July 2009, or with the World Federation of Haemophilia World Congress, held in Buenos Aires on July 2010. We did accept and encourage presentation of similar papers for the sake of discussion within the society, for prompting further research and for educational purposes. It has to be pointed out here that the number of associated increased by 18% in the last two years, and that more than 800 hundreds patients subscribed for the meeting, a never before reached goal.

On the other hand, the reader will find out that the majority of the paper presented are first time reports of first line research in all the hot topic areas of thrombosis and haemostasis. This is the main reason that prompted the Executive Committee to seek for a PubMed indexed, online available journal to host the proceedings of the Congress. They felt it useful to offer to a wider community of investigators the possibility to reach this interesting body of scientific evidence. Additionally, we were delighted to offer to our youngest member a chance to have their own work cited in their curricula, hoping this will be a good start of a bright scientific career. SISET is a small society, but we can proudly acknowledge many outstanding worldwide renowned scientists among our associates, and we have been the pleasure to see new ones coming on the stage.

My co-guest editor, Alfonso Iorio, Secretary to the Society, and myself did any efforts to clean up this supplement from any typos and we also tried to increase the consistency in affiliations and terminology, and finally provided indexing by author. Notwithstanding, the abstracts have to be considered published as submitted and, as it was clearly stated on the submission sheet, the authors are the only responsible for the content of their abstracts.

We do hope to have provided a valuable contribution to the scientific knowledge in the coagulation field.

We thank all the authors for sending their scientific contribution, the reviewers for their anonymous contribution, the members of the executive committee for their rele in refining the Congress programme.

We finally would like to warmly thank Claudio Velati, Editor in Chief of Blood Transfusion for hosting this supplement, Pasquale Pignatelli, Member of the SISET executive board, and Arianna Mariani, who managed the abstract submission website, and Francesca Fermi, at Blood Transfusion Editorial Office, for her invaluable support in editing the material and ultimately have this supplement completed.

Alfonso Iorio
SISET, Secretary

Gualtiero Palareti
SISET, President

# *COMUNICAZIONI ORALI*

## FIBRINOLISI

### OC001 TAFI AND FEED-BACK ACTIVATION OF FACTOR XI PLAY A MAJOR ROLE IN PLATELET-MEDIATED RESISTANCE TO FIBRINOLYSIS

Carrieri C., Semeraro F., Ammollo T.C., Semeraro N., Colucci M.
*Dept. Biomedical Sciences, Sect. of General & Experimental Pathology, Bari*

Platelet-rich thrombi are resistant to fibrinolysis. Because platelets promote thrombin formation it is surmised that they will also enhance TAFI (thrombin activatable fibrinolysis inhibitor) activation. It is unclear, however, if the augmented generation of activated TAFI (TAFIa) will add to the strong antifibrinolytic effect resulting from platelet-mediated clot retraction and PAI-1 release. We investigated the contribution of TAFI to platelet-mediated fibrinolysis resistance.
Platelet-poor (PP-WB, $<40x10^3$/ul) and platelet-rich (PR-WB, $>400x10^3$/ul) blood samples were obtained from normal human blood (N-WB, $150\text{-}220x10^3$/ul). Clot lysis time was measured by thromboelastography (Haemoscope), using recalcified blood supplemented with t-PA (100 ng/mL) and tissue factor (1/1,000 diluted Recombiplastin).
t-PA-induced lysis time increased proportionally to platelet concentration (from 21.5±8.9 to 60.1±18.4 min, in PP-WB and PR-WB, respectively). Neutralization of TAFIa activity (by PTCI) or inhibition of thrombin-mediated TAFI activation (by a specific monoclonal antibody) shortened lysis time by 52.3% in PR-WB and by 6.3% in PP-WB, suggesting that TAFI contribution was substantial only in the presence of platelets. Qualitatively similar results were obtained when clot retraction was prevented by cytochalasin D or when PAI-1 was inhibited by a neutralizing antibody. Assay of prothrombin F1+2 and TAFIa during clot lysis confirmed that platelets enhanced both thrombin generation and TAFI activation. Addition of a neutralizing anti-FXI antibody had little effect on clot formation but shortened the lysis time by 38% in PR-WB and by <5% in PP-WB. A significant shortening of lysis time (27%) was also observed when N-WB collected on CTI (corn trypsin inhibitor, a FXIIa inhibitor) was supplemented with the anti-FXI, but not with an anti-FXII antibody. Our data indicate that TAFI activation is one major mechanism whereby platelets make clots resistant to fibrinolysis and suggest that FXII-independent activation of FXI plays an important role in platelet-mediated enhancement of thrombin and TAFIa generation.

### OC002 EFFECT OF WARFARIN TREATMENT ON CLOT RESISTANCE TO FIBRINOLYSIS

Incampo F.[(1)], Carrieri C.[(1)], Galasso R.[(1)], Sciusco A.[(1)], Di Serio F.[(2)], Semeraro N.[(1)], Colucci M.[(1)]
[(1)] *Dept. Biomedical Sciences, Sect. of General & Experimental Pathology, Bari;* [(2)] *Dept. Clinical Pathology I, University-Hospital, Bari*

The amount of thrombin generated after fibrin formation is a major determinant of clot susceptibility to fibrinolysis. One of the major mechanisms behind this effect is the activation of TAFI by thrombin itself. Anticoagulants may or may not stimulate fibrinolysis depending on their effect on thrombin generation kinetics. We evaluated the influence of oral anticoagulant therapy (OAT) with warfarin on clot susceptibility to fibrinolysis.
Sixty-six consecutive patients under OAT (PT-INR =2.8±0.8) and 27 matched controls (PT-INR=1.1±0.13) were studied. Plasma clot formation induced by 1/1,000 thromboplastin and subsequent clot lysis by 45 ng/mL t-PA was monitored by a turbidimetric microplate assay.
OAT, despite its marked anticoagulant effect, displayed only a moderate profibrinolytic activity (lysis times in OAT and controls: 71±26 vs. 79±12 min, respectively, p=0.001), which was obviously less pronounced than that of an equipotent concentration of heparin (48±7.2 vs. 67±3.4 min, p<0.0001). Upon addition of PTCI (a selective inhibitor of activated TAFI) the lysis time was shortened by only 10±9.4% in OAT as compared to 30±5.8% in controls (p<0.0001), indicating very little activation of TAFI in the former. Surprisingly, however, the lysis time in the presence of PTCI (which reflects the TAFI-independent fibrinolysis) was longer in OAT than in controls (63±19.2 vs. 55±6.1 min, p=0.005), suggesting that warfarin treatment might also produce an antifibrinolytic effect. Spearman's analysis indicated that PT-INR was significantly correlated to plasma fibrinolysis in OAT (p=0.02) but explained <10% of the lysis time variability. Our data indicate that warfarin treatment reduces TAFI activation. However, the resulting profibrinolytic activity is lower than expected probably because it is partly blunted by other yet unknown effects of warfarin. It remains to be established whether fibrinolysis stimulation contributes to the antithrombotic effect of warfarin and whether clot lysis measurements may improve the monitoring of OAT.

### OC003 A HYPERCOAGULABLE AND HYPO-FIBRINOLYTIC STATE IS DETECTABLE BY GLOBAL METHODS IN PATIENTS WITH RETINAL VEIN OCCLUSION

Lami D.[(1)], Cellai A.P.[(2)], Sofi F.[(1)], Grifoni E.[(1)], Fedi S.[(1)], Rogolino A.[(2)], Sodi A.[(3)], Abbate R.[(1)], Prisco D.[(1)]
[(1)] *Department of Medical and Surgery, University of Florence, Florence;* [(2)] *Department of Heart and Vessel Thrombosis Centre, Azienda Osp., Florence;* [(3)] *Department of Oto-Neuro-Optha, University of Florence, Florence*

The pathogenesis of retinal vein occlusion (RVO), an important cause of permanent visual loss, 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 of hypercoagulability and hypofibrinolysis in these patients. Recently, new global tests have become available to improve the capability of studying the pathophysiology of this disease.
Aim of our study was to evaluate in RVO patients coagulation and fibrinolytic alterations by two global tests: Endogenous Thrombin Potential (ETP) and Clot Lysis Time (CLT). In addition, total and free Tissue Factor Pathway Inhibitor (TFPI) were examined.
We studied 81 RVO patients (39 males and 42 females; median age 61 years; range 18-70 years) and a control group matched for age and sex. The ETP was measured using a functional chromogenic assay (Dade Behring ETP) and results were expressed as Tlag (sec), Tmax (sec), Cmax (mA/min),

ETP % (U/mL). CLT (min) was determined by a plasma-based, tissue factor-induced clot lysis assay, in which a fibrin clot is lysed by exogenously added t-PA. TFPI total (ng/mL) and TFPI free (ng/mL) were determined by using ELISA commercial kits. Cmax, ETP, CLT, values were significantly higher in RVO patients than in control group (Cmax 135.68±23.57 vs. 127.56±21.73 sec, p=0.025; ETP 111.01±15.86 vs. 97.08±9.46 U/mL p<0.0001; CLT 75.02±13.49 vs. 69.96±14.71 sec, p=0.025). Instead, TFPI total and TFPI free were significantly lower than in control group (TFPI total 68.98±12.97 ng/mL vs. 74.14±18.30; TFPI free 10.03±4.58 vs. 12.01±5.50 ng/mL; p=0.043 and p=0.015, respectively).
Our results suggest that in RVO patients an increased thrombin generation exists which may lead to an impaired fibrinolysis, probably due to a decreased availability of TFPI.

## OC004 **HOMOCYSTEINE PLASMA LEVELS INFLUENCE IMPAIRED FIBRINOLITIC CAPACITY BUT NOT *IN VITRO* CLEAVAGE OF FIBRIN BY EXOGENOUS PLASMIN IN PATIENTS WITH PULMONARY EMBOLISM**

Cellai A.P.[(1)], Lami D.[(2)], Grifoni E.[(2)], Fiorillo C.[(3)], Becatti M.[(3)], Olimpieri B.[(1)], Poli D.[(1)], Miniati M.[(2)], Abbate R.[(2)], Prisco D.[(2)]
*[(1)] Department of Heart and Vessels, Azienda Ospedaliero-Universitaria Careggi, Thrombosis Centre, Florence; [(2)] Department of Medical and Surgical Critical Care, University of Florence, Firenze; [(3)] Department of Biochemical Sciences, University of Florence, Florence*

**Background** We recently demonstrated that fibrin resistance to lysis occurs in patients with prior pulmonary embolism (PE). Hyperhomocysteinemia (HHcy) is a marker of hypercoagulability and has been associated with impaired *in vitro* fibrinolysis. It is unclear whether impaired fibrinolysis reflects an alteration of fibrinogen or an abnormality in fibrinolysis. In VTE patients, mild HHcy was found to be associated with increased TAFI levels and reduced clot lysis time (CLT).
**Objective** To establish whether homocysteine levels are related to the extent of *in vitro* fibrin degradation induced by plasmin, and to plasma levels of fibrinolytic components.
**Patients** 41 patients with prior PE and no pulmonary hypertension 13 with Hhcy 28 with normal (NHcy) homocysteine levels.
**Methods** Plasma fibrinolytic potential was measured as lysis time of tissue factor (TF)-induced clots exposed to exogenous t-PA. The rate of *in vitro* plasmin-mediated cleavage of fibrin β-chain was assessed over a 6-hour period on fibrin clots, which were obtained by exposition to thrombin on purified fibrinogen. Homocysteine plasma levels were measured by Abbott Imx immunoassay, and HHcy was defined as levels of fasting Hcy>95$^{th}$ percentile of the values in healthy subjects.
**Results** Homocysteine levels correlated with CLT (p=0.005), but not with the impairment of plasmin-mediated lysis of fibrin β-chain. CLT was not significantly different between HHcy and NHcy patients. A significant difference was observed when the assay was performed in presence of the TAFIa inhibitor PTCI (p=0.04). No differences were found between the two groups in plasma levels of fibrinolytic components, nor in the rate of plasmin-mediated cleavage of fibrin β-chain.
**Conclusions** HHcy is related to a reduced fibrinolytic potential by influencing fibrinolysis but not to plasmin-mediated cleavage of fibrin. These results question the importance of Hcy-induced alterations of fibrinogen as the major determinant of the increased resistance of fibrin to lysis observed in PE patients.

## OC005 **INDUCIBLE NITRIC OXIDE SYNTHASE (INOS) REGULATION BY $CA^{2+}$/CALMODULIN-DEPENDENT PROTEIN KINASE II IN VASCULAR SMOOTH MUSCLE CELLS (VSMCS) FROM DIABETIC RATS**

Pipino C.[(1)], Di Pietro N.[(1)], Di Tomo P.[(1)], Giardinelli A.[(1)], Di Silvestre S.[(1)], Morabito C.[(2)], Formoso G.[(1)], Mariggiò M.A.[(2)], Consoli A.[(3)]
*[(1)] Department of Medicine and Aging, Aging Research Center, Ce.S.I, Chieti; [(2)] Department of Basic and Applied Medical Science, Aging Research Center , Ce.S.I, Chieti; [(3)] Department of Medicine and Aging, Aging Research Center, Ce.S.I, Chieti*

In diabetes, increased cytokine plasma levels could induce iNOS expression contributing to vascular damage. In addition to transcriptional regulation, iNOS activity may be posttranslationally regulated by palmitoylation and intracellular trafficking. It has been recently demonstrated that the multifunctional protein kinase CaMKII, a known contributor to vascular dysfunction in diabetes, may also play a significant role in iNOS-specific trafficking and activity following cytokine induction. Thus, aim of the present study was to investigate the relationships between cytokine increased iNOS activity and Ca2+/CaMKII/CaMKIIδ2 pathway involvement in vSMCs from diabetic rats (DR).
We measured iNOS expression (RT-PCR, Western Blot) and activity [conversion L-(3H)-arginine into L-(3H)-citrulline], intracellular Ca2+ levels (fluorescence video imaging), CaMKII phosphorylation (Western Blot), iNOS/CaMKIIδ2 co-immunoprecipitation (Western Blot) and nitrotyrosine levels (immunofluorescence) in cultures of aortic vSMCs from 10 diabetic (90% pancreatectomy, DR) and 10 control (sham surgery, CR) rats, after 24 hrs incubation with 20 micrograms/mL Lipopolysaccharide (LPS).
LPS increased iNOS expression to the same extent in CR and DR, while iNOS activity was about 7 folds greater in DR. Exposure to LPS led to an increase of intracellular calcium levels (1.9 folds increase) and $Ca^{2+}$-dependent CaMKII phosphorylation (3 folds increase) followed, as expected, by the absence of iNOS/CaMKIIδ2 co-immunoprecipitation. These LPS effects were associated to the greater induced iNOS-specific activity in DR. In CR, instead, LPS failed to affect these parameters, which were not different from basal levels after LPS stimulation. In addition, the increased iNOS activity in DR was accompanied by a significant increase in intracellular nitrotyrosine levels (1.5 folds increase).
Taken together, these results indicate for the first time that signal pathway involving CaMKII contributes to increase iNOS activity and nitrotyrosine generation in diabetes and might provide new insight in the mechanisms linking diabetes and atherosclerosis.

## MALATTIA DI VON WILLEBRAND

### OC006 TRANSIENT EXPRESSION AND INTRACELLULAR TRAFFICKING STUDY OF TWO NOVEL VON WILLEBRAND FACTOR (VWF) VARIANTS (A1716P AND C2190Y)

Castaman G.[1], Giacomelli S.[1], Haberichter S.L.[2], Obser T.[3], Rodeghiero F.[1], Schneppenheim R.[1]
*[1] Department of Cell Therapy and Hematology, Hemophilia and Thrombosis Center, San Bortolo Hospital, Vicenza; [2] Medical College of Wisconsin Department of Pediatrics Milwaukee, USA; [3] University Medical Center, Hamburg-Eppendorf Department of Pediatric Hematology and Oncology, Hamburg, Germany*

Von Willebrand Factor (VWF) is synthesized in endothelial cells and megakaryocytes. It is either constitutively released into plasma or initially stored in so called Weibel-Palade bodies of endothelium and released in response to an appropriate signal.
We have characterized two novel VWF variants in two different domains of the protein involved in collagen binding. Both patients present low VWF:Ag and activity (15-25%). The first mutation in exon 29 (c.5146 CGT>CCT) causes alanine to proline substitution at position 1716 (A1716P) in the A3 domain of VWF. The second in exon 37 (c.6569 TGC>TAC) causes the cysteine 2190 to tyrosine substitution (C2190Y) in the D4 domain. The effect of these two novel missense mutations was investigated by transient transfection experiments in 293 EBNA cells for functional studies and HEK293cells for cellular trafficking analysis.
The A1716P mutant shows a reduction in the synthesis of VWF (56%) and also its secretion in the medium (44%). When expressed as a ratio to the VWF secreted, the GP1b binding activity is normal, while collagen binding activity was reduced (0.58). The C2190Y variant is characterized by a significant reduced secretion of VWF in the medium, an almost normal GP1b binding activity while collagen binding activity is slightly reduced (0.7), probably due to the loss of cysteine in the D4 domain and to the introduction of a bulky and hydrophilic side chain of the tyrosine residue. In intracellular trafficking studies both variants show the absence of granules similar to Weibel-Palade bodies and the retention of VWF in the endoplasmatic reticulum.

### OC007 PREVALENCE AND DETERMINANTS OF BLEEDING IN SEVERE VON WILLEBRAND DISEASE TYPE 3: RESULTS OF RETRO/PROSPECTIVE STUDIES IN A COHORT OF 105/52 ITALIAN PATIENTS

Federici A.B.[1], Bucciarelli P.[2], Iorio A.[3], Castaman G.[4], Mazzucconi M.G.[5], Morfini M.[6], Rocino A.[7], Schiavoni M.[8], Rodeghiero F.[4], Mannucci P.M.[2]
*[1] Divisione di Ematologia, Dip. Medicina Interna, Milano; [2] Dep of Internal Medicine A. Bianchi Bonomi Hemophilia Thrombosis Center, Milano; [3] Centro Emofilia e Trombosi, Dip. Medicina, Perugia; [4] Divisione Ematologia, Ospedale S. Bortolo, Vicenza; [5] Divisione di Ematologia, Università di Roma, Roma; [6] Centro Emofilia, Ospedale Careggi, Firenze; [7] Centro Emofilia, Ospedale Pellegrini, Napoli; [8] Centro Emofilia, Policlinico, Bari*

Although rare, von Willebrand disease type 3 (VWD3) is of major interest because of its severe symptoms, the need for VWF concentrates and the risk of anti-VWF inhibitor. To determine the prevalence and determinants of bleedings requiring VWF concentrates, data were collected from the Italian registry and VWD3 patients were followed up for one year prospectively.
VWD3 was diagnosed when VWF antigen was undetectable and factor VIII (FVIII) reduced. Bleeding score (BS) was calculated at enrollment. Gene deletions and mutations were searched for in available DNA. Bleeding-free survival was computed with the Kaplan-Meier method and a Coxs proportional hazard model (HR) was used. 105 VWD3 patients (5.7%) were identified among the 1,850 Italian VWD (1.75 per million) with the following demographic, clinical and laboratory parameters (median, range): gender (M/F)=50/55; age=37 (3-65); BSS=18 (3-35); FVIII=4 (2-18); anti-VWF inhibitors=7 cases (6.7%) from 3 families. 65/105 cases had the following gene defects (pt-n): large deletions (7); small deletions and insertions (23); nonsense (9); splice site (8) and missense mutations (17). Mucosal bleedings (64%) were more frequent than hematomas and hemarthrosis (24%). At enrollment 95/105 (91%) VWD3 had been already exposed to VWF concentrates. 52 VWD3 patients could be followed-up and 46 (88%) showed 118 bleeds and 27 surgeries requiring 192 injections of concentrates. BSS>10 (6.8, 3.8-12.3) and FVIII<10 U/dL (4.1, 2.4-7) were associated with high risk of bleeding. The bleeding-free survival showed 4 different KM patterns with the following results: 89% (BSS=5-10 & FVIII>10U/dL); 64% (BSS>10 & FVIII=5-10U/dL); 59% (BSS=5-10 & FVIII<10 U/dL); 18% (BSS>10 & FVIII<5 U/dL). Based on these data we can show that also VWD3 is very heterogeneous. BSS and FVIII are good predictors of bleeding. Multicenter studies should be organized to identify additional modifiers of bleeding risk in VWD3.

### OC008 DOMINANT-NEGATIVE VON WILLEBRAND FACTOR GENE DELETION: MOLECULAR MECHANISM AND CORRECTION APPROACHES

Casari C.[1], Pinotti M.[1], Lancellotti S.[2], Adinolfi E.[3], Casonato A.[4], De Cristofaro R.[1], Bernardi F.[1]
*[1] Biochemistry and Molecular biology, University of Ferrara, Ferrara; [1] Institute of Internal Medicine, Catholic University School of Medicine, Roma; [3] Department of Experimental Medicine, University of Ferrara, Ferrara; [4] Department of Cardiologic, Thoracic and Vascular Science, University of Padua, Pavova*

Understanding molecular mechanisms leading to the dominant inheritance of von Willebrand disease (VWD) would improve our knowledge on pathophysiological aspects underlying its high prevalence. We produced a cellular model of severe type 2 VWD, caused by a heterozygous deletion in the VWF gene, to investigate the altered biosynthesis. Co-expression of the VWF in-frame deleted cDNA (p.P1105_C1926delinsR) impaired wild type vector-driven protein secretion and function (VWF collagen-binding 1.9 0,5% of wt), which mimicked the patient's phenotype.
Protein studies and cell immunostaining delineated the highly efficient dominant-negative mechanism. The deleted VWF

was synthesized in large amounts and, through a correctly encoded cysteine knot domain, formed heterodimers and heterotetramers with wild type VWF. Impaired multimerization was associated with reduced amounts of VWF in late endosomes.
The key role of heterodimers as multimer terminators was further supported by introduction of the dimerization mutation C2773R in the deleted construct, which resulted in a quantitative defect with normal multimer size.
Targeting the mRNA breakpoint by allele-specific siRNA selectively inhibited the in-frame deleted VWF expression, and restored secretion of multimers with improved function (28.0 to 3.3% of wt). This provided a novel tool to explore mutation-specific gene therapy in a severe form of dominant VWD.

OC009 **BLOOD GROUP SIGNIFICANTLY INFLUENCES VON WILLEBRAND FACTOR INCREASE AND HALF-LIFE AFTER DESMOPRESSIN IN VON WILLEBRAND DISEASE VICENZA**

Castaman G.[(1)], Tosetto A.[(1)], Eikenboom J.C.[(2)], Rodeghiero F.[(1)]
*[(1)] Department of Cell Therapy and Hematology, Hemophilia and Thrombosis Center, San Bortolo Hospital, Vicenza; [(2)] Leiden University Medical Center Einthoven, Laboratory for Experimental Vascular Medicine, Department of Thrombosis and Hemostasis, Leiden, The Netherlands*

**Background** Normal subjects with O blood group show reduced survival of von Willebrand factor (VWF) after desmopressin and it has been suggested that this pattern is attributable to the ABO-dependent glycosylation of VWF. Reduced survival of VWF has been also reported in some types of von Willebrand disease (VWD).
**Objectives** We investigated the influence of ABO blood group on the magnitude of response and the factor VIII (FVIII) and VWF survival after desmopressin administration in 19 patients with VWD Vicenza, the epitome of VWD types characterized by increased clearance of VWF.
**Results** Half-life of FVIII and VWF was approximately twice longer and maximal increase over baseline significantly greater in non-O compared to O-blood group patients. This effect was particularly evident with Ristocetin Cofactor activity of VWF (Half-life, min 54.4±14.4 in O vs. 116.7±35.2 in non-O blood group; P=0.0005). No difference was evident concerning VWF propeptide increase and its half-life was evident.
**Conclusions** ABO blood group significantly influences the pharmacokinetics of FVIII and VWF after desmopressin even in patients with accelerated VWF clearance.

OC010 **A PROSPECTIVE EVALUATION OF BLEEDING TENDENCY AND EFFICACY OF ANTIHEMORRHAGIC TREATMENTS IN PATIENTS WITH INCREASED VON WILLEBRAND FACTOR CLEARANCE**

Castaman G.[(1)], Tosetto A.[(1)], Federici A.B.[(2)], Rodeghiero F.[(1)]
*[(1)] Department of Cell Therapy and Hematology, Hemophilia and Thrombosis Center, San Bortolo Hospital, Vicenza; [(2)] University of Milano Institute of Internal Medicine, IRCCS Maggiore Hospital, Milano*

We prospectively evaluated the clinical history of 60 patients with von Willebrand disease Vicenza (VWD-VI) carrying R1205H von Willebrand factor (VWF) mutation and 23 with C1130F mutation, both characterized by increased clearance. During 71 months of follow-up, 35% of patients with VWD-VI and 39% with C1130F did not require treatment. The rate of spontaneous bleeding requiring consultation/treatment was 9.5/100 years/patient in patients with C1130F mutation vs. 2.1/100 years/patient in those with R1205H (p=0.0025, log-rank test). This difference persisted also by multivariate analysis adjusted for sex, age and blood group (HR=3.1 for C1130F, p=0.033) and females were at greater risk of bleeding (HR=3.2, p=0.049) because of menorrhagia. Only 3/15 (20%) women in fertile age with VWD-VI compared to 8/9 (89%) with C1130F mutation required consultation/treatment for menorrhagia (iron supplementation, combined oral estroprogestinic pill, tranexamic acid). Almost all dental extractions, minor surgeries and deliveries occurring during follow-up were successfully covered with desmopressin. Major surgery required FVIII/VWF concentrates, but a few cases benefited from desmopressin. Desmopressin maintains a major therapeutic role in patients with increased VWF clearance as for patients with type 1 VWD. Patients with VWD-VI have lower rate of spontaneous bleeding compared with those carrying C1130F mutation.

## *PIASTRINE: BIOCHIMICA E FISIOLOGIA*

OC011 **HIGH GLUCOSE RAPIDLY UPREGULATES CYCLOOXYGENASE-(COX)-2 IN HUMAN MEGA-KARYOCYTES**

Corazzi T.[(1)], Bury L.[(2)], Bolli G.B.[(3)], Gresele P.[(2)]
*[(1)] Dept. of Internal Medicine, University of Perugia, Perugia; [(2)] Dept. of Internal Medicine, Section of Internal and Cardiovascular Medicine, Perugia; [(3)] Dept. of Internal Medicine, Endocrinology and Metabolism, Perugia*

Type 2 diabetes mellitus (T2DM) is a risk factor for ischemic cardiovascular disease. Aspirin reduces the risk of thrombotic events in a broad range of patients by inhibiting platelet COX-1; however, many diabetic patients are poorly responsive to aspirin. The expression of COX-2 in platelets of diabetics has been claimed as one of the reasons for aspirin non-responsiveness.
Aim of our study was to assess whether the short-term exposure to high glucose affects COX-2 expression in human megakaryocytes (MK).
Circulating human stem-cell-derived MK were incubated with 22 mM glucose or with 22 mM mannitol (osmolarity control) for 24 hours. COX-2 was measured by western blotting in cell lysates and by immunofluorescence microscopy. TxB2 in MK supernatants was determined by RIA.
COX-2 was undetectable in MK cultured in glucose-free medium, but was strongly expressed in MK exposed to high glucose (0.14±0.02 ng/microg total protein, $p<0.05$ vs. control), but not to mannitol. Immunofluorescence confirmed COX-2 in MK.
A significant increase of COX-2 (0 to 0.18±0.01 ng/microg total protein, $p<0.05$ vs. control) was observed already after 4 hours of incubation with high glucose; further incubation in 5.5 mM (normal) glucose-containing medium reduced COX-2

to 0.11±0.01 ng/microg total protein ($p<0.01$) after 4 hours, and to its disappearance after 20 hours.
Preincubation with a glucose transporter inhibitor (cytocalasin B, 1-3 microM) completely suppressed high glucose-induced COX-2 expression.
Arachidonic acid (10 microM)-induced TxB2 production was significantly higher in high glucose-treated MK than in mannitol treated cells (15.0±2.8 ng/$10^6$ cells vs. 9.0±0.5ng/$10^6$ cells; $p<0.01$). Preincubation with aspirin (10 microM) inhibited TxB2 production significantly less in high glucose incubated MKs (5.8±2.5 ng/$10^6$ vs. cells 2.2±0.6 ng/$10^6$ cells; $p<0.05$), while the COX-2 inhibitor NS398 completely inhibited it.
High glucose induces a fast upregulation of MK COX-2, which is rapidly reversible upon prompt correction to normal glucose; COX-2 may be responsible for the high residual TxB2 production in the presence of aspirin.

## OC012 ATORVASTATIN INHIBITS OXIDATIVE STRESS VIA ADIPONECTIN-MEDIATED NADPH OXIDASE DOWN REGULATION IN HYPERCHOLESTEROLEMIC PATIENTS

Carnevale R.[(1)], Pignatelli P.[(1)], Di Santo S.[(1)],Bartimoccia S.[(1)], Calabrese C.[(1)], Mitrugno A.[(1)], Napoleone L.[(1)], Tanzilli G.[(2)], Basili S.[(1)], Violi F.[(1)]
*[(1)] Medicina Sperimentale, Sapienza, I Clinica Medica, Roma; [(2)] Cuore e Grandi Vasi, Sapienza, Roma*

**Background** Interventional treatment with atorvastatin lowered the circulating levels of the catalytic core of NADPH oxidase, namely sgp91phox, but the underlying mechanism is still undefined.
**Aim** To test the hypothesis that the inhibitory effect on oxidative stress, induced by Atorvastatin, could be mediated by adiponectin.
**Methods and Results** We compared 36 patients with polygenic hypercholesterolemia and 18 healthy subjects. Patients were randomized to either a diet (Group A) or atorvastatin 10 mg/day (Group B) for 30 days. Lower serum adiponectin levels and higher lipid profile, gp91phox serum levels, urinary isoprostanes, platelet oxygen free radicals, characterized patients. After 30 days of treatment, group B showed higher levels of adiponectin which is inversely correlated to reduced levels of sgp91phox, urinary isoprostanes and platelet oxygen free radicals ($p<0.001$).
In *in vitro* model adiponectin, dosages between 5 and 10 ng/mL, inhibited p47phox translocation to gp91phox and soluble gp91phox cleavage indicating its ability in inhibiting the assembly of NADPH oxidase subunits on cell membrane and in turn the enzymatic system activation.
**Conclusion** This study provides the first evidence that higher adiponectin serum levels are associated with gp91phox down-regulation in hypercholesterolemic patients and that atorvastatin exerts an antioxidant effect via adiponectin-mediated NADPH oxidase inhibition.

## OC013 A NOVEL ROLE FOR SEROTONIN IN PLATELET CALCIUM SIGNALING UNDER FLOW

Battiston M.[(1)], Cozzi M.R.[(1)], Deana R.[(2)], Mazzucato M.[(1)], Ruggeri Z.M.[(1)], De Marco L.[(1)]
*[(1)] Department of Laboratory Medicine, CRO National Cancer Institute I.R.C.C.S., Aviano (PN); [(2)] Department of Biological Chemistry, University of Padova, Padova*

**Background** Serotonin (5-hydroxytryptamine, 5-HT) has previously shown to play an important role on platelets function. Platelets contain three serotoninergic components: 5HT2A receptor, SERT and VMAT2. Intact platelets take up 5-HT by the SERT and store the monoamine in vescicules by VMAT2. After platelet activation, secreted 5-HT, binding to 5-HT2A, stimulates Ca++ mobilization through PLCb activation.
**Aim** The aim of this work was to study the role of platelet 5-HT in eliciting intracellular calcium signalling under flow.
**Methods** Platelet adhesion, activation and aggregate formation onto a WVF substrate were monitored at 1,500 $sec^{-1}$ using a real-time video microscopy. We analysed concurrently the instantaneous velocity and [Ca++]i in single platelets loaded with FLUO 3-AM or treated with serotonin (1 microM), ketanserine (5-HT2A antagonist, 5 μM), fluoxetine (SERT inhibitor, 1-100 μM), metilamine or ADP receptor antagonist MRS 2179 and ARC-69931 MX.
**Results** Platelets adhering to VWF under high shear rate demonstrate [Ca++]i elevation and several Ca++ flashes propagating along flow direction with a decreasing intensity depending on the distance from the point of origin. We have identified 5-HT as the key mediator of ICC (Intercellular Calcium Communications) since ketanserine completely abolishes its occurrence; similar findings were also found with R96544, a different 5-HT2A antagonist. On the contrary, fluoxetine was shown to have no effect of these events. By increasing the concentration of platelet 5-HT we observed an increased intensity and frequency of ICC. Furthermore, 5-HT2A blockage was accompanied by a decreased surface coverage (35±0.92 versus 58±6.1 of the control) and size and volume of platelet aggregates.
**Conclusion** Our results demonstrated that platelet 5-HT, through its binding to the 5-HT2A receptor, plays a pivotal role in the generation of intercellular calcium signals at high shear rate and contributes to platelet calcium cross-talk. The amplification and temporal modification of calcium signals regulate platelet adhesion and aggregate formation.

## OC014 DETERMINANTS OF PLATELET PARAMETERS IN THE HEALTHY POPULATION OF THE MOLI-SANI PROJECT

Santimone I.[(1)], Di Castelnuovo A.[(1)], De Curtis A.[(1)], Spinelli M.[(2)], Cugino D.[(1)], Donati M.B.[(1)], Cerletti C.[(1)], de Gaetano G.[(1)], Iacoviello L.[(1)]
*[(1)] Laboratori di ricerca, Università Cattolica del Sacro Cuore, Campobasso; [(2)] Laboratori di Ricerca, Università Cattolica del Sacro Cuore, Termoli*

**Background** Platelets are essential for primary haemostasis and repair of the endothelium, and play a key role in the development of acute cardiovascular disease. We investigated the association between four platelet indices namely, count (PLT), mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW) -with inflammatory markers, biochemical and environmental determinants in an apparently healthy population from the Moli-sani project.

**Methods** The Moli-sani Project is an epidemiological cohort study in men and women, aged ≥35 years, randomly recruited from the Molise region, Italy. From March 2005 to December 2009, 23,806 subjects were enrolled. After the exclusion of incomplete questionnaires or subjects with a history of CVD, cancer, hepatitis B or C, hematological disease or missing PLT values, 15,415 subjects were analyzed (53% women, mean age 56±12). Emochromocytometric analyses were performed in the centralized Moli-sani laboratory on fresh samples, using an automatic analyzer (Beckman Coulter, IL, Milan, Italy).
**Results** A large number of variables were associated with platelet measures in univariate analysis. However, in multivariate analyses PLT was lower in men [odds ratio (OR) for increasing tertiles of PLT: 0.43, 95% CI: 0.40-0.47] and in elderly (OR=0.49, 95% CI: 0.43-0.55), but higher in subjects with elevated total cholesterol (OR=1.46, 95% CI: 1.17-1.88), C-reactive protein (OR=1.36, 95% CI: 1.22-1.51) or white blood cell count (WBC) (OR=4.36, 95% CI: 3.91-4.87). Similar results were found for PCT. MPV was lower in men (OR=0.65, 95% CI: 0.60-0.71) but higher in smokers and in hypertensive subjects and increased with WBC. There was no association between any platelet indices and anti-platelets drugs.
**Conclusions** A large panel of environmental variables/CVD risk factors explain a small portion only of platelet parameters variability (area under roc curve <70%), the strongest determinants being age, sex, CRP, WBC. Platelet parameters modifications might contribute to the link between inflammation and CVD risk.

OC015 **MATRIX METALLOPROTEINASE (MMP)-2 ENHANCES PLATELET-ADHESION UNDER FLOW CONDITIONS**

Appolloni V., Guglielmini G., Falcinelli E., Gresele P.
*Section of Internal and Cardiovascular Medicine, Department of Internal Medicine, Perugia*

Platelets contain and release several MMPs, enzymes involved in the degradation of extracellular matrix, and one of these, MMP-2, potentiates platelet aggregation. Platelet adhesion is the first, step in the interaction between circulating platelets and a damaged vascular wall, starting the processes leading to thrombus formation. Little is known about the effects of MMP-2 on platelet adhesion over thrombogenic surfaces. We first studied the effect of MMP-2 on shear-dependent platelet activation using the O'Brien filtration system, in which anticoagulated whole blood is forced at 40 mmHg through a filter and platelets get activated and retained so blocking the filter. In the O'Brien's system, MMP-2 (50 ng/mL) reduced the filter occlusion time (ctrl= 52.5±4.2sec, MMP-2= 40±2.7sec, $p<0.05$) and increased retained platelets (ctrl= 72.3±2.3%, MMP-2= 81.1±1.8%, $p<0.05$). The coincubation of MMP-2 with the MMP-2 specific inhibitor, Inhibitor II, increased filter occlusion time and reduced retained platelets to values comparable to control (occlusion time= 592.4sec, retained platelets= 72.9±2.6%). The concentration of MMP-2 found in plasma after the O'Brien filtration test was significantly higher as compared to basal (+112±41.64 ng/mL, $p<0.05$). In the parallel-plate perfusion chamber MMP-2 increased dose-dependently (from 0.5 to 50 ng/mL) platelet deposition on type 1 fibrillar collagen under flow conditions, with a maximal increase at 50 ng/mL (+14%±5.85%, $p<0.006$).
The potentiating effect was time-dependent, with a maximal effect between 2 and 10 minutes of pre-incubation, progressively decreasing thereafter.
Platelet adhesion was increased by MMP-2 (10 ng/mL) at all the shear rates tested (250 $sec^{-1}$=+8.8±3.6%; 1,000 $sec^{-1}$= +7.8±3.9%; 3,000 $sec^{-1}$=+8.7±4.1%). In samples coincubated with the MMP-2 inhibitor the potentiating effect of MMP-2 on, platelet adhesion disappeared (MMP-2 +Inhibitor II 250 $sec^{-1}$=−0.38±23.6%; 1,000 $sec^{-1}$=–3.3±3.4%; 3,000 $sec^{-1}$ =−0.93±4.1% compared to control).
In conclusion, MMP-2 potentiates shear induced platelet activation, and in particular deposition onto immobilized type I collagen. Potentiation of platelet adhesion by MMP-2 may contribute to its prothrombotic activity *in vivo* (Momi et al., J Exp Med 2009;206:2365).

## *REGISTRI*

OC016 **ITALIAN REGISTRY OF ADVERSE PREGNANCY OUTCOMES AND VENOUS THROMBOEMBOLIC DISEASE IN PREGNANCY**

Tormene D., Grandone E., De Stefano V., Palareti G., Tosetto A., Margaglione M., Legnani C., Martinelli I., Castaman G., Simioni P.
*Department of Cardiologic, Thoracic and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua, Italy*

Venous thromboembolic disease (VTE) in pregnancy is an important cause of maternal morbidity and mortality. In women over half of all venous thromboembolic events occurring in the reproductive age are related to pregnancy and about 15% of maternal death are related to pulmonary embolism. Several conditions have been identified as important risk factors for the development of VTE during pregnancy and post-partum: a previous episode of VTE, thrombophilia, operative delivery, increasing maternal age, obesity and prolonged immobilisation.
Obstetric complications occur in at least 10% of pregnancies. Several studies in the last few years have found an association between the presence of thrombophilia and adverse pregnancy outcomes. However, the size of this increased risk and the mechanism which explain this association are not clarified yet.
(LMW)Heparin is the anticoagulant most often used in pregnancy for treatment and prophylaxis. Disadvantages include the risk of uncontrolled bleeding, allergic reactions, heparin-induced osteoporosis and heparin-induced thrombocytopenia.
At present there are no clear guidelines about doses and duration of treatment in pregnant women who develop a VTE or need a thromboprophylaxis.
Moreover, whether anticoagulant therapy will improve the outcome in women with pregnancy complications is unknown.
**Primary objective** To collect data about thromboembolic events and obstetric complications during pregnancy, with particular regards to the thromboprophylaxis/anticoagulant therapy used.
**Design** Registry (observational cohort study).
**Inclusion and exclusion criteria** Women who develop a

thrombotic (venous or arterial) event or an obstetric complication during pregnancy will be eligible for study entry.
**Primary outcomes** To collect data on: treatment of VTE in pregnant women, thromboprophylaxis, obstetric complications ad anticoagulant prophylaxis, correlation with thrombophilia.
**Secondary outcomes** Side effects of anticoagulant treatment or prophylaxis pregnancy outcomes should be collected during an observational period of 45 months.

## OC017 THE ITALIAN REGISTRY OF CHILDHOOD THROMBOSIS (REGISTRO ITALIANO TROMBOSI INFANTILE - RITI)

Gentilomo C.[1], Saracco P.[2], Agostini M.[3], Bagna R.[4], Bassi B.[5], Giordano P.[6], Laverda A.M.[7], Molinari A.C.[8], Ramenghi L.A.[9], Simioni P.[10]
*[1] Pediatria, Ospedale dell'Angelo - Università di Padova, Venezia Mestre; [2] Oncoematologia Pediatrica, OIRM-S. Anna, Torino; [3] Pediatria A/B DEA, OIRM-S. Anna, Torino; [4] Dipartimento di Scienze Pediatriche e dell'Adolescenza, Università di Torino, Torino; [5] Neuropsichiatria Infantile, Università di Torino, A.S.O. OIRM-S. Anna, Torino; [6] Oncoematologia Pediatrica, Dipartimento di Biomedicina dell'Età Evolutiva, Università di Bari, Bari; [7] Pediatria, Università di Padova, Padova; [8] Centro Emostasi e Trombosi, Dipartimento Ematologia-Oncologia, Ospedale Pediatrico Giannina Gaslini IRCCS, Genova; [9] Patologia Neonatale, Policlinico Mangiagalli-IRCCS, Milano; [10] Dipartimento di Scienze Cardiologiche, Toraciche e Vascolari, Università di Padova, Padova*

Thromboembolism (TE) in newborns and children is becoming a rapidly growing condition burdened by high mortality and morbidity. A dramatic increase in VTE (from 34 to 58 cases/10,000 admissions) has been reported in 40 tertiary care hospitals in US in 2001-07. Risk factors, clinical features and prognosis are age-dependent as well as optimal treatment strategy; however RCT are not available and most current recommendations are extrapolated by adult studies. In 2008 a multi-centre research network of Italian investigators has developed a national prospective on-line registry of childhood TE (RITI-www.trombosinfantili.it). The project has been supported by ALT-onlus. Primary aim, inclusion criteria, data collected: to enrol most cases of childhood (birth -18 yrs) TE (systemic venous and arterial TE and stroke) since 1/1/2007 and to collect data on risk factors, clinical, laboratory and radiographic findings, dosing, safety and failure rate of antithrombotic treatments and acute and long-term patient outcome. Recurrences, perceived quality of life, number of hospital admissions and procedures for TE sequelae are included, providing information on the social cost of pediatric TE.
**Results** RITI has completed the pilot phase and started the final launching by March 2010. It includes more than 100 pts, confirming results from other countries, primarily a different approach to TE among various pediatric centres and a poor prognosis in more than half of patients. Neonates and adolescents are the most representative age groups; cardiac and oncological disease and central vascular line the most frequent associated conditions; stroke cases are overrepresented (45%) as most of original investigators being pediatric neurologists; LMWH has been the first choice anticoagulant in 90%.
RITI will represent an opportunity for both Italian paediatricians and investigators of other registries to collaborate on multiple studies on risk factors, diagnostic investigations and outcome of childhood TE as well as on clinical trials.

## OC018 SEASONAL AND MONTHLY VARIABILITY IN THE INCIDENCE OF VENOUS THROMBO-EMBOLISM: A SYSTEMATIC REVIEW AND A META-ANALYSIS OF THE LITERATURE

Dentali F.[1], Ageno W.[1], Rancan E.[1], Donati A.[1], Galli L.[1], Squizzato A.[1], Venco A.[1], Manfredini R.[2]
*[1] Medicina Clinica, Università dell'Insubria, Varese; [2] Medicina Clinica e Sperimentale, Università di Ferrara, Ferrara*

**Background** Many studies showed that the occurrence of cardiovascular and cerebrovascular events exhibits a seasonal and monthly variation. On the other hand, evidences on the existence of a seasonal and monthly variation in the incidence of venous Thromboembolism (VTE) are more conflicting. Therefore, we conducted a systematic review and a meta-analysis of the literature to assess the presence of an infradian rhythm of this disease.
**Methods** MedLine and Embase databases were searched up to January 2010. Monthly and seasonal variation in the incidence of VTE were analyzed.
**Results** Nineteen studies for a total of about 40,000 patients were included in our systematic review. Thirteen studies (34,557 patients) analyzed the seasonal variation and 10 studies (22,825 patients) the monthly variation of VTE. Our results showed a significantly increased incidence of VTE in winter in comparison to the other seasons (chi-square 158.86, $p<0.001$). Thus, in winter there is a RR of VTE of 1.116 (99% CI [1.115, 1.117]) and an absolute increased risk of 11.62% (99% CI [11.52, 11.72]) in comparison to the other seasons.
Furthermore, our analysis showed a significantly increased incidence of VTE in January in comparison to the other months (chi-square 232.57, $p <0.001$). Thus, in January there is a RR of VTE of 1.194 (99% CI 1.186, 1.203) and an absolute increased risk of 19.46% (99% CI, [18.64, 20.28]) in comparison to the other months. Subgroup analyses including only idiopathic venous thromboembolic events confirmed the results of principal analyses.
**Conclusions** Our data support the presence of a infradian pattern in the incidence of venous thromboembolic events, with a significantly higher risk in winter and in January. Future studies are needed to better clarify the mechanisms behind this pattern.

## OC019 RISK OF BLEEDING IN LOW-RISK ATRIAL FIBRILLATION (AF) PATIENTS ON WARFARIN WAITING FOR ELECTIVE CARDIOVERSION

Poli D.[1], Antonucci E.[2], Grifoni E.[2], Paoletti O.[3], Rancan E.[4], Dentali F.[4], Testa S.[3]
*[1] Dept of Heart and Vessels Thrombosis Centre, Azienda Ospedaliero-Universitaria Careggi, Florence; [2] Dept. of Medical and Surgical Critical Care, University of Florence, Florence; [3] Haemostasis and Thrombosis Centre A.O. Istituti Ospitalieri of Cremona, Cremona; [4] Dept of Clinical*

*Medicine, University of Insubria, Varese*

Systemic embolism is the most serious complication of cardioversion of AF and the immediate post-cardioversion period is associated with increased risk for thrombus formation. For this reason VKAs treatment 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 multicentre study on 242 low-risk AF patients (CHADS2 score 0-1) starting warfarin for elective cardioversion. 178 were males (73.6%), mean age 63.9±9.8 years, 60 patients (25%) were aged ≤59 years. Patients with CHADS2 score=0 were 73 (30%), those with CHADS2 score=1 169 (70%); 142 (58.7%) were hypertensive, 2 (0.8%) had diabetes mellitus, 7 (2.9%) suffered from heart failure, and 18 (7.4%) were ≥75 years. Patients were on VKA treatment for a median time of 159 days (range 30-631), total follow-up period 127 patient/years (pt-yrs). Quality of anticoagulation and occurrence of bleeding events were recorded. Patients spent 23%, 64% and 8% of time below, within and above the intended therapeutic range, respectively. When we observed the INR levels, we found that 62 patients (25.6%) had INR>4.5 at least in one occasion, and 23 (9.5%) in ≥2. During follow-up 2 patients had major bleeds (rate 1.6% pt-yrs), one fatal.

In conclusion, our results show that low-risk AF patients for whom elective cardioversion is planned are exposed to VKA treatment, in many occasions also for long periods, with a not irrelevant risk of bleeding. Of note, only 2.9% had of our patients had haemodynamic indication to cardioversion that was performed essentially for rhythm control. Efforts are required to adequately select patients who could benefit from this procedure limiting time of warfarin exposure.

## OC020 **VKA TREATMENT AND BLEEDING RATE OF PATIENTS AGED ≥80 YEARS: RESULTS FROM A PROSPECTIVE COLLABORATIVE STUDY. ON BEHALF OF THE AD HOC STUDY GROUP OF FCSA**

Poli D.[(1)], Antonucci E.[(2)], Grifoni E.[(2)], Di Gennaro L.[(3)], Falanga A.[(4)], Falco P.[(5)], Grasso M.V.[(6)], Marongiu F.[(7)], Pedrini S.[(8)], Pignatelli P.[(9)], Scortechini A.R.[(10)], Benedetti R.[(11)], Testa S.[(12)], Ageno W.[(12)], Palareti G.[(13)]

*[(1)] Dept of Heart and Vessels Thrombosis Centre, Azienda Ospedaliero-Universitaria Careggi, Florence; [(2)] Department of Medical and Surgical Critical Care, University of Florence, Florence; [(3)] Haemostasis Research Center, Catholic University School of Medicine, Roma; [(4)] Department of Oncology/Hematology, Ospedali Riuniti of Bergamo, Bergamo; [(5)] Thrombosis Centre, Hospice San Marco, Latina; [(6)] UOC Internal Medicine, Lastaria Hospital, Latina; [(7)] Department of Medical Sciences "Mario Aresu", University of Cagliari, Cagliari; [(8)] Laboratory Service, Hospital of Brescia, Brescia; [(9)] Department of Experimental Medicine, Sapienza University, Roma; [(10)] Haematology Clinic, Umberto I Hospital, Ancona; [(11)] Dept. Internal Medicine 1, Civic Hospital S. Andrea, La Spezia; [(12)] Haemostasis and Thrombosis Centre, AO Istituti Ospitalieri, Cremona; [(13)] Angiology and Blood Coagulation, University Hospital of Bologna, Bologna*

The increasing number of very old patients on treatment with vitamin K antagonists (VKAs) requires a better knowledge of the risks associated with this treatment in elderly. We performed a prospective collaborative study among Centres affiliated to FCSA to assess the adverse events of VKAs in patients who started treatment after 80 years of age. Patients ≥80 years suffering from atrial fibrillation (AF) or venous thromboembolism (VTE) were prospectively followed-up from the start of treatment. Quality of anticoagulation and adverse events occurring during follow-up were recorded. Twenty-seven Centres participated to the study, 13 (48%) in the north, 9 (33%) in the centre and 5 (19%) in Southern Italy. The total number of patients recruited was 4,067 (Males 43%), median age 84 (range 80-102) years, total follow-up period was 9,571 patient/years; 3,015 patients (74.1%; 7,620 patient/years; mean time of follow-up 2.52 years) were on VKA for AF and 1,052 (25.9%; 1,951 patient/years; mean time of follow-up 1.85 years) for VTE. During follow-up 395 patients (9.5%) died, 21 (5.3%) for bleeding complications, rate 0.21x100 patient/years.

The total quality of anticoagulation measured as time spent within, above and below the international normalized ratio therapeutic range was 62%, 11% and 24%, respectively [IQR for time in therapeutic range (TTR) 49-75]. AF patients showed a better quality of anticoagulation (TTR 63%) vs. VTE patients (TTR 59%) p=0.000. During follow-up 180 major bleeding events were recorded (rate 1.88 x100 patient/years); 21 (11.6%) fatal; 133 among AF patients (1.75x100 patient/years) and 47 among patients with VTE (2.40x100 patient/years); RR 1.4 (0.96-1.93) p=0.06.

In conclusion, among very old patients on VKA treatment we observed a trend to a higher rate of bleeding among VTE patients in comparison to AF patients. However, the rate of bleeding events is acceptably low, probably due to the good quality of anticoagulation recorded.

## *TEV: D-DIMERO*

## OC021 **D-DIMER AND RESIDUAL VEIN OBSTRUCTION AS RISK FACTORS FOR RECURRENCE DURING AND AFTER ANTICOAGULATION WITHDRAWAL IN PATIENTS WITH A FIRST EPISODE OF PROVOKED DEEP VEIN THROMBOSIS**

Cosmi B., Legnani C., Cini M., Guazzaloca G., Palareti G.
*Divisione di Angiologia e Malattie della Coagulazione M. Golinelli, Università di Bologna, Ospedale S. Orsola-Malpighi, Bologna*

**Background/Aims** D-dimer (D-d) and residual venous obstruction (RVO) have been separately shown to be risk factors for recurrent VTE after a first episode of unprovoked proximal deep vein thrombosis (DVT). The aim of the study was to assess the predictive value of D-d and RVO, alone and in combination, for recurrence after provoked DVT of the lower limbs.

**Materials and Methods** Consecutive patients with a first episode of symptomatic provoked proximal DVT were evaluated at a university hospital in Bologna, Italy. On the day of anticoagulation withdrawal (T0), RVO was determined by compression ultrasonography. D-d levels (cut-off: 500 ng/mL) were measured at T0 and after 30+10 days (T1). The main

outcome was recurrent VTE during a 2-year follow-up.
**Results** 296 patients were evaluated. D-d was abnormal in 11% (32/276) and 31% (85/276) of subjects at T0 and at T1, respectively. RVO was present in 45% (132/294) of patients. Recurrence rate was 5.1% (15/296; 95% CI: 3-8%; 3% patient-years; 95% CI: 2-5%). An abnormal D-d either at T0 or at T1 was associated with an adjusted hazard ratio (HR) for recurrence of 4.2 (95% CI: 1.2-14.25; p=0.019) and 3.8 (95% CI: 1.2-12.1; p=0.023), respectively, when compared with normal D-d. The HR for recurrence associated with RVO was not significant and RVO did not increase the recurrence risk associated with an abnormal D-d either at T0 or T1.
**Conclusions** An abnormal D-d during anticoagulation or at one month after anticoagulation withdrawal is risk factor for recurrence in patients with provoked DVT, while RVO at the time of anticoagulation withdrawal is not.

## OC022 SEX, AGE AND NORMAL POST-ANTI-COAGULATION D-DIMER AS RISK FACTORS FOR RECURRENCE AFTER IDIOPATHIC VENOUS THROMBOEMBOLISM IN THE PROLONG STUDY EXTENSION

Cosmi B.[1], Legnani C.[1], Tosetto A.[2], Pengo V.[3], Ghirarduzzi A.[4], Testa S.[5], Prisco D.[6], Poli D.[6], Tripodi A.[7], Palareti G.[1]
[1] *Div. di Angiologia e Malattie della Coagulazione M. Golinelli, Università di Bologna, Ospedale S. Orsola-Malpighi Bologna;* [2] *Divisione di Ematologia, Ospedale S. Bortolo, Vicenza;* [3] *Dipartimento di Medicina Clinica e Sperimentale, Divisione di Cardiologia Clinica, Università di Padova, Padova;* [4] *Dipartimento di Medicina Interna I, Angiologia, Arcispedale Santa Maria Nuova, Reggio Emilia;* [5] *Centro Emostasi e Trombosi Ospedale Generale di Cremona, Cremona;* [6] *Dept of Medical and Surgical Critical Care, Centro Trombosi, Università di Firenze, Azienda Ospedaliero Universitaria Careggi, Firenze;* [7] *Angelo Bianchi Bonomi Hemophilia & Thrombosis Center, Dept. Internal Medicine, University & IRCCS Maggiore Hospital, Milano*

**Background** The PROLONG randomized study showed that patients with an abnormal D-dimer (D-d) after anticoagulation suspension for a first unprovoked episode of venous thromboembolism (VTE) benefited from anticoagulation resumption. In patients with normal D-dimer, anticoagulation optimal duration remains uncertain as the recurrence rate was 4.4 per 100 patients-years.
**Objectives** To assess whether sex and age, in combination with normal D-dimer, are risk factors for VTE recurrence in patients enrolled in the PROLONG study extended follow-up. Methods: D-dimer was measured at one month after anticoagulation suspension. Patients with a normal D-dimer did not resume anticoagulants, while patients with an abnormal D-dimer were randomized either to resume or not anticoagulants. Primary outcome was recurrent VTE.
**Results** After excluding patients resuming anticoagulants, recurrences were higher in males than females (17.8% - 47/263 vs. 11% - 27/244; hazard ratio-HR=1.5; p=0.042) and in patients aged above 65 than younger (20% - 50/249 vs. 9.3% - 24/258; HR=2.07; p=0.0017). In patients with a normal D-dimer and younger than 65 years, recurrences were higher in males than females (5% vs. 0.4% patient-years; adjusted HR=13.3; p=0.0012). Both females and males older than 65 years had more recurrences (6.6% and 8.1% patient-years, respectively, adjusted HR: 18.7; p=0.006 and 21.7; p=0.003, respectively) than females younger than 65 years.
**Conclusions** In patients with a normal D-dimer at one month after anticoagulation suspension for a first episode of idiopathic VTE, females younger than 65 years have a very low risk of recurrence and may not warrant prolonged anticoagulation.

## OC023 THE PREDICTIVE VALUE OF D-DIMER ON THE RISK OF RECURRENT VENOUS THROMBO-EMBOLISM IN PATIENTS WITH MULTIPLE PREVIOUS EVENTS: THE PROLONG PLUS STUDY

Ageno W.[1], Cosmi B.[2], Ghirarduzzi A.[3], Santoro R.[4], Bucherini E.[5], Poli D.[6], Prisco D.[7], Alatri A.[8], Pengo V.[9], Palareti G.[2], Galli L.[1]
[1] *Dipartimento di Medicina Clinica, Università dell'Insubria, Varese;* [2] *U.O. Angiologia e Malattie della Coagulazione, Università di Bologna, Bologna;* [3] *Angiologia, Ospedale di Reggio Emilia, Reggio Emilia;* [4] *U.O.C Emofilia, Emostasi e Trombosi, Azienda Ospedaliera di Catanzaro, Catanzaro;* [5] *Medicina Vascolare, Ospedale di Faenza;* [6] *Dipartimento Cuore e Vasi, AOU Careggi, Firenze;* [7] *Area Critica Medico Chirurgica, Università di Firenze, Firenze;* [8] *Centro Emostasi e Trombosi, Istituti Ospitalieri, Cremona;* [9] *Cardiologia, Università di Padova, Padova*

**Background** Optimal duration of oral anticoagulant therapy (OAT) in patients with recurrent venous thromboembolism (VTE) is poorly established. Recent studies in patients with a single episode of VTE have suggested that D-dimer (DD) may identify patients at low risk of recurrence. Aim of this prospective, multicenter, cohort study was to assess the negative predictive value of DD in patients with recurrent VTE.
**Patients and Methods** Patients with at least two episodes of objectively documented VTE on treatment with OAT for at least 6 to 12 months were eligible. Patients were excluded in case of a previous massive pulmonary embolism (PE) or if the last episode was an isolated, unprovoked PE; if two or more VTE episodes were unprovoked; in the presence of permanent risk factors. First DD was measured while on OAT: treatment was withheld if DD was negative and continued if DD was positive. Negative DD patients underwent repeated measurement after 7, 15, and 30 days; OAT was resumed if DD turned positive. If DD remained negative, OAT was permanently stopped and all patients underwent a 1 year clinical follow up. Primary end-point was the occurrence of recurrent VTE. For this pilot study, a sample of convenience of 100 patients was chosen.
**Results** The study was prematurely interrupted after the enrolment of 73 patients due to the high rate of recurrences. Thirty-five patients (47.9%) had persistently negative DD levels at day 30. Of them, 7 (20%) had a recurrent VTE event during follow up, 5 before day 90 and 2 after day 90.
**Conclusions** Further studies are needed to assess whether extending DD measurement from 30 days to 90 days after OAT is stopped improves the selection of patients who are eligible for interruption of the treatment after an initial 6-12 months course.

OC024 **DIFFERENT CUT-OFF VALUES OF QUANTITATIVE D-DIMER (DD) ASSAYS TO ESTABLISH DURATION OF ORAL ANTICOAGULATION TREATMENT (OAT) AFTER VENOUS THROMBO-EMBOLISM (VTE)**

Legnani C., Cosmi B., Cini M., Boggian O., Frascaro M., Abate C., Cavallaroni K., Palareti G.
*Dept. Angiology and Blood Coagulation, University Hospital S. Orsola-Malpighi, Bologna*

The PROLONG study showed that continuing OAT in patients with abnormal DD (Clearview Simplify D-dimer) resulted in a significant VTE recurrence reduction. If DD measurement has to be used to stratify the VTE recurrence risk, each DD assay should be independently assessed in a management study. Nevertheless, several clinicians have already adopted the DD-based risk stratification and implementation of OAT based on the PROLONG results, using quantitative DD assays in the absence of method-specific validated cut-off values. The present study retrospectively analyzed a subgroup of patients enrolled in the PROLONG study to define cut-off values of six quantitative DD methods to predict the risk of VTE recurrence.
DD levels were measured in plasma aliquots sampled 30±10 days after OAT cessation in 321 patients enrolled in the PROLONG study. During follow-up (542 y), 25 (7.8%) pts had recurrent VTE. Since DD levels increase with age and are significantly higher in females than in males, we calculated method specific cut-off values according to age and gender. The criteria used to select these cut-off values were: 1) % of altered DD between 36-40%, 2) % of VTE recurrence as low as possible in patients with normal DD. The selected cut-off values (males ≤70 y->70 y, females ≤70 y->70 y; ng/mL) were: VIDAS D-dimer Exclusion (Biomerieux): 490-1,050, 600-1,300; Biopool AutoDimer (Biopool): 90-190, 111-240; HemosIL D-dimer (Instrumentation Laboratory): 205-300, 225-330; HemosIL D-dimer HS (Instrumentation Laboratory): 170-345, 215-430; Innovance D-dimer (Siemens): 500-950, 550-1,150; STA Liatest D-dimer (Diagnostica Stago): 340-700, 450-1,050.
These data suggest that different cut-off values according to methods, patient's age and gender are indicated to identify patients at higher VTE recurrence risk. Furthermore, they are different than those used to rule out acute VTE. These method specific cut-off values are being evaluated in the ongoing prospective management multicenter DULCIS study.

OC025 **D-DIMER TO DETERMINE RISK FOR DISEASE RECURRENCE AFTER UNPROVOKED VENOUS THROMBOEMBOLISM: ADDRESSING UNANSWERED QUESTIONS WITH A LARGE INDIVIDUAL PATIENT META-ANALYSIS**

Iorio A.[(1)], Douketis J.[(2)], Marcucci M.[(1)], Baglin T.[(3)], Cushman M.[(4)], Eichinger S.[(5)], Palareti G.[(6)], Poli D.[(7)], Tait C.[(8)], Tosetto A.[(9)]
[(1)] *Medicine, Internal Vascular Medicine, Perugia;* [(2)] *Medicine, McMaster University, Hamilton, Canada;* [(3)] *Hematology, Addenbrookes Hospital, Cambridge, UK;* [(4)] *Medicine, University of Vermont, Colchester, USA;* [(5)] *Medicine, University of Vienna, Vienna;* [(6)] *Medicine, University of Bologna, Bologna;* [(7)] *Thrombosis Centre, Azienda Ospedaliera-Universitaria Careggi, Firenze;* [(8)] *Hematology, Royal Infirmary, Glasgow, UK;* [(9)] *Hematology, S. Bortolo Hospital, Vicenza*

**Background** In patients with a first unprovoked venous thromboembolism (VTE), an elevated D-dimer after anticoagulant therapy stopping is a risk factor for recurrent VTE. Questions remain about the effect of the timing of D-dimer testing, patient age and the D-dimer cut-point on the ability of D-dimer to distinguish risk for recurrent disease.
**Materials and Methods** We did a patient-level meta-analysis of prospective studies in patients with a first unprovoked VTE who had D-dimer testing after anticoagulation stopping and were followed for recurrent VTE. Kaplan-Meier analysis was used to determine the cumulative incidence of recurrent VTE in patients with a negative or positive D-dimer according to timing of D-dimer testing (<3 weeks, 3-5 weeks, or >5 weeks post-anticoagulation) and patient age (≤65 years, >65 years, or >75 years). We compared risk for recurrence first according to D-dimer status as defined in the source studies then using a pre-specified cut-point (500 µg/mL). We used the log-rank test to compare the risk for recurrent VTE according to D-dimer status (negative or positive) and the Cox regression analysis to adjust for potential confounders.
**Results** We studied 1,818 patients with a first unprovoked VTE who had follow-up for a mean (standard deviation [SD]) of 26.9 (19.1) months. After 3 years, the cumulative incidence of recurrent VTE was significantly higher after a positive D-dimer (25.4%; 95% confidence interval [CI]: 21.3-30.4) than after a negative D-dimer (9.3%; 95% CI: 7.1-12.1; hazard ratio, 2.5; 95% CI 1.9-3.3), irrespective of timing of post-anticoagulation D-dimer testing, patient age and D-dimer cut-point.
**Conclusion** In patients with a first unprovoked VTE who have D-dimer measured after stopping anticoagulation, the timing of D-dimer testing, patient age and the D-dimer assay cut-point used do not affect the ability of D-dimer to distinguish patients at higher or lower risk for recurrent VTE.

## *TEV: TERAPIA*

OC026 **TREATMENT OF VENOUS THROMBO-EMBOLISM IN ELDERLY PATIENTS AND PATIENTS WITH MODERATE RENAL IMPAIRMENT: SUBGROUP ANALYSES OF THE MATISSE CLINICAL TRIALS (ON BEHALF OF THE MATISSE INVESTIGATORS)**

Piovella F.[(1)], Barone M.[(1)], Beltrametti C.[(1)], Buller H.[(2)]
[(1)] *Angiologia - Malattie Tromboemboliche, Fondazione IRCCS Policlinico San Matteo, Pavia;* [(2)] *Academic Medical Center AMC, Amsterdam, The Netherlands*

**Background** The MATISSE trials demonstrated that fondaparinux was at least as effective and safe as standard therapies in the treatment of deep-vein thrombosis (DVT) and/or pulmonary embolism (PE).
**Objectives** To analyze the MATISSE data in the subpopulations of patients aged ≥75 years and with moderate renal impairment, respectively.
**Patients/Methods** In both MATISSE trials, fondaparinux was administered at a once-daily subcutaneous dose of 7.5 mg (5.0 mg if bodyweight <50 kg and 10.0 mg if >100 kg). In patients

with acute symptomatic DVT, fondaparinux was compared with twice-daily subcutaneous enoxaparin (1 mg/kg). In patients with acute symptomatic PE, it was compared with adjusted-dose intravenous unfractionated heparin. The primary efficacy and safety outcomes were recurrent venous thromboembolism (VTE) at three months and major bleeding during the study drug treatment period.
**Results** Of the 4,418 patients included in the pooled MATISSE studies, 25.9% (n=1,143) were aged ≥75 years and 13.7% (n=605) exhibited moderate renal impairment. In patients aged ≥75 years, the rates of symptomatic recurrent VTE were 4.6% and 4.2% with fondaparinux and heparins, respectively (absolute difference: 0.5%; 95% CI: –1.9 to 2.8).
In patients with moderate renal impairment, the respective rates were 4.7% and 4.9% (absolute difference: –0.2%; 95% CI: –3.6 to 3.2). No differences in the incidences of major bleeding and death between the treatment groups were seen in either subgroup.
**Conclusions** Once-daily fondaparinux is at least as effective and safe as standard heparin therapies in the initial treatment of VTE in elderly patients or patients with moderate renal impairment.

## OC027 BLEEDING AS A PREDICTOR OF MORTALITY IN PATIENTS WITH VENOUS THROMBOEMBOLISM

Prandoni P.[(1)], Dalla Valle F.[(1)], Piovella C.[(1)], Pesavento R.[(1)], Trujillo-Santos J.[(2)], Monreal M.[(3)]
*[(1)] Scienze Cardiotoraciche e Vascolari, Università di Padova, Padova; [(2)] Internal Medicine, Hospital Universitario Santa Mara del Rosell, Cartagena, Spain; [(3)] Internal Medicine, Hospital Universitari Germans Trias i Pujol, Badalona, Spain*

While there is emerging evidence that bleeding is a strong predictor of mortality in patients with acute arterial thrombosis receiving antithrombotic therapy, whether a similar association between bleeding and mortality exists also in patients with venous thromboembolism (VTE) is unknown. Out of 29,903 consecutive patients who were enrolled in the multicentre European RIETE registry following an episode of acute VTE, had conventional anticoagulation and were followed-up for at least three months, 888 (3.0%) suffered an episode of major bleeding. The most common sites of bleeding were gastrointestinal (38%), brain (16%), muscle (9%), and urinary tract (9%). Patients who bled were significantly older, and had a significantly higher prevalence of cancer, renal insufficiency, anemia and recent bleeding than those who did not. Of the 888 patients who bled, 411 (46%) died before the completion of the follow-up (of whom 222 as direct implication of the bleeding), as compared with 3,092 of the 29,015 (11%) who did not experience bleeding. After adjusting for baseline predictors of bleeding, the adjusted hazard ratio (HR) for death in patients who bled as compared with those who did not was 5.7 (5.0 to 6.3; p<0.001). After removing deaths directly related to bleeding, the value remained statistically significant (HR=2.6; 95% CI, 2.2 to 3.0; p<0.001). We conclude that major bleeding in patients with acute VTE is a strong predictor of mortality.

## OC028 ENDOVASCULAR THROMBOLYSIS IN ACUTE MESENTERIC VEIN THROMBOSIS. A 3-YEAR FOLLOW-UP WITH THE RATE OF SHORT AND LONG-TERM SEQUAELAE IN 32 PATIENTS

Di Minno M.N.D., Cerbone A.M., Tufano A., Cimino E., Guida A., Quintavalle G., De Gregorio A.M., Di Palo M.G., Di Minno G.
*Department of Clinical and Experimental Medicine, "Federico II" University, Naples*

**Background** Mesenteric vein thrombosis (MVT) is a rare, often lethal, entity that accounts for 10-15% of all cases of mesenteric ischemia. Current indications for surgery in patients with acute MVT include peritonitis, bowel infarction, hemodynamic instability. In all other cases, anticoagulation is of choice. At variance with anticoagulation, thrombolysis leads to a rapid re-opening of vessels, with immediate tissue reperfusion.
**Aim** To evaluate mortality and the development of portal hypertension in patients with MVT treated with percutaneous transhepatic thrombolysis and mechanical thrombectomy, and then warfarin, compared with warfarin alone.
**Methods** We have prospectively followed-up for 3 years 32 patients with acute MVT documented by CT-scan. None of them had indications for surgical interventions.
After 1-week low-molecular-weight-heparin therapy (LMWH), 14 patients (controls) received warfarin (INR 2-3). The other 18 patients (treated group) underwent percutaneous-transhepatic thrombolysis and mechanical thrombectomy before starting warfarin. Mean age, sex ratio, risk factors, the American Society of Anesthesiologist score on admission, localization of thrombosis and the duration of symptoms were similar in both groups.
**Results** 30-d mortality rate was similar in the two groups: because of co-morbid conditions (sepsis, pneumonia, myocardial infarction), 3 patients (16.6%) died in the treated group and 2 (14.2%) in the control group (p=0.998). Bowel resection was needed in 1 patient (5.5%) in the treated group (bleeding from a recent colo-rectal anatomosis) and in 5 patients (35.7%) in the control group (bowel ischemia with peritoneal signs) (p =0.022). A significant difference (p= 0.043) was also found as to development of portal hypertension (7/14 patients in the control group, 50%; 2/18 in the group receiving thrombolysis, 11.1%).
**Conclusion** In spite of the technical complexity and the bleeding risk, when administered promptly, percutaneous transhepatic thrombolysis and mechanical thrombectomy is a valuable mean of preventing bowel ischemia and long-term portal hypertension in patients with MVT.

## OC029 DIFFERENT DOSES AND DURATION OF TREATMENT OF SUPERFICIAL VEIN THROMBOSIS WITH PARNAPARIN: PRELIMINARY RESULTS OF THE RANDOMIZED STEFLUX CLINICAL TRIAL

Cosmi B.[(1)], Filippini M.[(1)], Tonti D.[(2)], Ghirarduzzi A.[(3)], Avruscio G.[(4)], Imberti D.[(5)], Andreozzi G.M.[(6)], Camporese G.[(6)], Palareti G.[(1)]
*[(1)] Div. Angiologia e Malattie della Coagulazione, Università di Bologna, Ospedale S. Orsola-Malpighi, Bologna; [(2)] Angiologia, Ospedale Bufalini, Cesena; [(3)] Dept. Internal Medicine I, Angiology, Arcispedale Santa Maria Nuova, Reggio Emilia; [(4)] Dept. Angiology, S. Antonio Hospital, Padua; [(5)] Dept. Internal Medicine, G. da Saliceto Hospital, Piacenza; [(6)] Unit of Angiology, University*

*Hospital of Padua, Padua; on behalf of the STEFLUX Investigators*

**Background/Aims** The optimal treatment of superficial vein thrombosis (SVT) is still uncertain. The aim of the SteFlux (Superficial Thromboembolism Fluxum) study was to compare different doses and duration with low molecular weight heparin (parnaparin) in the treatment of SVT.
**Materials and Methods** A double blind placebo controlled randomized phase III study was conducted in 16 Italian centres. Patients with SVT of at least 4 cm in length of the internal or external saphenous veins or their collaterals were randomized to receive either parnaparin 8,500 UI aXa od for ten days followed by placebo for additional 20 days or parnaparin 8,500 UI aXa od for ten days followed by parnaparin 6,400 UI aXa od for 20 days or parnaparin 4,250 UI aXa od for 30 days.
Primary outcomes were the composite of symptomatic and asymptomatic deep vein thrombosis (DVT), pulmonary embolism (PE), and recurrence or extension of the SVT in the first 30 days with a follow-up of 90 days. The calculated sample size was 382 per arm.
**Results** Preliminary results show an overall rate of events of 15.6% (81/519; 17 DVTs, 1 PE, 25 new SVT, 38 extension of SVT), 27 of which occurred between 30 and 90 days (5.2%). One or more risk factors were present in 64/81 (79%) of subjects with an event and in 302/438 without events (70%). There is a trend for a higher rate of events in subjects with risk factors for SVT (64/366=17.4%) than in subjects without risk factors (17/153= 11.1%) (p=0.08).
**Conclusions** Subjects with risk factors for SVT have a trend for higher rates of events than patients without and may require a longer duration of treatment.

## OC030 **D-DIMER AND ULTRASOUND TO ESTABLISH THE OPTIMAL DURATION OF ANTICOAGULATION FOR VENOUS THROMBOEMBOLISM: PRELIMINARY RESULTS OF THE D-DIMER AND ULTRASOUND IN COMBINATION ITALIAN STUDY (DULCIS)**

Cosmi B.[(1)], Legnani C.[(1)], Ghirarduzzi A.[(2)], Testa S.[(3)], Prandoni P.[(4)], Pengo V.[(5)], Palareti G.[(1)]
*[(1)] Div. Angiologia e Malattie della Coagulazione M. Golinelli, Ospedale S. Orsola Malpigli, Università di Bologna, Bologna; [(2)] Divisione Medicina Interna I, Angiologia, Arcispedale Santa Maria Nuova, Reggio Emilia; [(3)] Centro Emostasi e Trombosi, Ospedale Generale di Cremona, Cremona; [(4)] Dipartimento di Scienza Toraciche e Vascolari, Unità Tromboembolismo Venoso, Università di Padova, Padova; [(5)] Dipartimento di Medicina Clinica e Sperimentale, Divisione di Cardiologia Clinica, Università di Padova, Padova*

**Background/Aims** The purpose of this study is to evaluate the efficacy and safety of a procedure employing the evaluation of residual vein obstruction (RVO) and D-dimer to establish the individual risk of recurrence and thus the necessity to prolong or stop anticoagulation after deep vein thrombosis (DVT) and/or pulmonary embolism (PE). The aims of the study are: i) to obtain a recurrence rate <5% per year in the first and second year after anticoagulation is suspended according to the procedure, ii) to allow that after treatment for the first episode of venous thromboembolism, anticoagulation suspension in feasible in at least 40% of all subjects included in the study.
**Materials and Methods** Multi-centre management study in which patients with RVO is less <4 mm in case of a previous DVT and/or normal pulmonary arterial pressure with echocardiography in case of previous PE and in those who have undergone additional 6 months of therapy for previously altered RVO, D-dimer is measured during anticoagulation. If D-dimer is below age and gender cut-offs, anticoagulation is interrupted and D-dimer is then re-assessed after 15, 30, 60 and 90 days. If all the D-dimer measurements are below the cut-offs, anticoagulation is definitely interrupted and patients are followed-up for two years. If one of these D-dimer measurement is above the cut-off , anticoagulation is resumed for at least 6 months and patients are re-evaluated.
**Results** Up to date, 206 out of 539 screened patients (38%) have been enrolled. Of these 123 (60%) have stopped anticoagulation because of a normal D-dimer and 6 had a recurrent event (4.9%). In 83 subjects anticoagulation was resumed and 1 major bleeding event was observed (1.2%). Additional data will be available in the near future.

## *TEV: PROFILASSI 1*

## OC031 **THROMBIN GENERATION (TG) AND FVIII PLASMA LEVELS IN PATIENTS WITH PORTAL VEIN THROMBOSIS WITH AND WITHOUT LIVER CIRRHOSIS**

Rossetto V.[(1)], Senzolo M.[(2)], Sartori M.T.[(1)], Dabrilli P.[(1)], Zerbinati P.[(1)], Fadin M.[(1)], Gavasso S.[(1)], Bon M.[(1)], Simioni P.[(1)]
*[(1)] Clinica Medica II, Dip. Scienze Cardiologiche, Toraciche e Vascolari, Ospedale Un., Padova; [(2)] Gastroenterologia, Dip. Scienze Chirurgiche e Gastroenterologiche, Ospedale Un., Padova*

Portal vein thrombosis (PVT) is rare in the absence of liver cirrhosis or cancer but presents with an incidence of 0.6%-16% in cirrhotics. An association between factor VIII (FVIII) and PVT has been demonstrated in non cirrhotic and cirrhotic patients.
**Aim and methods** To evaluate risk factors associated with PVT in cirrhotic and non cirrhotic patients, with attention to thrombin generation (TG) and FVIII levels.
69 patients with PVT, 43 with and 26 without cirrhosis, 24 cirrhotics and 33 controls were included; they underwent thrombophilia screening and TG performed with and without thrombomodulin. ETP ratio (ETP with thrombomodulin/basal ETP) was calculated too.
**Results** PVT was associated with chronic myeloproliferative disease in patients without cirrhosis (30.7%); a high prevalence of genetic thrombophilia was seen in cirrhotics (20.9%); (chi-square=20.37, p<.0001). PVT patients without cirrhosis showed higher FVIII than controls (162.5±78% vs. 119.3±40%, p=.021). FVIII was associated with thrombosis (chi-square=6.8, p=.009), particularly when involving more than one vessel (chi-square=3.86, p=.049). ETP ratio was higher than controls (0.67±0.2 vs. 0.41±0.14, p=.001) and correlated with FVIII levels. In cirrhotics with PVT, FVIII was higher than either controls (180.13±75% vs. 119.3±40%, p<.001) or PVT patients without cirrhosis. Cirrhotics also showed a significant reduction of Protein C compared to either controls or PVT without cirrhosis. Moreover they showed higher ETP ratio (0.80±0.17) than both controls and patients with only PVT

(p=.01). ETP ratio was even higher in those with underlying genetic thrombophilia. Classifying cirrhotics with Child-Pugh score, FVIII was persistently increased, Protein C decreased and ETP ratio increased by worsening of hepatic function.
**Conclusions** PVT is associated with an increased ETP ratio in parallel with an increase in FVIII compared to controls, especially in cirrhotic population. However TG does not seem to differ in cirrhotic patients with or without PVT despite it is markedly increased in those with thrombophilia.

## OC032 NATURAL HYSTORY OF CEREBRAL VEIN THROMBOSIS: A LARGE MULTICENTER STUDY

Dentali F.[(1)], Poli D.[(2)], Palareti G.[(3)], De Stefano V.[(4)], Malato A.[(5)], Scoditti U.[(6)], Di Minno D.[(7)], Grandone E.[(8)], Testa S.[(9)], Ageno W.[(1)]
*[(1)] Medicina Clinica, Università dell'Insubria, Varese; [(2)] Heart and Vessels, Azienda Ospedaliero-Universitaria Careggi, Firenze; [(3)] Angiologia, Ospedale Universitario S. Orsola Malpighi, Bologna; [(4)] Ematologia, Università Cattolica, Roma; [(5)] Ematologia, Azienda Ospedaliera Universitaria Policlinico P. Giaccone, Palermo; [(6)] Neurologia, Ospedale di Parma, Parma; [(7)] Centro di riferimento regionale, Policlinico Federico II, Napoli; [(8)] Atherosclerosis and Thrombosis Unit, I.R.C.C.S. Casa Sollievo della Sofferenza, San Giovanni Rotondo; [(9)] Haemostasis and Thrombosis Centre, Ospedale di Cremona, Cremona*

**Background** Cerebral vein thrombosis (CVT) has been considered, until a few years ago, an uncommon disease with significant long-term morbidity and high mortality rate. New non-invasive diagnostic techniques have increased the frequency with which this disease is diagnosed. Only a few study with a relatively short follow up have evaluated the natural history of CVT.
**Methods** In a multicenter study (19 centers) we evaluated the long term prognosis of a large cohort of patients with a first episode of CVT. Only patients with a objectively diagnosed CVT and with a follow up of at least 6 months were considered. Patients were contacted locally by each center. Information was collected in a computerized database. End points were recurrent CVT, other venous thromboembolic events (VTE) and mortality.
**Results** 512 patients (73% female) with CVT were included. About 40% of patients were idiopathic. Patients were followed for a total follow-up of 2,218 patients year. Mean follow-up was 4.3 years (range 6 months, 21 years). Twelve patients were lost at follow-up (2.3%). Almost all patients were treated with oral anticoagulation, the mean duration of treatment was 12 months, and the mean time of follow-up after anticoagulation discontinuation was 3.2 years. CVT recurred in 17 patients (3.3%), and 32 patients (6.3%) had another clinical manifestation of VTE for an overall incidence of recurrence of 22.1 events/1,000 patients year. Many events occurred after anticoagulation discontinuation for an incidence of recurrence in this group of 29.1 events /1,000 year patients.
**Conclusions** In this large retrospective multicenter study with a very long follow up, the risk of CVT recurrence and of incidence of other venous thromboembolic events appear to be low. Further analyses will explore if some subgroups have a higher risk of recurrence.

## OC033 ARE MEN AT HIGHER RISK FOR DISEASE RECURRENCE THAN WOMEN?

Marcucci M.[(1)], Tosetto A.[(2)], Douketis J.[(3)], Baglin T.[(4)], Cushman M.[(5)], Eichinger S.[(6)], Palareti G.[(7)], Poli D.[(8)], Tait C.[(9)], Iorio A.[(1)]
*[(1)] Internal Vascular Medicine, Medicine, Perugia; [(2)] Hematology, S. Bortolo Hospital, Vicenza; [(3)] Medicine, McMaster University, Hamilton, Canada; [(4)] Hematology, Addenbrookes Hospital, Cambridge, UK; [(5)] Medicine, University of Vermont, Colchester, USA; [(6)] Medicine, University of Vienna, Vienna, Austria; [(7)] Medicine, University of Bologna, Bologna; [(8)] Thrombosis Centre, Azienda Ospedaliera Universitaria Careggi, Firenze; [(9)] Hematology, Royal Infirmary, Glasgow, UK*

**Background** In patients with a first episode of venous thromboembolism (VTE), the purported higher risk for recurrent disease in men may be spurious and attributable to a lower risk in women, some of whom have a low recurrence risk after hormonal therapy (HT)-associated VTE.
**Materials and Methods** We did a patient-level meta-analysis of prospective studies in patients with a first VTE who were followed after anticoagulation was stopped for symptomatic recurrent VTE. We used Kaplan-Meier analysis to determine the cumulative incidence of recurrent VTE and multivariable Cox regression to adjust for patient sex and HT.
**Results** We studied 2,554 patients with a first VTE who had follow-up for a mean (standard deviation) of 27.1 (19.6) months. The 3-year cumulative incidence of recurrent VTE was 9.1% (95% CI 7.3-11.3) in women and 19.7% (95% CI 16.5-23.4) in men. Among patients with unprovoked VTE index event, women with a previous HT-associated VTE had a hazard ratio (HR) for recurrence versus HT non user women equal to 0.5 (95% confidence intervals [CI] 0.3-0.8). The HR for VTE recurrence for men versus women was 2.2 (95% CI 1.7-2.8) without adjusting for HT-associated VTE, and 1.8 (95% CI 1.4-2.5) after adjusting. Among patients with provoked VTE index event, the HR for men was 1.2 (95% CI 0.6-2.4) versus all women and very similar (1.2; 95% CI 0.6-2.3) versus HT non user women.
**Conclusion** In patients with a first unprovoked VTE, men had a 2.2-fold higher risk for recurrent VTE; this risk remained 1.8-fold higher in men than women after the exclusion of women with HT-associated VTE. These results were not confirmed in patients with a first provoked VTE.

## OC034 NADROPARIN FOR THE PREVENTION OF THROMBOEMBOLIC EVENTS IN AMBULATORY PATIENTS RECEIVING CHEMOTHERAPY WITH METASTATIC OR LOCALLY ADVANCED CANCER: A POST-HOC ANALYSIS OF THROMBOEMBOLIC RISK RELATED TO TYPE CHEMOTHERAPY IN THE PROTECHT STUDY

Verso M.[(1)], Gasparini G.[(2)], Barni S.[(3)], Labianca R.[(4)], Mandalà M.[(4)], Passalacqua R.[(5)], Cavanna L.[(6)], Tonato M.[(1)], Gussoni G.[(7)], Agnelli G.[(1)]
*[(1)] Perugia; [(2)] Roma; [(3)] Treviglio; [(4)] Bergamo; [(5)] Cremona; [(6)] Piacenza; [(7)] Milano*

**Background** The PROTECHT study (NCT 00951574) have demonstrated that the use of nadroparin was associated with a

reduction in venous and arterial thrombotic events (TE) from 3.9% to 2% (interim-adjusted p value=0.024, relative risk reduction 49.6%, NNT=50.5), in ambulatory patients with metastatic or locally advanced cancer who are receiving chemotherapy.

**Aim of the study** The aim of this post-hoc analysis was to evaluate the risk of TE according to type of chemotherapy drug.

**Methods** Ambulatory cancer patients were randomly assigned in a double-blind manner to receive subcutaneous injections of nadroparin (3,800 IU anti-Xa once a day) or placebo, in a 2:1 ratio. The primary endpoint was symptomatic TE, as adjudicated by an independent committee. TE incidence was assessed according to the type of chemotherapy (cisplatin, carboplatin, oxaliplatin, gemcitabine, taxotere and vinca alkaloids).

**Results** The two treatment study groups were well balanced for demographic characteristics, cancer site, concomitant medication and chemotherapy. Among the 1,150 evaluable patients the most common tumours were gastrointestinal 36.5% (420/1,150), lung 24.3% (279/1,150), Breast 14.3% (165/1,150) and Ovarian 12.4% (143/1,150). Chemotherapy distribution was as follows: cisplatin 22.9% (263/1,150), carboplatin 15.1% (174/1,150), oxaliplatin 20.2% (232/1,150), taxotere 18.2% (209/1,150) and vinca alkaloids 10.4% (120/1,150). The results according to chemotherapy are shown in the Table.

| Chemotherapy | Nadroparin % (Events/pts) | Placebo % (Events/pts) | RRR | P-value (two tailed) |
|---|---|---|---|---|
| **Cisplatin** | 2.3 (4/177) | 7 (6/86) | 67.1% | 0.06 |
| **Carboplatin** | 0.8 (1/199) | 5.5 (3/55) | 85.4% | 0.05 |
| **Oxaliplatin** | 0.7 (1/143) | 1.1 (1/89) | 36.4% | 0.73 |
| **Gemcitabine** | 2.6 (4/156) | 8.1 (7/86) | 67.9% | 0.04 |
| **Taxotere** | 1.4 (2/142) | 4.5 (3/67) | 68.9 | 0.17 |
| **Vinca alkaloids** | 2.2 (2/90) | 3.7 (1/27) | 40.5% | 0.66 |

The adjunct of gemcitabine, taxotere or vinca alkaloids to a regimen containing platinum compound increased the risk of TE (RRI - Relative Risk Increase) of 126.7%, 4.0% and 44.4% respectively.

**Conclusion** In this exploratory post-hoc analysis, nadroparin reduces the TE risk in cancer patients receiving cisplatin, carboplatin or gemcitabine.

## OC035 EFFICACY OF NADROPARIN IN PREVENTING THROMBOEMBOLIC EVENTS IN AMBULATORY CANCER PATIENTS RECEIVING CHEMOTHERAPY STRATIFIED ACCORDING TO KORANA RISK SCORE: A POST-HOC ANALYSIS OF PROTECHT STUDY

Verso M.[(1)], Agnelli G.[(1)], Labianca R.[(2)], Gasparini G.[(3)], Barni S.[(4)], Mandalà M.[(2)], Cavanna L.[(5)], Passalacqua R.[(6)], Gussoni G.[(7)], Tonato M.[(1)]
*[(1)] Perugia; [(2)] Bergamo; [(3)] Roma; [(4)] Treviglio; [(5)] Piacenza; [(6)] Cremona; [(7)] Milano*

**Background** Korana et al validated a risk model to stratify patients at high or low risk for developing thrombotic events while receiving chemotherapy. The PROTECHT study (NCT 00951574) have demonstrated that the use of nadroparin was associated with a reduction in venous and arterial thrombotic events (TE) from 3.9% to 2% (interim-adjusted p value=0.024, relative risk reduction 49.6%, NNT=50.5), in ambulatory patients with metastatic or locally advanced cancer who are receiving chemotherapy.

**Aim of the study** The aim of this post-hoc analysis was to evaluate the efficacy of nadroparin in reducing TE in the PROTECHT population stratified according to Korana risk score.

**Methods** Ambulatory cancer patients were randomly assigned in a double-blind manner to receive subcutaneous injections of nadroparin (3,800 IU anti-Xa once a day) or placebo, in a 2:1 ratio. The primary endpoint was symptomatic TE, as adjudicated by an independent committee. In the Korana risk model, cancer patients are stratified according to five variables (site of cancer, platelet count haemoglobin level, leukocyte count and body mass index) Patients with a score ≥3 are at high risk to develop TE.

**Results** Among the 1,150 evaluable patients 115 patients (70 nadroparin, 45 placebo) were at high risk of TE (Korana score ≥3), therefore 1,035 patients (699 nadroparin, 336 placebo) were at low/intermediate risk of TE (Korana score <3).

The results of this analysis are shown in the Table according to the Korana score.

| Variable Korana Score | Nadroparin % (Events/pts) | Placebo % Events/pts) | RRR | P-value |
|---|---|---|---|---|
| **High risk ≥ 3** | 4.3 (3/70) | 11.1 (5/45) | 61.3% | 0.16 |
| **Intermediate/ Low risk < 3** | 1.7 (12/699) | 3 (10/336) | 43.3% | 0.19 |

**Conclusion** In this exploratory post-hoc analysis, nadroparin reduces the TE risk in cancer patients who are at high risk (Korana score ≥3) of TE according to Korana score.

## *ATEROSCLEROSI: MODELLI SPERIMENTALI*

## OC036 PLATELET-DERIVED MATRIX METALLO-PROTEINASE (MMP)-2 CONTRIBUTES TO ATHEROSCLEROSIS IN STRONGLY HYPER-CHOLESTEROLEMIC MICE

Momi S., Cecchetti L., Gresele P.
*Dept. Internal Medicine, Sect. Internal and Cardiovascular Medicine, Perugia*

MMPs represent a family of zinc dependent endopeptidases that are involved in atherosclerosis, inflammatory cell recruitment and neointima formation, plaque rupture and. Platelets contain, and release upon activation, MMP-2 that in turn potentiates the platelet response to stimuli. MMP-2 accelerates breakdown of extracellular matrix associated with the vascular remodelling accompanying atherosclerosis and neointima formation. MMP-2 knockout mice (MMP-2-/-) were previously reported to display reduced intimal hyperplasia as compared with wild-type mice in a carotid artery injury model.

Aim of the present study was to evaluate whether blood cell derived, largely platelet-released, MMP-2 plays a role in atherogenesis *in vivo*.

We generated chimeric mice by cross-transplanting bone marrow from MMP-2-/- into LDLR-/- mice to obtain mice with MMP-2-/--derived blood cells and wild-type vessels. Mice were fed an atherogenic diet for 16 weeks and the presence of atherosclerotic lesions in the aorta was assessed by oil red O staining. Chimeric mice (MMP2-/-/LDLR-/-) had a smaller area

of the aorta covered by lipid lesions than LDLR-/- (total aorta: 22±2.3% vs. 37±7%, p<0.001; aortic root: 21±7.3% vs. 79±11%, p<0.01). To investigate whether the blood cell source of MMP-2 participating in atherogenesis were platelets, we rendered LDLR-/- mice thrombocytopenic by busulfan and reconstituted their circulating platelets by injecting activated platelet suspension. Each mouse received $50x10^6$ thrombin (0.05 U/mL)-stimulated platelets every 4 days for 6 weeks. Atherosclerotic lesions in aorta of LDLR-/- mice injected with MMP-2-/- platelets were reduced by 23% as compared with LDLR-/- mice transfused with LDLR-/- platelets, thus indicating a role of platelet-derived MMP-2 in atherosclerosis.
In conclusion, the lack of platelet-derived MMP-2 reduces atherosclerosis in hypercholesterolemic LDLR-/- mice. The development of drugs acting selectively on MMP-2 represents a new attractive approach to anti-atherothrombotic therapy.

## OC037 **THROMBOXANE AND PROSTACYCLIN BIOSYNTHESIS IN HEART FAILURE OF ISCHEMIC ORIGIN: EFFECTS OF DISEASE SEVERITY AND ASPIRIN TREATMENT**

Santilli F.[(1)], Basili S.[(2)], Lattanzio S.[(1)], Cavoni A.[(3)], Guizzardi G.[(3)], De Feudis L.[(4)], Traisci G.[(4)], Pettinella C.[(1)], Minuz P.[(5)], Ciabattoni G.[(1)], Davì G.[(1)]
*[(1)] Center of Excellence on Aging, G. d'Annunzio University Foundation, Chieti; [(2)] Sapienza University Department of Medicine, Rome; [(3)] Department of Geriatrics, Pescara Hospital, Pescara; [(4)] Department of Medicine, Pescara Hospital, Pescara; [(5)] Department of Medicine, University of Verona, Verona*

**Objectives** To characterize the rate of thromboxane and prostacyclin biosynthesis in chronic heart failure (HF) of ischemic origin, with the aim of dissecting out the influence of HF on platelet activation from the effects of the underlying vascular disease.
**Background** Thromboembolism is a relatively common complication of chronic HF and the place of antiplatelet therapy is uncertain.
**Methods** We compared urinary 11-dehydro-thromboxane (TX) B2, 2,3 dinor 6-keto-PGF1a, and 8-iso-prostaglandin (PG) F2a, and plasma N-terminal pro-brain natriuretic peptide (NT-pro-BNP), asymmetric dimethylarginine (ADMA), C-reactive protein (CRP), soluble CD40 ligand (sCD40L), and prothrombin fragment 1+2 (F1+2), in 84 patients with HF secondary to ischemic heart disease (IHD), in 61 patients with IHD without HF and 42 healthy subjects.
**Results** HF patients not on aspirin had significantly higher urinary 11-dehydro-TXB2 excretion as compared to healthy subjects (P<0.0001) and IHD patients not on aspirin (P=0.028). They also showed significantly higher 8-iso-PGF2a (P=0.018), NT-pro-BNP (P=0.021), ADMA (P<0.0001) than IHD patients not on aspirin. HF patients on low-dose aspirin had significantly lower 11-dehydro-TXB2 (P<0.0001), sCD40L (P=0.007) and 2,3-dinor-6-keto-PGF1a (P=0.005) than HF patients not treated with aspirin. HF patients in NYHA class III-IV had significantly higher urinary 11-dehydro-TXB2 than patients in I-II class, independently of aspirin treatment (p<0.05). On multiple linear regression analysis, higher plasma NT-pro-BNP levels (β Coefficient=0.32, SEM=0.067, P<0.0001), lack of aspirin therapy (β Coefficient= –0.73, SEM= 0.051, P<0.0001) and plasma sCD40L (β Coefficient=0.15, SEM= 0.081, P=0.024), predicted the excretion rate of 11-dehydro-TXB2 in HF patients ($R^2$=0.771).
**Conclusion** Persistent platelet activation characterizes patients with HF. This phenomenon is related to disease severity and is largely suppressable by low-dose aspirin. The homeostatic increase in prostacyclin biosynthesis is impaired, possibly contributing to enhanced thrombotic risk in this setting.

## OC038 **EFFECT OF OXIDATIVE STRESS ON VON WILLEBRAND FACTOR IN END STAGE RENAL DISEASE: ITS CONTRIBUTION TO INCREASED CARDIOVASCULAR RISK IN UREMIA**

Lancellotti S.[(1)], Oggianu L.[(2)], Gambaro G.[(1)], Sturniolo A.[(3)], Costanzi S.[(3)], De Filippis V.[(4)], Pozzi N.[(4)], De Cristofaro R.[(1)]
*[(1)] Medicina Interna e Scienze Mediche Specialistiche, Medicina Interna e Geriatria, Università Cattolica S. Cuore, Roma; [(2)] Section of Biology Applied to Human Health, Rome Tre University, Rome; [(3)] Haemodialysis and peritoneal dialysis Care Unit, Columbus Hospital, Rome; [(4)] Department of Pharmaceutical Sciences, University of Padua, Padova*

Patients with chronic kidney disease (CKD) have a dramatically high risk for cardiovascular disease (CVD) associated with an abnormally high oxidative stress (OS). Enhanced levels of hemostatic factors, such as fibrinogen, von Willebrand factor (VWF) and PAI, have been reported in CKD patients. VWF is a multimeric endothelial glycoprotein that, upon release from endothelial cells, is rapidly cleaved by ADAMTS13, a Zn-protease that hydrolyses the Tyr1605-Met1606 peptide bond in the A2 domain of the protein. In CKD a severe endothelium dysfunction is associated with increased VWF levels and platelet activation. The hemostatic function of VWF is directly proportional to its molecular weight and thus indirectly to ADAMTS-13 proteolysis of the Tyr1605-Met1606 peptide bond, highly sensitive to oxidative stress.
In this study one OS marker (plasma protein carbonyls) and several haemostatic and haematological parameters (VWF:Ag, VWF-RiCof, Fibrinogen, aPTT, PT, D-dimer, ADAMTS-13 activity and blood group) were measured in 20 patients under haemodialysis treatment for uremia and 20 sex- and age-matched healthy controls.
The mean level of protein carbonyls and VWF:Ricof was higher than in controls (0.6±0.2 nmol/mg vs. 0.07 nmol/mg, and 148±23% vs. 101±16%, respectively). In a multivariate analysis the ratio VWF: RiCof/VWF:Ag, expression of the presence of high molecular weight VWF multimers, was associated only with protein carbonyls (ANOVA, p=0.041). This finding was confirmed by multimer analysis of VWF by SDS-agarose electrophoresis. Purified VWF from uremics patients showed a higher carbonyl content than in controls (p<0.05). Thus, we hypothesize that OS in ESRD/ haemodialysis (HD) involves also VWF, impairing its proteolysis by ADAMTS-13.
This process could contribute to the accumulation of ultra-large VWF multimers with prothrombotic effects. This pathogenetic mechanism could have a relevant role in CV complications of CKD and indicate new antithrombotic strategies in this clinical setting.

## OC039 **ALX-0081, A SELECTIVE INHIBITOR OF THE**

**INTERACTION BETWEEN PLATELET GPIB AND VON WILLEBRAND FACTOR, PREVENTS ISCHEMIC STROKE IN THE GUINEA PIG: COMPARISON WITH THE THROMBOLYTIC RTPA**

Momi S.[(1)], Tantucci M.[(2)], Van Roy M.[(3)], Ulrichts H.[(3)], Holtz J.[(1)], Gresele P.[(1)]
*[(1)] Dept. Internal Medicine, Sect. Internal and Cardiovascular Medicine, Perugia; [(2)] Department of Experimental Medicine, University of Perugia, Perugia; [(3)] Pharmacology, Ablynx, Zwijnaarde, The Netherlands*

The interaction between platelet glycoprotein Ib (GPIb) and VWF triggers the initial step of platelet adhesion and arterial thrombus formation. Antiplatelet therapy is the cornerstone of treatment of acute cerebrovascular disease but is associated with risk of bleeding. New antiplatelet approaches with a better efficacy/safety ratio are therefore required. ALX-0081 is a Nanobody against the A1 domain of VWF that specifically blocks the GPIb binding site of VWF.
We have assessed the efficacy and safety of ALX-0081 in a photochemical injury-induced middle cerebral artery (MCA) thrombosis model in the guinea pig.
End points were: continuous measurement of blood flow in the MCA; skin bleeding time; brain damage (including ischemia and hemorrhage) by TTC staining and image analysis; haemoglobin content in the damaged hemisphere, an index of hemorrhage, expressed as % increase in the damaged vs. healthy hemisphere.
ALX-0081 (5 and 8.5 mg/kg) and rtPA (low dose: 0.032 mg/kg + 0.576 mg/kg/30min; high dose: 0.1 mg/kg + 0.9 mg/kg/30 min) were administered immediately after the total occlusion of the MCA.
ALX-0081 restored perfusion in the MCA at both doses while rtPA was effective only at the high dose. ALX-0081 did not modify the skin bleeding time at low dose (5 mg/kg: +39%, p=NS) while it did it at the high dose (8.5 mg/kg: +259%, p<0.01); rtPA prolonged bleeding time at both doses (low dose: +289%, p<0.01; high dose: +572%, p<0.001). The brain damage area was strikingly reduced in ALX-0081-treated guinea pigs (5 mg/kg: 3.64±2.15% of total, 8.5 mg/kg: 5.3±0.9%; vs. 14.6±1.2% in controls, p<0.001) and not in rtPA-treated guinea pigs (low dose: 141.3% of total; high dose: 15.7±4.5%). The haemoglobin content in the damaged hemisphere was markedly enhanced in rtPA-treated (controls: 13.8±4.3%; rtPA low dose: 40.4±7.2%; high dose: 64.5±17.3%, p<0.001 vs. controls) but not in ALX-0081-treated animals (15.3±1.7%).
The inhibition of the VWF-GPIb axis seems an effective and safe strategy for the treatment of thromboembolic disorders.

OC040 **DIFFERENTIAL EXPRESSION OF MATRIX METALLOPROTEINASES (MMPs) AND TISSUE INHIBITORS OF MMPs (TIMPs) IN PLATELETS AND MEGAKARYOCYTES**

Cecchetti L.[(1)], Tolley N.[(2)], Weyrich A.S.[(3)], Gresele P.[(1)]
*[(1)] Dept. of internal medicine, Division of Internal and Cardiovascular Medicine, Perugia; [(2)] Dept. Internal Medicine, Eccles Institute of Human Genetics, Salt Lake City, USA; [(3)] Dept. Internal Medicine, University of Utah, Salt Lake City, USA*

MMPs are a family of endopeptidases able to degrade extracellular matrix and are regulated by specific tissue inhibitors (TIMPs). Platelets store and secrete several MMPs/TIMPs and some of them, like MMP-1 and MMP-2, potentiate platelet activation.
In the last years it has emerged that platelets contain different mRNAs, some of which translate new protein upon platelet activation.
We have assessed the presence of mRNA for several MMPs and TIMPs in megakaryocytes and in CD45-depleted platelet preparations.
Human CD34+-derived megakaryocytes and platelets express mRNA for MMP-1 and -9 as well as TIMP-1, -2 and -3. Quantitative PCR shows that the expression level of such transcripts varies widely in platelets. mRNA for MMP-2 and 14 are present in megakaryocytes, but not in platelets, while mRNA for MMP-3 and TIMP-4 is absent from both cell types.
Unexpectedly, mRNA expression patterns did not uniformly predict the presence or the amount of their corresponding protein in platelets; e.g. MMP-2 mRNA is absent from platelets but its protein is present and secreted upon activation while platelets lack MMP-9 protein although they contain its mRNA.
TIMP-2 protein is absent from resting platelets despite the presence of mRNA but it accumulates upon thrombin activation (0.70.1 ng/mL vs. 21+4 ng/mL after 1h; P<0.05), an effect prevented by two protein synthesis inhibitors puromycin and cycloheximide, showing that activated platelets translate TIMP-2 mRNA into new protein. TIMP-2 de-novo synthesis was confirmed by 35S incorporation.
In contrast, platelets store and release upon activation MMP-1 and TIMP-1. Finally, TIMP-3 is stored but not secreted upon thrombin activation.
Taken together, these data demonstrate that megakaryocytes and platelets differentially express mRNAs and protein of MMP/TIMP family members and that platelets are able to de-novo synthesize TIMP-2 upon activation, providing another example of the growing repertoire of regulatory proteins that are under translational control in platelets.

OC041 **NOX2 UP-REGULATION IS ASSOCIATED WITH ARTERY DYSFUNCTION IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE**

Loffredo L., Carnevale R., Cangemi R., Angelico F., Augelletti T., Di Santo S., Perri L., Pignatelli P., Basili S., Violi F.
*Dipartimento di Medicina Sperimentale I Clinica Medica, Roma*

**Rationale** Peripheral arterial disease (PAD) is a clinical setting characterized by endothelial dysfunction but the underlying mechanism is still undefined.
**Objective** The aim of this study was to evaluate whether NADPH oxidase is implicated in determining reduced flow mediated dilation (FMD) in PAD.
**Methods and Results** We performed a cross-sectional study comparing FMD, oxidative stress by urinary excretion of isoprostanes, NO generation by serum levels of nitrite/nitrate (NOx), and serum NOX2, the catalytic core of NADPH oxidase, in a population of 50 PAD patients and 50 controls (mean age: 67±9 years). Also, we performed an interventional cross-over study to assess if propionyl-L-carnitine (PLC) (6 g/day), vs. placebo, was able to improve endothelial dysfunction, oxidative stress and serum NOX2 levels in PAD patients.

Compared with controls, patients with PAD had enhanced NOX2 (18.1±17.6 vs. 34.4±21.2 pg/mL; p<0.001) and urinary isoprostanes excretion (126.9±122.9 vs. 199.1±130.4 pg/mg creatinine; p=0.005), reduced NOx (22.0±6.07 vs. 13.8±6.6 uM; p<0.001) and lowered FMD (8.2±3.2 vs. 6.1±2.6%; p=0.001). Multiple linear regression analysis showed that the FMD was independently associated with NOX2.
General linear model analysis showed that PLC infusion was associated with a significant increase of FMD, with a reduction of NOX2 and urinary isoprostanes. No changes were observed after placebo treatment. *In vitro* study showed that platelet incubation with PLC was associated with inhibition of p47 phox translocation on cellular surface and consequently lowered NOX2 activation.
**Conclusions** This study provides the first evidence that in PAD patients NOX2 is over-expressed and may contribute to reduce FMD.

OC042 **OXIDATIVE STRESS IN TYPE 2 DIABETES MELLITUS: CONTRIBUTION OF VON WILLEBRAND FACTOR IN DIABETIC MICROANGIOPATHY**

De Cristofaro R.[(1)], Lancellotti S.[(1)], Oggianu L.[(2)], Pitocco D.[(3)], Zaccardi F.[(3)], Peyvandi F.[(4)], Palla R.[(4)], Mannucci P.M.[(4)], Pozzi N.[(5)], De Filippis V.[(5)]
*[(1)] Medicina Interna e Scienze Mediche Specialistiche, Medicina Interna e Geriatria, Università Cattolica S. Cuore, Roma; [(2)] Section of Biology Applied to Human Health, Rome Tre University, Roma; [(3)] Medicina Interna e Scienze Mediche Specialistiche, Patologia Medica e Semeiotica Medica, Università Cattolica S. Cuore, Roma; [(4)] A. Bianchi Bonomi Hemophilia and Thrombosis Center, University of Milan and Department of Medicine and Medical Specialties, IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Luigi Villa, Milano; [(5)] Department of Pharmaceutical Sciences, University of Padua, Padova*

An enhanced formation of reactive oxygen species and peroxynitrite occurs in several clinical settings including diabetes and cardiovascular diseases (CVD). Peroxynitrite oxidizes methionine and tyrosine residues to methionine sulfoxide (MetSO) and 3-nitrotyrosine (NT), respectively. Notably, ADAMTS-13 cleaves von Willebrand factor (VWF) exclusively at the Tyr1605Met1606 peptide bond in the A2 domain. In this study we assessed whether or not peroxynitrite can oxidize either or both of these amino acid residues, thus potentially affecting ADAMTS-13-mediated cleavage.
We tested our hypothesis synthesizing peptide substrates based on: (1) VWF Asp1596-Ala1669 sequence (VWF74) and (2) VWF Asp1596-Ala1669 sequence containing nitrotyrosine (VWF74-NT) or methionine sulfoxide (VWF74-MetSO) at position 1605 or 1606, respectively. The peptides were treated with human recADAMTS-13 and the cleaved peptides were analyzed by RP-HPLC. VWF74-MetSO was minimally hydrolyzed, whereas VWF74-NT was hydrolyzed efficiently and comparably to the native VWF74 peptide. Oxidation by peroxynitrite of purified VWF multimers inhibited ADAMTS-13 hydrolysis, but did not alter their electrophoretic pattern or their ability to induce platelet agglutination by ristocetin. Notably, VWF from 20 type 2 diabetic patients showed increased levels as both antigen and activity (Mann Whitney test, p<0.0001) and reduced activity/antigen ratio (p=0.04), compared to controls. Carbonyl content of plasma proteins of DM patients was significantly higher than in control subjects (0.6±0.1 nmol/mg vs. 0.09±0.02, p<0.0001). VWF multimers, purified from DM patients showed a relative resistance to ADAMTS-13 activity.
In conclusion, an exhalted oxidative stress in T2 DM may contribute to prothrombotic effects, hindering the proteolytic processing by ADAMTS-13 of high-molecular-weight VWF multimers, which have the highest ability to bind and activate platelets in the microcirculation. The reduced VWF:Act/ VWF:Ag ratio is consistent with an increased sequestration of VWF by platelets in these diabetic patients. These findings may pave the way to new antithrombotic strategies in this clinical setting.

## *EMOFILIA: TRATTAMENTO*

OC043 **DEEP INTRONIC MUTATIONS MAY CAUSE MILD HEMOPHILIA A WITH A POOR RESPONSE TO DESMOPRESSIN**

Castaman G.[(1)], Giacomelli S.H.[(1)], Mancuso M.E.[(2)], Sanna S.[(1)], Santagostino E.[(2)], Mannucci P.M.[(2)], Rodeghiero F.[(1)]
*[(1)] Department of Cell Therapy and Hematology, Hemophilia and Thrombosis Center, San Bortolo Hospital, Vicenza; [(2)] Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Medicine and Medical Specialties, University of Milan, IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Milano*

In about 2% of patients with severe Hemophilia A and 10-15% with mild HA no DNA mutation is detected after sequencing the whole F8 exons, their flanking splice site and the promoter region. mRNA analysis is useful to investigate the presence of nucleotide changes in the middle of intron regions that could create new cryptic splice site.
We performed FVIII mRNA studies in six patients with mild Hemophilia A and no apparent genomic mutation and a poor response to desmopressin. In five patients FVIII mRNA studies revealed the presence of an abnormal mRNA transcript in addition to normal FVIII mRNA. Sequencing of the abnormal transcripts revealed complex abnormalities which allowed the identification of three different intronic variation (c.2113+1152DelA, c.5587-93C>T and c.5999-277G>A) at DNA level, not present in 200 normal controls. These changes were also identified in relative female carriers. By in silico analysis, c.5587-93C>T and c.5999-277G>A caused the generation of new donor splicing sites with high score, leading to intron retaining and consequently to the creation of premature termination codons. These two predictions are perfectly in accordance with mRNA results. In contrast, in silico analysis of the c.2113+1152DelA variation predicts no particular change in the splicing score, leaving unexplained the introduction of intron 13 fragment at mRNA level in the patient and his mother. No mRNA abnormality was identified in the remaining patient.
Deep intronic variations may be responsible for mild hemophilia A and seem to predict a poor response to desmopressin. F8 mRNA analysis is a useful tool for the identification of deep intronic variation not detectable by standard DNA sequencing.

OC044 **INFLUENCE OF RECOMBINANT FVIIA ON CLOT STABILITY OF NORMAL AND HAEMOPHILIC BLOOD. SMALL CHANGES IN ASSAY CONDITIONS HAVE A GREAT IMPACT ON THE OUTCOME**

Galasso R.[(1)], Carrieri C.[(1)], Scaraggi A.[(1)], Marino R.[(3)], Semeraro N.[(1)], Colucci M.[(1)]
*[(1)] Dept. Biomedical Sciences Sect. of General & Experimental Pathology Bari; [(2)] MIDIM Hemostasis and Thrombosis Centre, Bari; [(3)] Policlinico Hemophilia Center Bari*

Recombinant FVIIa (Novoseven) has been shown to enhance clot resistance to lysis in haemophilic but not in normal plasma. We investigated the effect of Novoseven (100 U/mL) on tPA-induced lysis of blood clots from healthy subjects and haemophilia A patients with inhibitor under different conditions. Clotting and lysis times were measured by thromboelastography, using recalcified blood supplemented with tPA (100 ng/mL) and tissue factor (10-5 diluted Recombiplastin).
Novosen had no effect on clot formation and lysis of normal citrated blood. When blood was collected on corn trypsin inhibitor (40 ug/mL, CTI-blood) to avoid contact activation, Novosen markedly shortened clotting time (from 38±5.2 to 15±3.9 min, n=3, $p<0.05$), but had little effect on fibrinolysis ($p>0.05$). However, when a small amount of thrombin (0.2 U/mL) was added to blood to provide a constant initial thrombin burst, Novosen prolonged the lysis time by about 3-fold ($p<0.001$), an effect reversed by >80% by the TAFIa inhibitor PTCI.
In CTI-blood from haemophilic patients (2-25 y), an appreciable clot formation (within 120 min) occurred only in 2 out of 6 patients studied. On Novosen addition, clot formation occurred in all sample (10±2.8 min) and mean lysis time was 63±33 min. When thrombin (0.2 U/mL) was added to CTI-blood, clot formation occurred rapidly in all samples, and Novosen addition resulted in a marked prolongation of lysis time (from 32±11 to 64±19 min, $p<0.01$).
These data indicate that Novosen is able to improve clot stability in whole blood, largely though a TAFI-mediated mechanism, not only in haemophilic samples but also in normal blood, under conditions of weak clotting activation, suggesting the potential use of this haemostatic agent beyond clotting factor deficiencies.

OC045 **IMMUNE TOLERANCE INDUCTION WITH EMOCLOT: A SINGLE CENTER EXPERIENCE**

Mancuso M.E., Santagostino E.
*Centro Emofilia e Trombosi Angelo Bianchi Bonomi, Dipartimento di Medicina Interna, Milano*

Immune tolerance induction (ITI) is able to eradicate inhibitors in patients with hemophilia A (HA) and the type of FVIII product may influence the likelihood of success. Our aim was to assess the outcome of ITI after a prolonged follow-up period in a retrospective series of hemophiliacs with inhibitors treated with a plasma-derived FVIII concentrate containing VWF (Emoclot, Kedrion, Castelvecchio Pascoli, Italy).
Eleven patients with severe HA and high-responding inhibitors (median historical peak: 135 BU/mL, IQR: 31-475) started ITI at a median age of 17 years (IQR: 15-34). Median pre-ITI inhibitor titer was 2.0 BU/mL (IQR: 1.5-7.0) and median peak titer during ITI 43 BU/mL (IQR: 10-123). FVIII doses ranged from 50IU/kg every other day (7 patients) to 100 IU/kg/day. Median ITI duration was 15.6 months (IQR: 13.0-25.4). Overall, ITI was successful in 9 patients (82%): complete success (negative inhibitor titer and normal FVIII recovery and half-life) after a median of 15 months in 4 (36%) and partial success (inhibitor titer <5 BU/mL and/or abnormal FVIII recovery and/or half-life) after a median of 22.5 months in 5 (45%, only one with detectable inhibitors). During the post-ITI follow-up period (median 17years, IQR: 15-20) all 9 patients were successfully treated with Emoclot (2 on prophylaxis). At the end of follow-up 2 patients who previously had a partial success normalized FVIII recovery and half-life. Inhibitor relapse occurred in 2 patients (both partial successes) during on demand treatment with Emoclot: at low titer after 3 months in one who maintained high-dose FVIII on demand treatment and at high titer after 3 years in the other who underwent a second ITI with Emoclot achieving a partial success.
In this series, the success rate with Emoclot at low/medium doses was similar to that reported with other FVIII products and the long-term outcome was greatly satisfactory since 9/11 patients benefited from an effective FVIII replacement therapy.

OC046 **BIOEQUIVALENCE OF B-DOMAIN DELETED AND PLASMA DERIVED FVIII CONCENTRATES**

Morfini M.[(1)], Iorio A.[(2)], Mazzucconi M.G.[(3)], Cinotti S.[(1)], Zanon E.[(4)], Paladino E.[(1)], Marchesini E.[(2)]
*[(1)] Hemophilia Centre, Azienda Ospedaliera Universitaria Careggi, Florence; [(2)] Internal and Vascular Medicine, University of Perugia, Perugia; [(3)] Hemophilia Centre, La Sapienza University, Roma; [(4)] Hemophilia Centre, Padova Hospital, Padova*

**Introduction** AESPERT (Across factor Eight Switch Pharmacokinetic Evaluation of Recombinant Product Trial) was an Italian multicenter single dose pharmacokinetic study comparing a plasma derived (Emoclot) against a BBD (Refacto) factor VIII. The study was investigator driven and approved by the Ethical Committee of each participating Haemophilia Center.
**Methods** 14 patients, >18 yr, FVII<0.1, without FVIII inhibitors, >150 ED, were enrolled when switching to BDD-rFVIII. A single dose (25 U/kg) of both concentrates, according to the labelled potency of each product, was administered by bolus (5-10 min) 15 days apart, after 4 days of washout. Blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, 10, 12, 24, 36 and 48 hours FVIII:C was assayed in duplicate on three dilutions by one stage clotting method with product specific calibration curves. The FVIII concentration/time curves were analysed by model independent method (WinNonlin, Pharsight) to evaluate CMax, Recovery, AUC, AUMC, Clearance, MRT, terminal Half-Life and VD.
**Results** The concentration-time curve MLE fitting was very good for both products (r=0.9). AUC (U*h/L) of Refacto (113±5.30) was slightly higher than that of pd-FVIII (102±7.90) and consequently the Clearance (mL/kg/h) was smaller (3.14 vs. 3.47). Terminal HLs (hr) were similar (14.36

vs. 14.36) while Cmax (IU/dL) was higher (71.6 vs. 60.2) and VDA (mL/kg) smaller (56.3 vs. 66.7) for BBDrFVIII, in agreement with higher AUC and IVR (U/dL/U/kg) (2.86 vs. 2.41, BBD-rFVIII vs. Emoclot).
**Conclusions** PK characteristics of the BBD-FVIII resulted very similar to that of a plasma-derived naive high purity FVIII concentrate.

## OC047 EFFECT OF PLASMA-DERIVED OR RECOMBINANT FACTOR VIII ON INHIBITOR DEVELOPMENT. A SYSTEMATIC REVIEW

Iorio A.[(1)], Halimeh S.[(2)], Goldenberg N.[(3)], Kenet G.[(4)], Young G.[(5)], Brandao L.R.[(6)], Marchesini E.[(1)], Marcucci M.[(1)], Mannucci P.M.[(7)], Nowak-Gottl U.[(8)]
*[(1)] Internal and Vascular Medicine, University of Perugia, Perugia; [(2)] Hemophilia Centre, Duisburg Hospital, Duisburg, Germany; [(3)] Hemophilia Centre Denver, Aurora Hospital, Denver, USA; [(4)] Hemophilia Centre, Tel-Hashomer Hospital, Tel-Hashomer, Israel; [(5)] Hemophilia Centre, Los Angeles Hospital, Los Angeles, USA; [(6)] Hemophilia Centre, Toronto Hospital, Toronto, Canada; [(8)] Hemophilia Centre, Angelo Bianchi Bonomi Institute, Milano; [(8)] Hemophilia Centre Munster University Munster, Germany*

**Introduction** Different rate of inhibitors after plasma derived (pd) or recombinant (r) FVIII has been hypothesized and conflicting results were reported in the literature. Objectives of study were to systematically review the incidence rates (IR) of inhibitors in previously untreated patients (PUPs) with hemophilia A (HA) treated with either pd or rFVIII and to explore the role of study and patient characteristics as confounders.
**Methods** Summary IR (95% confidence interval, CI) from included studies was recalculated and pooled for pd or r FVIII. Sensitivity analysis was used to investigate the effect of study design, severity of disease and inhibitor characteristics. Meta-regression and analysis-of-variance were used to investigate the effect of covariates (testing frequency, follow up duration, intensity of treatment).
**Results** 2,094 patients (1,965 treated with pd, 887 with r FVIII; median age: 9.6 months) from 24 studies were included in the analysis. Pooled IR (CI) was 14.3% (10.4-19.4) for pd and 27.4% (23.6-31.5) for r; high-responding (HR) inhibitor IR was 9.3% (6.2-13.7) for pd and 17.4% (14.2-21.2) for rFVIII. In the multi-way ANOVA study design, study period, testing frequency and median follow up explained most of the variability while the effect of source of concentrate diminished and turned non significant. It was not possible to analyze effect of treatment intensity or trigger events like surgery.
**Discussion** These findings underscore the need for further studies, namely randomized clinical trials or IPD meta-analyses, to address whether or not the risk of inhibitor in PUPs with HA differs between r and pd FVIII.

## OC048 TYPE OF FACTOR VIII PRODUCT AS RISK FACTOR FOR INHIBITOR DEVELOPMENT IN PATIENTS WITH HAEMOPHILIA A AND NULL MUTATIONS

Mancuso M.E.[(1)], Mannucci P.M.[(1)], Rocino A.[(2)], Garagiola I.[(1)], Tagliaferri A.[(3)], Margaglione M.[(4)], Santagostino E.[(1)]
*[(1)] Centro Emofilia e Trombosi Angelo Bianchi Bonomi, Dipartimento di Medicina Interna e Specialità Mediche, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano; [(2)] Centro Emofilia, Ospedale San Giovanni Bosco, Napoli; [(3)] Centro Emofilia, Ospedale Universitario, Parma; [(4)] Istituto di Genetica Medica, Università di Foggia, Foggia*

This retrospective study evaluates risk factors for inhibitor development in severe/moderate hemophiliacs. Clinical data up to inhibitor development or 150 exposure days (EDs) were collected in 370 patients with FVIII<5% (FVIII<1%: 310, 84%) and median age of 21 years (IQR: 6-33) first treated with plasma-derived (pdFVIII, 68%) or recombinant products (rFVIII, 32%). Exposure to blood components >4 days was an exclusion criterion. The F8 genotype was known in 320 patients (86%), 57% with null mutations (nonsense mutations, inversions and large deletions). Inhibitors (titer >0.6 BU/mL on at least two consecutive occasions) were detected in 109 patients (29%) after a median of 17 EDs (IQR: 10-29), being high responding (peak titer >5 BU/mL) in 25%. By multivariate Cox regression model family history of inhibitors, intensive treatment and the use of rFVIII at first exposure were independent risk factors for inhibitor development (adjusted hazard ratios 2.5, 3.3 and 3.9, 95% CI 1.2-5.1, 1.5-7.2 and 1.4-11.0, respectively). Sub-analyses were performed in 334 patients who never switched class of product and in 296 patients who did not start prophylaxis: in both cases the use of rFVIII was associated with an increased inhibitor risk (adjHR 3.3, 95% CI 1.4-7.5 and 3.6, 95% CI 1.6-8.2, respectively). Similar results were obtained also excluding 97 patients (26%) who were first exposed to blood components (adjHR 4.0, 95% CI 1.7-9.7). Interestingly, patients with null mutations treated with rFVIII had a higher inhibitor risk than those first treated with pdFVIII (adjHR 2.6, 95% CI 1.2-6.0), while no difference was found in patients with non-null mutations treated with rFVIII (HR 1.3, 95% CI 0.5-3.6). These results were confirmed including only high-responding inhibitors in the analysis.
**Conclusions** Our results show the influence of the interaction between type of FVIII product and F8 genotype on inhibitor risk, being the use of rFVIII associated with a higher risk in patients with null mutations.

## OC049 RESCUE OF COAGULATION FACTOR VII MRNA PROCESSING AND PROTEIN FUNCTION BY ENGINEERED U1+5A SNRNA

Balestra D.[(1)],Cavallari N.[(1)], Maestri I.[(2)], Mari R.[(3)], Rizzotto L.[(1)], Pagani F.[(4)], Bernardi F.[(1)], Pinotti M.[(1)]
*[(1)] Biochimica e Biologia Molecolare, Università degli Studi di Ferrara, Ferrara; [(2)] Medicina Sperimentale, Università degli Studi di Ferrara, Ferrara; [(3)] Centro di Emostasi e Trombosi, Università degli Studi di Ferrara, Ferrara; [(4)] Human Molecular Genetics, International Centre for Genetics, Trieste*

Changes affecting mRNA processing represent a frequent cause of severe coagulation factor defects and of all inherited human diseases.
We extensively investigated the IVS7+5G/A mutation (9726+5A) in the coagulation factor VII (FVII) gene, occurring in the IVS7 donor splice site (5ss) in the first of six

highly homologous 37bp repeats containing several cryptic splice sites. This mutation is the most frequent cause of severe FVII deficiency in Central Italy.
A cellular model of this deficiency was created by producing a full length FVII splicing competent construct (pSCFVII-wt). This minigene drove in COS-1 cells the synthesis of properly processed FVII transcripts and of secreted functional FVII (23 ±4 ng/mL), which were virtually undetectable upon introduction of the mutation (pSCFVII-9726+5A). At the mRNA level the mutation caused exon 7 skipping and, to a less extent, activation of the 37-bp downstream cryptic site.
To attempt rescue of FVII expression, we have engineered the U1-snRNA, the spliceosome component selectively recognizing donor splice sites (5'ss), to re-direct recognition of the mutated donor splice site. Upon cotrasfection of the engineered U1-snRNA (pU1+5A) with pSCFVII-9726+5A, the splicing pattern and protein level were evaluated. At RNA level, the expression of U1+5A reduced from 80% to 40% the exon 7 skipping and increased recognition of the correct 5'ss, resulting in appreciable synthesis of normal transcripts (from hardly detectable to 20%). At protein level, we observed an increase of secreted protein levels in medium (5.0±2.8 ng/mL) and of the FVII coagulant activity, which reached 9.5±3.2% of pSCFVII-wt. The effects of engineered U1-snRNA were dose-dependent.
Altogether these results demonstrate for the first time the U1-snRNAmediated rescue at the mRNA and protein levels, thus highlighting its therapeutic implications in bleeding disorders, which would benefit even from tiny increase of functional levels.

## *GENETICA*

### OC050 **A NEW WARFARIN DOSING ALGORITHM INCLUDING 3730G/A VKORC1 POLYMORPHISM: COMPARISON WITH THE RESULTS OBTAINED BY OTHER PUBLISHED ALGORITHMS**

Cini M., Legnani C., Guazzaloca G., Valdrè L., Frascaro M., Poggi M., Bettini F., Lo Manto G., Palareti G.
*Dept. Angiology and Blood Coagulation "Marino Golinelli", University Hospital S. Orsola-Malpighi, Bologna*

Recently, several dosing algorithms which combine clinical and genetic parameters [polymorphisms in the gene for cytocrome P450 2C9 (CYP2C9) and in the gene for vitamin K epoxide reductase complex subunit 1 (VKORC1)] to predict the optimal warfarin dose have been developed.
We evaluated 45 patients (28 males, mean age 70 years, range 22-92 years), on long-term stable warfarin therapy whose mean therapeutic warfarin dose was 5.30 mg/day.
They were genotyped for detection of two CYP2C9 (CYP2C9*2 and CYP2C9*3) and three VKORC1 (-1639G/A, 1173C/T, 3730G/A) polymorphisms.
The accuracy of eight published algorithms (referred to as Gage, Sconce, Zhu, Wadelius, IWPC, Perini, Shelleman, Anderson) to predict stable warfarin dosage was retrospectively analyzed in our population. Six of the 8 algorithms tended to under-predict the dose, while the dosage was greatly over predicted by one algorithm (mean 13.35 mg/day, Gage). The accuracy in predicting warfarin dose was similar for three algorithms (Sconce, Wadelius, IWPC) with a mean absolute error (MAE) ranging from 1.49 to 1.91 mg/day and R2 from 0.72 to 0.74; the percentage of patients in range (range=predicted dose within + or – 1 mg/day of actual dose) varied from 44.4% to 51.1%, and the percentage of patients over predicted (predicted dose ≥2 mg/day of actual dose) from 0 to 2.2%.
We also evaluated a new algorithm developed by using a multiple linear regression equation including: age, gender, height, weight, smoking status, alcohol and vegetables consumption, history of venous thromboembolism, diabetes, CYP2C9, VKORC1 -1639G/A and 3730G/A genotype. This algorithm showed a mean predicted dose of 4.94 mg/day, with a MAE=0.85, $R^2$=0.90, 71.1% patients in range and 2.2% patients over predicted in our study group.
In conclusion, these data suggest that the inclusion of the 3730G/A VKORC1 genotype in the algorithm can improve the accuracy in warfarin dose prediction.

### OC051 **FACTOR VII DEFICIENCY: ANALYSIS OF THE GENOTYPES OF 19 UNRELATED PATIENTS**

Riccardi F.[(1)], Di Perna C.[(1)], Rivolta G.F.[(1)], Martorana D.[(2)], Angeri F.[(1)], Neri T.M.[(2)], Tagliaferri A.[(1)]
*[(1)] Regional Reference Center for Inherited Bleeding Disorders, University-Hospital of Parma, Parma; [(2)] Molecular Genetics Unit, University Hospital of Parma, Parma*

Congenital deficiency of factor VII (FVIID) is an autosomal recessive hemorrhagic disorder that occurs with an estimated incidence of 1 per 500,000 Caucasians. The phenotypes of affected patients vary considerably, ranging from those suffering fatal cerebral nervous system haemorrhages to asymptomatic patients, with a poor relationship between the FVII coagulant activity (FVII:C) and bleeding tendency.
The human factor VII gene (F7) is located at chromosome 13q34 and it comprises 9 exons.
The main objective of this study was to identify molecular defects in F7 gene in 43 Emilia-Romagna patients with suspected hereditary FVIID, in order to facilitate genetic counselling and prenatal diagnosis. The factor VII gene, including exons, flanking and promoter regions, were analysed by DHPLC and direct sequencing.
We found 16 different mutations in 19 unrelated patients.
Heterozygous amino acid changes were identified in 16 unrelated patients: 3 were novel mutations (Q312X, S269P, IVS1+4C>T) and 8 were previously described mutations (IVS1+5G>A, Y68C, R79W, R223W, D242N, R290C, R304Q, G331S).
Two patients were compound heterozygous; one with severe reduction of FVII (3 IU/dl) was characterized by the presence of two mutations (S339F and A244V). S339F was not previously described. The other patient, a woman with 0.7 FVII:C, had a recurrent mutation (C310F) and a synonymous amino acidic change (E35E) that need further investigations.
Finally, we identified the mutation T359M in an homozygous patient with severe form of FVIID (FVII:C = 0.9 IU/dl).

### OC052 **MOLECULAR BASES OF HEREDITARY ANTITHROMBIN (AT) DEFICIENCY: 14 NOVEL MUTATIONS IN THE ANTITHROMBIN GENE IN 27 PATIENTS**

Guida A.[1], Tufano A.[2], Cerbone A.M.[1], Tandurella I.[3], Ceglia C.[3], Di Minno M.N.D.[1], Ruocco A.L.[1], Castaldo G.[3], Di Minno G.[1]
*[1] Department of Clinical and Experimental Medicine, University "Federico II", Naples; [2] Regional Reference Centre for Thrombosis and Haemostatis, University "Federico II", Naples; [3] Dept of Biochemistry and Molecular Biology, University "Federico II", Naples*

**Background** Inherited AT deficiency is a major thrombophilic condition (prevalence 1:500-1:5,000). It is a heterogeneous disorder. The AT gene (SERPINC1, 7 exons) has been mapped on 1q23-25.
**Aim** To elucidate molecular basis of AT deficiency in 27 AT deficient patients.
**Materials and Methods** We screened 27 AT-deficient patients referred to our Centre (14 M/13 F); 21 type I, 3 type II, 3 unclassified, from 16 unrelated families. Twenty-four out of 27 had thrombotic events: 20/24 (82%) had venous thromboembolism (VTE); 3/24 experienced both arterial and venous thrombosis, one patient (1/24) only experienced arterial thrombosis. Among VTE patients, 10/20 (50%) experienced recurrences. The mutations of SERPINC1 and additional thrombophilic conditions have been analyzed.
**Results** By sequencing the amplified fragments corresponding to exons 1-7 of the SERPINC1 gene, 16 candidate causal mutations have been identified, only two of them previously reported (missense c.235C>T mutation, Arg79Cys in the exon 2, and a site splicing c.1218+1 G>A in exon 6). The remaining 14 (5 frameshift; 5 nonsense; 3 missense; 1 deletion inframe) were novel mutations. As a whole, 3 mutations in exon 2; 2 in exon 3; 1 in exon 4; 3 in exon 5; 3 in exon 6; 4 in exon 7, and 2 polymorphisms have been found, 1/3 of them being likely to produce truncated proteins. All were found to affect highly conserved residues in the SERPINC1 protein.
Among the subjects with type I deficiency, there were mutations occurring in exon 2 (heparin binding site, n=3) and exon 7 (thrombin binding site, n=3) as much as in exons 3, 4, 5 and 6, arguing for functionally relevant mutations, depending on the site and on the severity of the mutation.
**Discussion** We have identified a spectrum of unreported mutations that may be of value to unravel the role of specific regions of AT as to structure/function relationships.

## OC053 **TWO NOVEL MUTATIONS WITHIN AFIBRINOGENEMIC ITALIAN PATIENTS**

Pisanelli D.[1], Valdrè L.[2], Soli F.[1], Cappucci F.[1], Legnani C.[2], Palareti G.[2], Rizzo M.A.[4], Grandone E.[1], Margaglione M.[5]
*[1] Emostasi e Trombosi, I.R.C.C.S. Casa Sollievo della Sofferenza, San Giovanni Rotondo; [2] U.O. Angiologia e Malattie della Coagulazione, Policlinico S. Orsola-Malpighi, Bologna; [3] Genetica Medica, APSS-Azienda Provinciale per i Servizi Sanitari, Trento; [4] U.O. Laboratorio analisi, Ospedale San Carlo, Potenza; [5] Scienze Biomediche - Genetica, OO.RR. Ospedali Riuniti, Foggia*

Congenital afibrinogenemia (CAF, OMIM No.202400) is a rare autosomal recessive disorder characterized by bleeding manifestations ranging from mild to severe and by extremely low levels of both functional (Clauss) and antigenic fibrinogen.
Here we describe two novel mutations. The first case (FA) is a 41-years old afibrinogenemic patient who was diagnosed when he was a young man. For many years he received prophylactic infusions of fibrinogen (about 70 mg/kg body weight with target fibrinogen levels of about 100 mg/dL). At the age of 36 he showed a myocardial infarction and, 6 days later, an arterial thrombosis (right radial artery) and an acute ischemic stroke involving frontal area. At that time fibrinogen levels were undetectable.
Recently, another 40-years patient (CF) from Southern Italy with Clauss and antigenic fibrinogen levels undetectable and a cerebral haemorrhage was studied.
DNA sequencing of fibrinogen α, ß, and γ chains genes in proband allowed the identification of two novel missense mutations:
1) Ala277Ser substitution in the homozygous state within the exon 6 of fibrinogen β chain (FA);
2) Cys197Phe substitution in the homozygous state within the exon 4 of fibrinogen β chain (CF).

Alignment of the human fibrinogen partial protein sequence with those of other organisms showed that both the mutations occur in highly conserved amino acid residues among different species. The molecular modelling analysis showed the effects of mutations on the protein structure, suggesting that the Ala277 residue is crucial for the glycosilation and might impair the correct folding of the protein, while the Cys197Phe causes the rupture of a disulphide bond with the Cys139 in the gamma chain, giving rise to an aromatic ring. Thus, both the mutations seem to be critical for the correct folding of fibrinogen and could be affect the circulating plasma levels.

## OC054 **GENETIC DETERMINANTS OF PLATELET PARAMETERS IN FAMILIES WITH JUVENILE MYOCARDIAL INFARCTION**

Gianfagna F., Vohnout B., Tamburrelli C., Crescente M., Cutrone A., Di Castelnuovo A., Donati M.B., de Gaetano G., Cerletti C., Iacoviello L.
*John Paul II Centre for High Technology Research and Education in Biomedical Sciences, Catholic University Research Laboratories, Campobasso*

**Background** Platelets are involved in the pathogenesis of myocardial infarction (MI). Variations in platelet parameters among individuals could modulate the risk of thrombosis. This study aims at evaluating the heritability of some platelet parameters, the genetic correlations among such variables and the genetic correlations between platelet parameters and juvenile MI.
**Methods** The study population included 732 subjects (aged 43±19 SD, 44% men) from 54 extended pedigrees in 2-4 generations (23 families with familiarity for MI at young age and 31 matched control families). Blood cell count, whole blood platelet function, mixed platelet-leukocyte conjugates and markers of platelet and leukocyte activation (basal and ADP-collagen stimulated levels of P-selectin surface expression on platelets and L-selectin and CD11b on monocytes and PMNs) were measured. Heritability and genetic correlations were evaluated by variance component and bivariate linkage analysis performed by SOLAR software. Factor analysis and generalized estimating equations

methodology were used by SAS software to evaluate pleiotropism and association with disease or familiarity.
**Results** All traits had significant genetic components, ranging between 15.2% and 69.0% of the phenotypic variability (highest value for MPV). Shared household effects tended to be smaller (range 0-39.6%, highest value for P-selectin). Environmental covariates explained 0-13.7% of the trait variances, reaching 40.5% for basal platelet-PMNs aggregates when also biological covariates were added. Markers of cell activation and platelet-platelet or platelet-leukocyte aggregates were found to be the most genetically correlated traits. MI at young age showed heritability of 58.6% with no significant effect of household shared factors and environmental covariates. Low levels of mixed aggregates, in combination with different surface expression levels of proteins involved in cell aggregate formation showed significant genetic correlations among them (pleiotropism) and association with familiarity and disease.
**Conclusions** Genetically determined platelet phenotypes could contribute to the risk of MI at young age.
*Supported by Telethon GGP04198.*

### OC055 **IDENTIFICATION OF MOLECULAR BASIS OF ANTITHROMBIN DEFICIENCY: CLINICAL FEATURES OF 25 INVESTIGATED PROBANDS AND REPORT OF NINE NOVEL MUTATIONS**

De Stefano V., Marietti S., Rossi E., Za T., Ciminello A., Betti S., Chiusolo P., Leone G.
*Institute of Hematology, Catholic University, Rome*

More than 200 mutations cause type I (quantitative) or II (qualitative) antithrombin (AT) deficiency (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=SERPINC1). Type II is subclassified according to dysfunction of reactive site (RS) or heparin binding site (HBS) or pleiotropic effects; in type II HBS the thrombotic risk is low.
DNA of 18 unrelated patients with AT deficiency and venous thromboembolism (VTE) was sequenced according to Picard et al. (Thromb Haemost 2005;93:57). Seven asymptomatic women with AT deficiency identified by screening before pregnancy or contraceptives intake were also analyzed. Sixteen different mutations were identified in patients with VTE. Two novel (W307X, Y260_P352del -two cases) and five known (C-4X, A94V, R129X -two cases, R132X, R425QfsX8) mutations were found in nine heterozygous patients with type I deficiency. One novel (E265K) and one known (M251I) mutations were found in two heterozygous patients with type II RS deficiency; three known mutations (L270P, A404T, L409P -two cases) were found in four heterozygous patients with pleiotropic (quantitative-qualitative) AT deficiency. For two novel heterozygous mutations (E180K and E205K) the phenotype was not characterized. Finally, one patient with type I deficiency was double heterozygous for two novel mutations, L210PfsX43 and G2R; the father heterozygous for L210PfsX43 had type I deficiency, whereas heterozygous G2R mutation had null effect in the mother. Among the asymptomatic women, three with type I deficiency had two novel (S250IfsX16 and V303CfsX13) and one known (C-4X) heterozygous mutations. Four with type II HBS deficiency had three known heterozygous mutations (R47H, R47C, L99F -two cases). In four pregnant women (one type I and three type II HBS) antithrombotic prohylaxis was tailored according to the phenotype.
In conclusion, molecular bases of AT deficiency are heterogeneous; their identification can provide data to understand AT structure-function and give advice for antithrombotic prophylaxis tailored according to AT deficiency subtypes.

### OC056 **CYCLOOXYGENASE-1 HAPLOTYPE C50T/A-842G DOES NOT AFFECT PLATELET RESPONSE TO ASPIRIN**

Liani R., Pettinella C., Romano M., Stuppia L., Santilli F., Davì G.
*G. d'Annunzio University Foundation, Center of Excellence on Aging, Chieti*

COX-1 polymorphism C50T, in complete linkage disequilibrium with the other polymorphism A-842G, has been depicted as a determinant of pharmacological response to aspirin treatment.
Whether these polymorphisms exert an effect on response to aspirin both *in vitro* and *ex vivo* is still controversial.
We genotyped a population of 148 healthy individuals for the C50T/A-842G haplotype and among them 30 persons underwent low dose aspirin (100 mg daily) treatment for 4 weeks and were followed up for 7 days after withdrawal. In this subgroup we evaluated the thromboxane-dependence of biochemical and functional indexes used to monitor the antiplatelet effect of low-dose aspirin.
We found 10 heterozygous individuals among 148 subjects studied (6.7%) and only one homozygous (0.67%). In the group on low-dose aspirin serum thromboxane (TX) B2 as well as urinary 11-dehydro-TXB2 and arachidonic acid (AA) induced aggregation were uniformly suppressed in carriers and non carriers of the 50T/-842G haplotype with an increase until basal levels of all the parameters in 7 days after withdrawal.
We found no relationship between the 50T/-842G haplotype and the so called phenomenon of aspirin resistance. Platelet cyclooxygenase activity, as reflected by serum TXB2, is uniformly and persistently suppressed by low-dose aspirin in both groups of carriers and non carriers of these polymorphisms.

## *INFIAMMAZIONE E TROMBOSI*

### OC057 **GLYCOGEN SYNTHASE KINASE-3 NEGATIVELY REGULATES TISSUE FACTOR EXPRESSION IN MONOCYTES INTERACTING WITH ACTIVATED PLATELETS**

Evangelista V., Amore C., Manarini S., Di Santo A.
*Laboratorio di Biologia e Farmacologia Vascolare, Consorzio Mario Negri Sud, Santa Maria Imbaro*

At the site of vascular injury monocytes (MN) interacting with activated platelets (PLT) synthesize tissue factor (TF) and promote thrombus formation. Intracellular signals necessary for the expression of TF in MN, in the context of a developing thrombus, remain unknown.

The study was designed to investigate the role of glycogen synthase kinase 3 (GSK3), a serine-threonine kinase, down-stream insulin receptor pathway, on PLT-induced TF expression in MN. To this purpose we used a well characterized *in vitro* model of human MN-PLT interactions that allows detailed analysis of TF activity, TF protein and gene expression.
The results demonstrated that, in MN interacting with activated PLT: 1) TF activity, antigen and mRNA were low until 8-10 hours and dramatically increased thereafter, up to 24 hours. 2) According to the kinetics of TF expression in MN, GSK3β undergoes phosphorylation on serine 9, a process associated with down-regulation of enzyme activity. 3) Pharmacological blockade of GSK3 further increased TF expression and was accompanied by increased accumulation of NF-kB, in the nucleus. 4) Blockade of phosphoinositide-3-OH kinase (PI(3)K) by wortmannin inhibited PLT-induced TF expression. 5) According to the established role of GSK3 down-stream insulin receptor, insulin increased PLT-induced TF expression in a PI(3)K-dependent manner. GSK3 acts as molecular brake of the signalling pathway leading to TF expression in MN interacting with activated PLT. PI(3)K, through Akt-dependent phosphorylation of GSK3, relieves this brake and allows TF gene expression.
This study identifies a novel molecular link between thrombotic risk and metabolic disorders.

## OC058 PON2 SER311CYS POLYMORPHISM AS A PREDICTOR OF TOTAL AND CARDIOVASCULAR MORTALITY IN PATIENTS WITH ANGIO-GRAPHICALLY PROVEN CORONARY ARTERY DISEASE

Martinelli N.[(1)], Girelli D.[(1)], Trabetti E.[(2)], Malerba G.[(2)], Consoli L.[(1)], Micaglio R.[(1)], Friso S.[(1)], Pizzolo F.[(1)], Pignatti P.F.[(2)], Corrocher R.[(1)]
*[(1)] Medicina, Università di Verona, Verona; [(2)] Scienze di vita e riproduzione, Università di Verona, Verona*

**Background** Paraoxonase (PON) genes (PON1 and PON2) polymorphisms have been linked to cardiovascular risk with controversial results. Two PON1 polymorphisms (Leu55Met and Gln192Arg) have been recently associated with long-term clinical outcome in patients with coronary artery disease (CAD). The aim of this study was to evaluate the role of three PON genotypes (PON1 Leu55Met and Gln192Arg, and PON2 Ser311Cys) as possible predictors for total and cardiovascular mortality in the setting of established CAD.
**Materials and Methods** A cohort of 664 patients with angiographically proven CAD was prospectively followed after coronary revascularization (endovascular or surgical).
**Results** After a median follow-up of 57 months, 100 patients had died (15.1%), 65 because of cardiovascular causes (9.8%). PON2 Ser311Cys genotype was associated with total (Ser/Ser 16.9%, Ser/Cys 13.0%, Cys/Cys 3.3%) and cardiovascular mortality (Ser/Ser 11.0%, Ser/Cys 8.7%, Cys/Cys 0%) by Kaplan-Meier analysis (P=0.023 and P=0.044, respectively), while no significant association was found for the two PON1 polymorphisms. After adjustment for the other predictors of mortality at univariate analysis (i.e. age, myocardial infarction history, hs-CRP and creatinine), including also HDL-levels and PON1 polymorphisms, PON2 genotype significantly predicted both total and cardiovascular mortality, with minor allele 311-Cys associated with a lower risk of death (HR per allele copy: 0.61 with 95% CI 0.380.97 for total mortality; 0.53 with 95% CI 0.28±0.99 for cardiovascular mortality).
**Conclusions** Our results suggest that PON2 Ser311Cys polymorphism may predict mortality in CAD patients after coronary revascularization.

## OC059 PROSTACYCLIN REDUCTION REGULATES TISSUE FACTOR AND PREDISPOSES COX-2 KNOCKOUT MICE TO CAROTID ARTERY THROMBOSIS

Barbieri S.S.[(1)], Gianellini S.[(1)], Amadio P.[(1)], Zacchi E.[(1)], Howe L.R.[(2)], Weksler B.B.[(3)], Mussoni L.[(1)], Tremoli E.[(4)]
*[(1)] Dept. Pharmacological Sciences, University of Milan, Milan; [(2)] Dept. of Cell and Developmental Biology, Weill Cornell Medical College, New York, USA; [(3)] Division of Hematology and Medical Oncology, Weill Cornell Medical College, New York, USA; [(4)] Dept. Pharmacology and Cardiologic Center IRC University of Milan, Milan*

Selective inhibitors of cyclooxygenase-2 (COX-2) increase the risk of myocardial infarction and athero-thrombotic events, but the mechanisms responsible for these effects are not fully understood. To assess the role of COX-2 in arterial thrombosis, a well-studied, chemically-induced model of arterial injury was applied to COX-2 knockout mice (COX-2KO) and data were compared with those obtained in wild-type (WT) mice. We found that thrombus formation in response to ferric chloride injury of the carotid arteries was significantly increased in COX-2-null mice compared to WT mice. To explain this observation, we first investigated the role of platelets in our model. Platelets isolated from blood of COX-2KO mice showed enhanced response to collagen and ADP, in terms of aggregation together with increased ability to produce thromboxane compared to WT platelets. Interestingly, cyclooxygenase-1 and thromboxane synthase protein levels were similar in both animal groups. However, using cross-transfusion experiments, we excluded the likelihood that COX-2KO platelets were responsible for the increased arterial thrombus formation in COX-2-null mice. Importantly, we found that the activity of tissue factor (TF), initiator of blood coagulation, was elevated in concentrated microparticles derived from plasma as well as in leukocytes and in the carotid arteries of COX-2KO mice. Increased levels of TF mRNA correlated with reduced levels of prostacyclin synthase mRNA and the decreased prostacyclin production in the arterial wall of COX-2-null mice. In addition, treatment with CA10441, an antagonist of prostacyclin receptor (IP), increased TF activity in the carotid arteries of WT mice. Conversely, carbacyclin, a stable IP agonist, reduced TF activity in the carotid arteries of COX-2KO mice. These findings reveal for the first time that the propensity to thrombosis observed in association with COX-2 inhibition is consequent to the impairment in the generation of prostacyclin by vascular endothelium, which in turn results in increased TF expression and activity.

## OC060 DOWNREGULATION OF TISSUE FACTOR EXPRESSION IN HUVEC AND TUMOR CELLS BY BETAINE

Napoleone E.[(1)], Cutrone A.[(1)], Cugino D.[(1)], Santimone I.[(1)], Iacoviello L.[(1)], Grossi E.[(2)], de Gaetano G.[(1)], Donati M.B.[(1)], Lorenzet R.[(1)]
*[(1)] Laboratori di Ricerca, Università Cattolica, Campobasso; [(2)] Direzione Medica, Bracco SpA, Milano*

**Background** Betaine, an organic compound used in clinical practice to lower homocysteine levels with a potential benefit for cardiovascular health, has been implicated in the etiology of cancer. A methyl donor in the methionine cycle, betaine maintains normal DNA methylation pattern in tumours with epigenetic alterations.
Tissue factor (TF), the trigger of blood coagulation, is often expressed by tumour-associated endothelial and inflammatory cells, as well as by cancer cells themselves, and plays a predominant role in tumour growth, metastasis formation and angiogenesis.
**Aim** To evaluate the effect of betaine on TF expression in human monocytes (MN), human umbilical vein endothelial cells (HUVEC) and the metastatic breast carcinoma cell-line MCF7.
**Methods** MCF7 cells were grown in 10% FCS DMEM until confluency. MN were obtained from whole blood collected from healthy donors by Lymphoprep sedimentation. HUVEC were grown in 15% FCS 199/DMEM until confluency. Cells were then incubated with betaine 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 mRNA was assessed by real-time RT-PCR.
**Results** Betaine downregulated the constitutive TF activity of MCF7 in a dose-dependent way. The decrease was paralleled by a reduction in TF mRNA levels.
A similar inhibition was observed when endotoxin-stimulated HUVEC were cultured in the presence of betaine. At odds with these observation, TF expression by MN exposed to endotoxin, as well as to TNF-α or IL-1β, was unaffected by betaine.
**Conclusions** These data support the hypothesis that betaine, by its downregulation of TF, may play a role in inflammatory processes underlying both cancer and vascular cell disorders.

## OC061 ASSOCIATION BETWEEN TUMOR NECROSIS FACTOR ALPHA AND GP91PHOX EXPRESSION IN PATIENTS WITH HEART FAILURE. EFFECT OF A PILOT STUDY WITH N-3 SUPPLEMENTATION

Cangemi R., Celestini A., Pignatelli P., Carnevale R., Proietti M., Calabrese C., Russo R., Loffredo L., Basili S., Violi F.
*Medicina Sperimentale, I Clinica Medica, Sapienza Università di Roma, Roma*

**Objectives** We investigated if NADPH oxidase is enhanced in the peripheral circulation of patients with heart failure and if its circulating levels could be influenced by n-3 polyunsaturated fatty acids supplementation.
**Background** NADPH oxidase is the major source of ROS in the vascular system, but its regulation and interplay with TNFα and the pro-inflammatory protein sCD40L has never been investigated in heart failure (HF).
**Methods and Results** Serum gp91phox, the catalytic core of NADPH oxidase, plasma TNFα and sCD40L were measured in 120 HF patients and in 60 healthy subjects, matched for sex and age. One-gram n-3 polyunsaturated fatty acids treatment was administrated to 22 HF patients to assess the behaviour of serum gp91phox, plasma TNFα and sCD40L after 21 and 60 days of treatment. Compared with healthy subjects, HF patients had higher serum of gp91phox ($p<0.001$), plasma TNFα ($p<0.001$) and sCD40L ($p<0.001$), with a progressive increase from NYHA I to NYHA IV classification. In HF patients gp91phox were associated with TNFα ($r=0.54$, $p<0.001$), and sCD40L ($r=0.56$; $p<0.001$).
Compared to baseline levels, gp91phox were lowered at 21 (from 32.5±17.1 to 15.8±8.6 pg/mL; $p<0.01$) and 60 days of treatment (16.1±8.6 pg/mL; $p<0.01$), returning to values similar to the baseline after treatment suspension (23.6±17.1 pg/mL). A similar behaviour was observed for TNFα and sCD40L.
**Conclusions** The study provides evidence that circulating gp91phox is increased in HF likely as a consequence of the underlying inflammatory process. Therapeutic approach by n-3 polyunsaturated fatty acids supplementation may represent a tool to modulate the expression of gp91phox.

## OC062 DETERMINANTS OF THROMBOXANE BIOSYNTHESIS IN RHEUMATOID ARTHRITIS: ROLE OF RAGE AND OXIDANT STRESS

Vazzana N.[(1)], Ferrante E.[(1)], Santilli F.[(1)], Di Cicco M.[(2)], Lauriti C.[(2)], Di Battista L.[(2)], Ciabattoni G.[(3)], Di Matteo L.[(2)], Davì G.[(1)]
*[(1)] G. d'Annunzio University Foundation Center of Excellence on Aging, Chieti; [(2)] Department of Rheumatology, Pescara Civil Hospital, Pescara; [(3)] Department of Drug Sciences, School of Pharmacy, G. d'Annunzio University, Chieti*

**Objective** The biosynthesis of thromboxane (TX) by platelets and other cells by inflammatory triggers may provide a molecular link between chronic inflammatory disease and atherothrombosis. In the present study, our aims were to evaluate: (i) the rate of TX biosynthesis in patients with rheumatoid arthritis (RA); (ii) the role of lipid peroxidation and receptor for advanced glycation end-products (RAGE) hyperactivation as determinants of altered TX biosynthesis in this setting, and (iii) possible modulation of these biochemical abnormalities by anti-tumour necrosis factor (TNF) agents.
**Methods** Fifty-four patients with RA and 20 healthy subjects were recruited and a cross sectional comparison of urinary 11-dehydro-TXB2, 8-iso-PGF2a and plasma esRAGE levels was performed between patients and controls.
**Results** Urinary 11-dehydro-TXB2 was significantly higher in RA patients than in healthy controls. Furthermore, urinary 8-iso-PGF2a and plasma esRAGE were higher and lower, respectively, in patients than in controls. A direct correlation was found between urinary 11-dehydro-TXB2 and 8-iso-PGF2a only in patients not on anti-TNF therapy. Conversely, patients on anti-TNF therapy showed significantly lower urinary 8-iso-PGF2a but not 11-dehydro-TXB2 than anti-TNF treated subjects, with esRAGE as the only independent predictor of 11-dehydro-TXB2 in this group of patients.
**Conclusion** We provided biochemical evidence of enhanced TX biosynthesis in patients with RA, driven, at least in part,

by lipid peroxidation. Treatment with anti-TNF agents may blunt isoprostane generation in the absence of significant effects on TX biosynthesis. We suggest that RAGE hyperactivity may escape TNF blockade thus contributing to persistent TX biosynthesis in this setting.

OC063 **PHOSPHODIESTERASE -TYPE IV-cAMP-PKA AXIS REGULATES SFK-PYK2 PATHWAY, PMN/ PLATELET ADHESION AND PMN ACCUMULATION AT THE SITE OF VASCULAR INJURY**

Totani L.[(1)], Pamuklar Z.[(2)], Piccoli A.[(1)], Pecce R.[(1)], Dell'Elba G.[(1)], Federico L.[(2)], Martelli N.[(1)], Manarini S.[(1)], Evangelista V.[(1)]
*[(1)] Laboratorio di Biologia e Farmacologia Vascolare, Unità di Biomarcatori Vascolari, Consorzio Mario Negri Sud, Santa Maria Imbaro; [(2)] The Gill Heart Institute, The University of Kentucky, Lexington, USA*

Pharmacological modulation of granulocytes (PMN) recruitment on platelets (PLT) 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 established that Src family kinase (SFK) activity is required for PMN-PLT adhesion, and suggested the focal adhesion kinase, Pyk2, as downstream effector of SFK. Aims of this study were: 1) to definitely establish the role of Pyk2 in PMN recruitment at the site of vascular injury 2) to explore the hypothesis that pharmacological modulation of cAMP by PDE inhibitors may intercept SFK-Pyk2 pathway and prevent PMN-PLT adhesion. Using a flow adhesion assay we found that inhibition of Pyk2 activity by Tyrphostin A9 dose-dependently reduced firm adhesion of PMN on adherent platelets. In agreement, fewer PMN accumulated at the site of guidewire-induced de-endothelization of femoral artery, in Pyk2-null, respect to wild-type mice (0.5±0.05 and 13±6 PMN/microscopic field). Four weeks after endothelial damage, the intimal/media ratio, was reduced in Pyk2-null, respect to wild-type mice (0.07±0.01 and 0.7±0.13). Inhibitors of PDE-IV (rolipram, RO 201724 and zardaverine), but not of PDE-III and V, dose-dependently reduced PMN-PLT adhesion in the flow assay and Pyk2 phosphorylation. These effects were prevented by PKA inhibitors. Immunoblot analysis with an antibody recognizing specific PKA-phosphorylated substrates (RRXS/T) revealed that COOH-terminal Src-kinase (CSK) underwent phosphorylation in PMN treated with PDE-IV inhibitors indicating that in PMN, PDE-cAMP-PKA axis negatively regulates SFK activity through activation of CSK.
After vascular injury, fewer PMN accumulated at the site of endothelial denudation, in rolipram (10 mg/kg i.p.)-treated, respect to untreated mice (2.2±0.5 and 347 PMN/field). PDE-IV inhibitors may be a novel pharmacological approach to reduce vascular response to injury and restenosis.

## *PIASTRINE: ALTERAZIONI QUALITATIVE E QUANTITATIVE*

OC064 **CHARACTERIZATION OF THROMBIN GENERATION (TG) BY RESTING AND ADP ACTIVATED PLATELETS ACTIVATED IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV)**

Panova-Noeva M.[(1)], Marchetti M.[(1)], Leuzzi A.[(1)], Russo L.[(1)], Finazzi G.[(2)], Rambaldi A.[(2)], Barbui T.[(3)], ten Cate H.[(4)], Falanga A.[(1)]
*[(1)] Division of Immunohematology and Transfusion Medicine & Hemostasis and Thrombosis Center, Department of Oncology-Hematology, Ospedali Riuniti di Bergamo, Bergamo; [(2)] Division of Hematology, Department of Oncology-Hematology Ospedali Riuniti di Bergamo, Bergamo [(3)] Direzione Scientifica, Fondazione Ricerca, Ospedale Maggiore, Bergamo [(4)] Department of Internal Medicine, Maastricht University, Maastricht, The Netherlands*

ADP is a known activator of platelet adhesion and aggregation. Once activated, platelets can induce generation of thrombin, which in a positive loop further activate the platelets. In this study we investigated the TG capacity of platelets from 50 ET and 40 PV patients, stimulated by ADP at low (2 microl) and high concentration (10 microl) compared to platelets from 59 control subjects. The role of phosphatidilserine (PS) exposed by platelets in resting and ADP stimulated conditions in thrombin formation was also explored. The platelet-associated TG was measured by the Calibrated Automated Thrombogram (CAT) assay in platelet rich plasma (PRP) after addition of ADP and/or Annexin V (PS biding protein). The results showed that platelets from patients (ET: 163±42; PV: 178±59 nM) generated significantly more thrombin when stimulated with ADP compared to controls platelets (135±30 nM). Platelets from patients wild type for JAK2V617F mutation showed no significant differences in TG when stimulated by different ADP concentrations. Differently, platelets from patients carriers of JAK2V617F mutation showed significantly ($p<0.001$) higher TG when stimulated by high ADP concentration (185±47 nM) compared to basal TG (138±44 nM) and TG at low ADP concentration (146±45 nM). The addition of Annexin V in PRP samples, significantly ($p<0.001$) decreased TG by 99.8% in controls, 85% in ET patients and 76% in PV patients. Both in the absence and the presence of ADP, the TG was significantly ($p<0.001$) less inhibited by Annexin V in those subjects who presented higher TG in basal conditions (in the absence of Annexin V) (R=0.77; R=0.65; respectively) and in presence of ADP (R=0.56; R=0.81; respectively). In agreement with other studies, our results suggest that platelets from ET and PV patients are more reactive to physiological platelet agonist that contributes to an increased TG.

OC065 **PROSTAGLANDIN E2 BIOSYNTHESIS IN PLATELETS FROM PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA**

Pascale S.[(1)], Dragani A.[(1)], Petrucci G.[(2)], Habib A.[(3)], Davì G.[(1)], Rocca B.[(2)], Patrono C.[(2)]
*[(1)] Centro Malattie Emorragiche e Trombotiche, Ospedale Civile Spirito Santo, Pescara; [(2)] Dipartimento di Farmacologia Università Cattolica del Sacro Cuore Roma; [(3)] Dipartimento di Biochimica, Università di Beirut, Beirut, Lebanon*

Activated human platelets physiologically synthesize

prostaglandin (PG)E2, although at much lower rates than thromboxane (TX)A2. It has been previously shown that newly released platelets express cyclooxygenase (COX)-2 (Rocca et al., PNAS 2002; Dragani et al., Blood 2010), and that platelet COX-2 co-localizes with PGE2 synthase (Rocca et al PNAS 2002). PGE2 has been shown to potentiate platelet aggregation (Gross et al. J Exp Med, 2007). Essential Thrombocythemia (ET) is a myeloproliferative neoplasm characterized by high platelet output and risk of arterial thrombosis. We have studied PGE2 biosynthesis from ET platelets and the contribution of COX-2 activity to its production.

Blood was obtained from 34 ET patients (13 males, age 54.27±12.24 years) all on aspirin therapy (100 mg once daily) according to current recommendations. PGE2 and TXB2 were measured by immunoassay in sera after whole blood clotting at 37°C. TXB2 averaged 2,132 ng/mL (mean and standard deviation), while PGE2 was 2.5±5.6 ng/mL serum. The two metabolites were positively and significantly correlated (rho=0.7, p<0.0001, n=34). The ratio between TXB2 and PGE2 was 2320 and was significantly lower than the ratio previously reported in healthy subjects (43±19) (Rocca et al., PNAS 2002). In a subgroup of 14 patients, a selective COX-2 inhibitor, NS-398 (1 microM final concentration) or aspirin (50 microM) were added *in vitro* to whole blood samples. NS-398 inhibited more efficiently PGE2 as compared to TXB2 (39±13% versus 59±23% versus vehicle treated samples). Aspirin added *in vitro* further inhibited TXB2 almost completely (91±6.6%). PGE2 was more inhibited as compared to NS-398, but to a lesser final extent than TXB2 (74±20%).

In conclusion, PGE2 production is preferentially COX-2-dependent in platelets from ET patients. The ratio between the two platelet-derived prostanoids indicates also a relative increase of PGE2 in ET. PGE2 might contribute to platelet activation and vessel homeostasis in ET.

## OC066 MARKED EMPERIPOLESIS AND INCREASED P-SELECTIN EXPRESSION ON MEGAKARYOCYTES SUGGEST A MYELOFIBROSIS-LIKE PHENOTYPE IN A NOVEL CASE OF GRAY PLATELET SYNDROME

De Candia E.[(1)], Pecci A.[(2)], Larocca L.[(3)], Balduini C.[(4)]
*[(1)] Medicina Interna e Geriatria, Università Cattolica del Sacro Cuore, Roma; [(2)] Medicina Interna, Policlinico San Matteo-Università di Pavia, Pavia; [(3)] Anatomia Patologica-Università Cattolica del Sacro Cuore-Roma; [(4)] Medicina Interna, Università di Pavia, Pavia*

**Introduction** The gray platelet syndrome (GPS) is a rare inherited platelet disorder, featuring mild bleeding diathesis, macrothrombocytopenia, defect of α-granule proteins, myelofibrosis, and spleen enlargement. In a novel family of GPS new morphological and functional abnormalities of platelets were described in a 14-year-old boy and his father (De Candia et al JTH 2007;5:551-9). Morphological and immunohystochemical evaluations on the bone marrow (BM) of GPS patients have anecdotally been reported.

**Methods** Immunohystochemical labelling for the following megakaryocytic lineage specific antigens were performed on the patients BM biopsy sections: i) α-granule proteins thrombospondin (TSP), platelet factor 4 (PF4) and P-selectin (P-sel), ii) membrane thrombin receptors PAR1 and PAR4, iii) c-mpl, in addition to specific staining for reticulin.

**Results** The BM of the GPS patient displayed hypercellularity and marked emperipolesis, with more >80% megakaryocytes (MK) containing 2 to 4 neutrophils engulfed within the cytoplasm. Specific staining for reticulin showed marked fibrosis. Despite the severe reduction of TSP, PF4 and P-sel on GPS peripheral platelets, MKs displayed only a slight reduction of TSP and PF4, and a surprisingly increased immunolabelling for P-sel, with respect to controls. PAR1 and PAR4 staining on the MK membrane was reduced. Accordingly, in this patient platelet responsiveness to agonists of PAR1 and PAR4 was defective. C-mpl expression on MKs was reduced, similarly to myeloproliferative disorders. Interestingly, emperipolesis was found in some MKs of the father's BM, confirming that a mild GPS status can be identified in this family.

**Conclusions** In the BM of the present GPS patient abnormal P-selectin expression is associated with emperipolesis and with local PARs disruption. Abnormal neutrophil-MKs interaction and activation of PARs in the BM may account for many of the features of GPS: decreased surface expression of PAR1, decreased responsiveness to PARs agonists, α-granules mobilization and hence gray platelets and myelofibrosis.

## OC067 IMPACT OF A NOVEL INTEGRIN BETA3 MUTATION (DEL647-686), ASSOCIATED WITH A GLANZMANN'S VARIANT HEREDITARY PLATELET DEFECT, ON GPIIb/IIIa EXPRESSION AND SIGNALLING

Bury L., Cecchetti L., Giannini S., Corazzi T., Appolloni V., Gresele P.
*Dept. Internal Medicine, Sect. Internal and Cardiovascular Medicine, Perugia*

We recently described a family with an inherited autosomal dominant mucocutaneous bleeding disorder associated with a novel ITGB3 mutation leading to del647-686 and to the loss of part of the β Tail Domain (βTD) of integrin GPIIIa (Gresele et al., Haematologica 2009; 94:663-669): a variant form of Glanzmann Thrombasthenia (GT).

Characteristics of this family are moderate macrothrombocytopenia, impaired platelet aggregation to all agonists, a mildly reduced expression of GPIIb/IIIa, normal adhesion to fibrinogen but reduced spreading, slightly enhanced fibrinogen and PAC-1-binding to resting platelets and a constitutively phosphorylated FAK.

Given that a mild reduction of GPIIb/IIIa, like in heterozygous GT, does not normally associate with defective platelet function, and that no previous deletions of GPIIIa βTD were described, we decided to express the mutation in CHO cells to assess its impact on GPIIb/IIIa function and signalling.

cDNAs coding for GPIIb, normal GPIIIa and del647-686 GPIIIa were cloned in expression vectors and transfected in CHO cells.

Western blotting, flow cytometry and fluorescence microscopy showed that the mutant GPIIb/IIIa is expressed on CHO cells, although slightly less than wild-type GPIIb/IIIa.

Mutant integrin-bearing CHO cells bind fibrinogen and PAC-1 and aggregate spontaneously in the presence of fibrinogen, differently from CHO cells expressing wild type GPIIb/IIIa which need DTT stimulation.

Western blotting showed a constitutively phosphorylated FAK in CHO cells expressing mutant GPIIb/IIIa, similar to patient's platelets.
Mutant CHO cells adhered to fibrinogen as well as wild type cells, but spreading was defective.
In conclusion, a βTD domain-deleted GPIIb/IIIa complex is expressed in a constitutively active conformation and exerts a dominant negative effect on the normal receptor, leading to defective outside-in signalling.
This novel autosomal dominant macrothrombocytopenia associated with platelet dysfunction raises interesting questions about the role of integrin GPIIIa, and its βTD, in platelet function.

OC068 **MUTATIONS RESPONSIBLE FOR MYH9-RELATED THROMBOCYTOPENIA RESULT IN A REDUCED SDF-1 AND TYPE I COLLAGEN INDUCED MIGRATION OF IMMATURE MEGAKARYOCYTES**

Pecci A.[(1)], Bozzi V.[(1)], Panza E.[(2)], Barozzi S.[(1)], Seri M.[(1)], Balduini C.L.[(1)]
*[(1)] Dept. of Internal Medicine, IRCCS Policlinico San Matteo, Pavia; [(2)] Dept. of Internal Medicine, University of Bologna, Bologna*

**Background** MYH9-related disease (MYH9-RD) is an autosomal-dominant thrombocytopenia caused by mutations of the gene for the non-muscle myosin heavy chain IIA (NMMHC-IIA). We reported that thrombocytopenia of MYH9-RD derives from altered proplatelet formation, but it is unknown whether MYH9 mutations impair another crucial step of megakaryopoiesis, i.e. the migration of immature megakaryocytes from the bone marrow osteoblastic niche to vascular sinusoids. Moreover, it is debated whether MYH9 mutations results in haploinsufficiency or a dominant-negative effect.
**Methods** We investigated migration of differently manipulated Dami cells, a CD61+(high)/CD42b+(low) megakaryoblastic cell line, toward a SDF-1 gradient by a modified Transwell assay. Localization of transfected wild-type or mutant proteins was studied by immunofluorescence for the respective N-terminal fused tag epitopes.
**Results-Conclusions** Migration of Dami cells to SDF-1 was specifically promoted by interaction with type I collagen (Col-I), a major component of the bone marrow osteoblastic niche. NMMHC-IIA had a key role in Col-I induced migration to SDF-1 since blocking of its ATPase activity by blebbistatin resulted in a significant impairment of this process, while overexpression of NMMHC-IIA by transfection of wild-type MYH9 almost doubled the extent of migration. Transfection of Dami cells with NMMHC-IIAs harbouring the three most frequent mutations of MYH9-RD (R702C, D1424H or R1933X) resulted in a reduction of Col-I induced migration compared to cells transfected with wild-type protein. Immunofluorescence analysis of cells adhering to Col-I demonstrated that transfected mutant NMMHC-IIAs failed to reorganize with either endogenous or transfected wild-type proteins into NMMHC-IIA clusters ordered with regularity along actin filaments. Moreover, cells transfected with mutant NMMHC-IIAs showed a comparable Col-I induced migration with respect to mock transfected cells.
These findings indicate that NMMHC-IIA plays an essential role in migration of a megakaryoblastic cell line and suggest that MYH9 mutations affect also megakaryocyte migration by functional insufficiency of mutant protein.

OC069 **INHERITED HUMAN GP 91PHOX DEFICIENCY IS ASSOCIATED WITH IMPAIRED ISOPROSTANE FORMATION AND PLATELET DYSFUNCTION**

Di Santo S.[(1)], Pignatelli P.[(1)], Carnevale R.[(1)], Bartimoccia S.[(1)], Sanguigni V.[(2)], Nocella C.[(1)], Finocchi A.[(2)], Soresina A.R.[(3)], Plebani A.[(3)], Violi F.[(1)]
*[(1)] Medicina Sperimentale, Sapienza I Clinica Medica, Roma; [(2)] Medicina Interna, Tor Vergata Clinica Medica, Roma; [(3)] Pediatria, Università di Brescia, Brescia*

Platelet isoprostane 8-iso-PGF2α, a pro-aggregating molecule, is believed to derive from non enzymatic oxidation of arachidonic acid. We hypothesized that NADPH is implicated in isoprostane formation and platelet activation.
We studied 8-iso-PGF2α in platelets from 8 male patients with hereditary deficiency of gp91phox, the catalytic subunit of NADPH oxidase, and 8 male controls. Upon stimulation platelets from controls produced 8-iso-PGF2α, that was inhibited –8% by aspirin and –58% by a specific inhibitor of gp91phox. Platelets from patients with gp91phox hereditary deficiency had normal thromboxane A2 formation but marked 8-iso-PGF2α reduction compared to controls. In normal platelets incubated with a gp91phox inhibitor or with SQ29548, a thromboxaneA2/Isoprostanes receptors inhibitor, platelet recruitment, an *in vitro* model of thrombus growth, was reduced –66% and –75% respectively; a lower effect (–17%) was seen with aspirin. In gp91phox-deficient patients agonist-induced platelet aggregation was within the normal range while platelet recruitment was reduced compared to controls. Incubation of platelets from X-CGD with 8-iso-PGF2α dose-dependently (1-100 pmol/L) increased platelet recruitment by mobilizing platelet $Ca^{2+}$ and activating gpIIb/IIIa; a further increase of platelet recruitment was detected by platelet co-incubation with L-NAME, an inhibitor of NO synthase.
This study provides the first evidence that platelet 8-iso-PGF2α maximally derives from gp91phox activation and contributes to platelet recruitment via activation of gpIIb/IIIa.

## *TEV: FATTORI DI RISCHIO E MANAGEMENT*

OC070 **IN MEDICAL INPATIENTS INCIDENCE AND PROGNOSIS OF ASYMPTOMATIC DISTAL DEEP VEIN THROMBOSIS; THE IMPACT STUDY**

Ciuti G.[(1)], Grifoni E.[(1)], Pavellini A.[(1)], Zanasi L.[(1)], Abbate R.[(2)], Prisco D.[(2)], Moggi Pignone A.[(1)]
*[(1)] SOD Medicina Interna ad Orientamento all'Alta Complessità Assistenziale 3, AOU Careggi, Firenze; [(2)] AOU Careggi SOD Malattie Aterotrombotiche Firenze*

**Background** Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and pulmonary embolism, is the third cause of mortality. DVT of the lower limbs is the more frequent clinical manifestation of VTE and can involve proximal or distal veins (dDVT). dDVT is often asymptomatic and data

about its incidence and prognosis are scanty, especially in high risk medical inpatients. Therefore, there is a lack of consensus on the value of detecting and treating them.
**Aim** To prospectively evaluate incidence, characteristics and prognosis of asymptomatic isolated dDVT in an internal medical setting.
**Study design** Consecutive patients hospitalized for acute medical illnesses, in whom VTE was not the admission diagnosis, underwent Doppler Ultrasound (US). Clinical evaluation of lower limbs, Ddimer (DD) measurement, and hereditary and transient VTE risk factors assessment were also performed. When a dDVT was found, US characteristics (number of dDVTs, distance from popliteal cavity, dDVT diameter and length) were recorded. For all patients with dDVT a standard 6-week treatment with therapeutic doses of low molecular weight heparin or fondaparinux was planned. Follow-up visits were scheduled at 1, 6, and 12 weeks.
**Preliminary results** Until now 97 patients (45 males, 52 females), mean age 77±14 (range 19-104) years, admitted to our internal medicine unit were enrolled. Eleven asymptomatic dDVTs were found (11.3%). A non-statistically significant difference in the incidence of dDVT was found according to sex (p=0.093) and age (p=0.159). Clinical signs of DVT and difference in legs diameter were not related to dDVT. Immobilization was significantly associated to dDVT (p=0,024). Finally, increased DD levels were not associated with dDVT.
**Conclusions** We found a high incidence of clinically silent dDVTs in medical inpatients. Among risk factors, only immobilization is a strong risk factor for dDVT. The study is still ongoing and no prospective data are available yet.

## OC071 **CIRCULATING MICROPARTICLES AND RISK OF VENOUS THROMBOEMBOLISM**

Bucciarelli P., Martinelli I., Artoni A., Previtali E., Passamonti S.M., Merati G., Tripodi A., Mannucci P.M.
*Dip. di Medicina e Specialità, C. Emofilia e Trombosi A. Bianchi, Milano*

**Background** Circulating microparticles (MPs) may trigger and sustain a hypercoagulable state, leading to thrombotic complications. Data on their association with venous thromboembolism (VTE) are few and inconsistent.
**Aims and Methods** We carried out a case-control study on 186 patients with a first episode of VTE and 418 healthy controls, in order to investigate whether or not high levels of platelet-derived circulating MPs are associated with an increased risk of a first VTE. MPs were measured by flow cytometry.
**Results** Patients had higher median plasma levels of platelet-derived MPs than controls (1,942/miL vs. 1,519/miL, p<0.0001). MPs were higher in individuals with thrombophilia (particularly antiphospholipid antibodies and hyperhomocysteinemia) than in those without, while no significant difference was found in relation to sex, age and body mass index nor between oral contraceptive users and non-users. The risk of VTE increased progressively with increasing MPs levels, with a linear dose-response effect. Individuals with MPs levels above the 90$^{th}$ percentile of the distribution among controls (P90=2,947/miL) had a 5-fold increased risk of VTE than those with MPs levels <10$^{th}$ percentile of controls (P10=786/L), independently of sex, age, body mass index, thrombophilia, and plasma levels of factor VIII [adjusted odds ratio: 4.60 (95% CI: 1.79-11.8)]. Using the 95$^{th}$ percentile of controls as a cut-off point (P95=3,633/miL), the adjusted odds ratio of VTE for individuals with MPs above the 95$^{th}$ percentile was 2.52 (95% CI: 1.16±5.49) compared with those having MPs <95$^{th}$ percentile. After exclusion of individuals with antiphospholipid antibodies and hyperhomocysteinemia, the adjusted odds ratio was only marginally lower [2.28 (95% CI: 1.01-5.29)]. The interaction between MPs levels >95$^{th}$ percentile and thrombophilia increased the VTE risk from 2.42 (95% CI: 0.95-6.17) to 6.25 (95% CI: 1.06-37.0).
**Conclusions** Elevated levels of circulating MPs are associated with an increased risk of VTE.

## OC072 **THE METABOLIC SYNDROME AND THE RISK OF VENOUS THROMBOSIS: RESULTS OF AN INDIVIDUAL LEVEL PATIENT META-ANALYSIS**

Ageno W.[(1)], Ay C.[(2)], Jang M.J.[(3)], Di Minno M.N.D.[(4)], Pabinger I.[(2)], Oh D.[(3)], Di Minno G.[(4)], Squizzato A.[(1)], Grandi A.M. .[(1)], Dentali F.[(1)]
[(1)] *Medicina Clinica, Università dell'Insubria, Varese;* [(2)] *Medicine, Medical University of Vienna, Vienna, Austria;* [(3)] *Internal Medicine, Pochon CHA University, Sungnam, Corea;* [(4)] *Dipartimento di Medicina Sperimentale e Clinica, Università Federico II, Napoli*

**Background** The metabolic syndrome (MS) is a cluster of interrelated risk factors that identify patients at increased risk of cardiovascular events. Recent studies also suggested an association between MS and venous thromboembolism (VTE). However, the role of the individual features of MS and whether MS and its features are more important than obesity alone to predict VTE remains controversial.
**Methods** We performed an individual patient level meta-analysis of case-control studies comparing the prevalence of MS between patients with unprovoked VTE and controls. MEDLINE, EMBASE databases, and abstract books were searched up to January 2010. Odds ratios (OR) and 95% confidence intervals of pooled results were calculated. The influence of individual variables (age, sex, BMI and MS) on the likelihood of VTE was compared using logistic regression analysis. Multivariate analysis was subsequently performed including the individual components of MS in the place of MS. The impact of increasing number of individual components of MS on the risk of VTE was investigated.
**Results** Four studies were identified and analyzed, for a total of 1,332 patients (479 cases and 833 controls). Mean age was 53.3 and 52.7, respectively (p=n.s.), 49.5% cases and 42.4% controls were males (p=0.0003), 38.8% and 30.0% were obese (p=0.0001). MS was significantly associated with VTE (OR 1.97, 1.57-2.47), and the association linearly increased with the number of MS features (p for trend <0.001). At multivariate analysis, MS but not obesity remained associated with VTE (OR 1.92, 1.50-2.46 and 1.14, 0.88-1.47, respectively). All individual features of MS, but HDL cholesterol, were independently associated with VTE.
**Conclusions** The results of this meta-analysis confirm the association between MS and VTE and suggest that MS (and visceral obesity defined by increased waist circumference) could be a more important predictor of VTE than obesity defined by BMI.

OC073 **THROMBIN GENERATION ASSAY (TGA) FOR PREDICTING RISK OF VENOUS THROMBO-EMBOLISM (VTE) IN CARRIERS OF FACTOR V LEIDEN (FVL) OR G20210A PROTHROMBIN (PT) MUTATIONS**

Legnani C., Lani E., Frascaro M., Boggian O., Cini M., Cosmi B., Valdrè L., Guazzaloca G., Palareti G.
*Dept. Angiology and Blood Coagulation Marino Golinelli, University Hospital S. Orsola-Malpighi, Bologna*

Though FVL and PT mutations are risk factors for VTE, most of the heterozygous carriers remain asymptomatic. A number of other factors contribute to hypercoagulability, making it difficult to predict which carriers will ultimately suffer from VTE. Assuming that some heterozygous carriers of FVL or PT mutations are more prone to hypercoagulability than others, aim of this study was to evaluate if TGA is useful to identify those who are at higher VTE risk.
TGA was performed in 140 healthy subjects without thrombophilic alterations (68 males; 19-86 years), in 140 (47 males; 18-81 years) asymptomatic heterozygous carriers of FVL (n= 64) or PT (n=76) mutations, and in 130 (46 males; 18-81 years) heterozygous carriers of FVL (n= 78) or PT (n=52) mutations with a personal history of VTE (n=113 with deep vein thrombosis and n=17 with isolated pulmonary embolism). The presence of other thrombophilic alterations was excluded in all heterozygous carriers. TGA was performed using an automated TGA (Technothrombin TGA RC low, Technoclone), with and without thrombomodulin (TM) addition (Biochemicals & Reagents, 4 nM final concentration).
No significant differences were found for all TGA parameters between healthy subjects and asymptomatic carriers, either with or without TM addition. In comparison with healthy controls, symptomatic heterozygous carriers showed higher: thrombin peak (without TM: 151.8±68.7 vs. 127.5±58.4 nM, p=0.002; with TM: 130.8±74.1 vs. 99.5±48.4 nM, p=0.001), nM/min thrombin (without TM: 23.9±22.1 vs. 17.6±13.5 nM/min, p=0.004; with TM: 23.6±22.8 vs. 15.6±12.9 nM/min, p=0.004), and AUC (without TM: 1787±296 vs. 1,650±355 nM, p=0.001; with TM: 1366±411 vs. 1,116±342 nM, p<0.0001). Statistically significant differences were also found between asymptomatic and symptomatic carriers (data not shown).
Further investigation are needed to establish if TGA can be used to evaluate the individual risk of VTE in subjects genetically predisposed, as suggested by these preliminary data.

OC074 **ABNORMAL PROTAC-INDUCED COAGULATION INHIBITION CHROMOGENIC ASSAY RESULTS ARE ASSOCIATED WITH AN INCREASED RISK OF RECURRENT VENOUS THROMBOEMBOLISM**

Chantarangkul V.[(1)], Tripodi A.[(1)], Legnani C.[(2)], Lemma L.[(1)], Cosmi B.[(2)], Palareti G.[(2)], Mannucci P.M.[(1)]
[(1)] *Angelo Bianchi Bonomi Hemophilia Thrombosis Center, Department of Internal Medicine, Milano;* [(2)] *Department of Angiology and Blood Coagulation M. Golinelli, University Hospital Sant'Orsola Malpighi, Bologna*

Evaluation of the risk of recurrent venous thromboembolism (VTE) is required to determine the optimal duration of secondary prophylaxis. Application of global assays reflecting the pro-vs.-anti-coagulant balance *in vivo* would be desirable.
We aimed at investigating the relationship between recurrent VTE and the Protac- induced coagulation inhibition (PICI) assay, which is based on tissue factor-induced thrombin generation measured by a chromogenic substrate.
One-hundred-ninety patients were followed-up after a first episode of unprovoked, objectively documented VTE for 2.7 years after stopping treatment with vitamin K antagonists (VKA). PICI was measured one month after stopping treatment as the percentage of the OD values recorded without or with Protac. The lower the PICI%, the greater the pro- vs. anti-coagulant imbalance. The study outcome was objectively-documented symptomatic recurrent VTE. Patients with PICI% <74% had crude hazard-ratios (HR) (95% CI) for recurrent VTE of 2.86 (1.01-8.12) as compared to those with PICI% >87%. After adjustment for age, gender, type of index event, VKA duration and normal/abnormal D-dimer, HR (95% CI) were substantially unchanged [2.91 (1.01-8.38)]. The corresponding values after further adjustment for the above variables plus the absence/presence of the most frequent thrombophilic alterations were 3.38 (1.16-9.84). These HR values compare favourably with those obtained in a previous study investigating the thrombin generation test performed in the presence of thrombomodulin.
In conclusion, the measurement of PICI helps to identify patients at higher risk of VTE recurrence. Advantages of PICI over thrombin generation tests are easy performance in general clinical laboratories, easy standardization and no special equipment.

OC075 **WELLS RULE AND D-DIMER FOR THE DIAGNOSIS OF ISOLATED DISTAL DEEP VEIN THROMBOSIS**

Sartori M., Cosmi B., Legnani C., Valdrè L., Rodorigo G., Guazzaloca G., Palareti G.
*U.O. Angiologia e Malattie della Coagulazione Marino Golinelli, Policlinico S. Orsola-Malpighi, Bologna*

**Background** Wells and colleagues developed a diagnostic rule to estimate the probability of the presence of proximal symptomatic deep venous thrombosis (DVT). The accuracy of the Wells rule has not been validated for use in primary care patients in whom symptomatic isolated distal deep venous thrombosis (IDDVT) is suspected.
Objective To validate the diagnostic accuracy of the Wells rule, and D-dimer testing for IDDVT.
**Design, Setting, and Patients** Cross-sectional study with data collection from 1 September 2009 to 1 April 2010, including 270 consecutive outpatients who were referred by the emergency department or by a primary care physician to our ultrasound laboratories. All patients underwent history-taking and physical examination to calculate the Wells rule score, D-dimer testing, and a comprehensive real-time B-mode and colour Doppler ultrasonography examination of both legs by a vascular medicine physician. The proximal deep veins were examined first, then, only in patients with normal proximal findings, the calf veins were evaluated, including the axial (peroneal and posterior tibial) and the muscular veins.
**Results** The prevalence of IDDVT was 11.5%. 18 patients

(10.2%) in the low-risk group according to Wells rule had IDDVT, whereas 13 patients (13.8%) in the high-risk group had IDDVT. The Wells rule had a sensitivity of 42%, a specificity of 66% with a predictive negative value of 89% (CI 95%: 69-100%). D-dimer was higher in patients with IDDVT versus those without IDDVT (1,380±280 vs. 670±20 ng/mL, p<0.001). Three patients with negative results on a D-dimer test (<500 ng/mL) had IDDVT. Sensitivity and specificity of D-dimer were 84% and 57%, with a predictive negative value of 97% (CI 95%: 81-100%).
**Conclusion** The Wells rule does not guarantee estimation of risk in patients in whom IDDVT is suspected. D-dimer <500 ng/mL alone does not exclude the presence of IDDVT.

OC076 **CANCER-RELATED VENOUS THROMBOSIS: RESIDUAL VEIN THROMBOSIS IMPROVES SCREENING FOR OCCULT CANCER**
Malato A.[(1)], Lo Coco L.[(1)], Saccullo G.[(1)], Abbadessa V.[(2)], Siragusa S.[(1)]
[(1)] *U.O.S. Interdipartimentale di Emostasi, Policlinico Universitario di Palermo, Palermo;* [(2)] *Ematologia, Policlinico Universitario di Palermo, Palermo*

**Introduction** Clinical advantage of extensive screening for occult cancer in patients with idiopathic Deep Vein Thrombosis (DVT) is unknown. We have demonstrated that a Residual Vein Thrombosis (RVT)-based screening for occult cancer improves early detection as well as cancer-related mortality (Siragusa S et al. Blood 2007;110(699):OC).
**Aim** 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 tumour 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.
**Results** Over a period of 8 years, 537 patients were included in the analysis: first cohort included 346 patients (Group A), second cohort 191 (Group B). Clinical characteristics between groups were homogenous. During the follow-up, 8.3% of patients developed overt cancer in group A; in group B, 7.8% of patients had diagnosed cancer at the moment of extensive screening while one new case (0.7%) occurred during the follow-up. The sensitivity of this approach was 92.1% (95% confidence intervals: 75.2-104.2). Cancer-related mortality was 7.5% in group A and 3.6% in group B (p< 0.001) (Figure).
**Conclusions** At the long-term follow-up n 537 patients, the RVT-based screening for occult cancer shows high sensitivity for improving early detection as well as cancer-related mortality.

## *TROMBOSI: FISIOPATOLOGIA E CLINICA 1*

OC077 **CANCER-RELATED DISSEMINATED INTRA-VASCULAR COAGULATION: FIRST LINE THERAPY WITH PLASMA-DERIVED PROTEIN C**
Malato A., Saccullo G., Lo Coco L., Siragusa S.
*U.O.S. Interdipartimentale di Emostasi, Policlinico Universitario di Palermo, Palermo*

**Background** Cancer-related disseminated intravascular coagulation (DIC) is a rare but life-treating condition. Acute management is mainly based on administration of fresh-frozen plasma (FFP) and Antithrombin (AT) with the aim of restoring coagulation factors deficiency. Plasma-derived PC has a self-limiting process in determining anticoagulation thus it seems more suitable than a-rPC in patients at high risk for bleeding, such as cancer patients.
**Objective** To describe the efficacy and safety of PC concentrate to restore physiological values in adult cancer patients with overt DIC. Study Design. Not controlled clinical trial (NCCT).
**Materials and Methods** Adult cancer patients affected by DIC, having PC plasma concentration less than 50%, were treated with PC concentrate (Ceprotin, Baxter) as an adjusted bolus of 30 to 50 UI/kg/die to restore normal PC values (70-120%). Clinical outcomes (bleeding, thrombosis and mortality) were recorded up to a follow-up of 28 days from the initial diagnosis of DIC. PC activity, WBC, platelets, D-dimer, fibrinogen, PT, aPTT, AT and DIC score were measured after 12, 24, 48, 7 and 10 days.
**Results** Twenty-two patients were included over a period of 3 years; among them 16 had solid cancer and 6 had haematological cancer. All patients had advanced/metastatic neoplasm. PC concentrate normalized PC activity in all patients within 48 h and remained upper the lower normal value for the following days. Baseline PC levels were lower in non-survivors than in survivors although this difference was non-significant. During the study period, there was a significant increase of platelets, fibrinogen, PT, AT, and a significant decrease of D-dimer, aPTT and DIC score. No bleeding or thromboses were observed; mortality at 28 days was 35%.
**Conclusions** Our investigation shows that PC concentrate is safe and normalizes laboratory variables in cancer patients with overt DIC.

OC078 **PORCINE ENDOTHELIAL CELLS TRANSGENIC FOR HUMAN ENDOTHELIAL PROTEIN C RECEPTOR AND/OR THROMBOMODULIN PRODUCE DIFFERENT LEVELS OF HUMAN ACTIVATED PROTEIN C**
Bulato C.[(1)], Radu C.M.[(1)], Gavasso S.[(1)], Galli C.[(2)], Spiezia L.[(1)], Cozzi E.[(3)], Simioni P.[(3)]
[(1)] *Department of Cardiologic, Thoracic and Vascular Sciences, University of Padua, Padua;* [(2)] *Laboratorio di Tecnologie della Riproduzione, Istituto Sperimentale Italiano Lazzaro Spallanzani, Cremona;* [(3)] *Department of Surgical and Gastroenterological Sciences "Pier Giuseppe Cevese", University of Padua, Padua*

**Introduction** When transplanted into primates, pig organs are

subject to an acute vascular rejection characterized by endothelial cell activation and platelet aggregation, followed by thrombin generation and fibrin deposition, finally leading to microthrombosis. Microvascular thrombosis are probably associated to the inability of porcine thrombomodulin (TM) and porcine endothelial protein C receptor (EPCR) to activate the natural primate anticoagulant PC adequately.
**Aim of the study** We assessed the ability of porcine aortic endothelial cells (PAEC), expressing human EPCR and/or human TM, to activate human PC by an *in vitro* assay.
**Methods** Human PC and thrombin were added to confluent cells to start the formation of activated PC (APC). The reaction was stopped by adding hirudin. The APC formed was measured by a chromogenic substrate. The concentration of APC was calculated using a standard curve of purified APC.
**Results** PAEC expressing human EPCR (hEPCR-PAEC) did not activate human PC. The amount of APC produced by PAEC expressing human TM (hTM-PAEC) after 1, 2, 3, and 4 hours of incubation with human PC and thrombin was 4.77±0.04 ug/mL, 8.88±0.45 ug/mL, 10.20±0.54 ug/mL and 10.50±0.40 ug/mL respectively. The amount of APC detected in PAEC coexpressing human EPCR and TM (hEPCR/hTM-PAEC), after exposure to human PC and thrombin for 1, 2, 3 and 4 hours, was 4.83±0.45 ug/mL, 7.75±0.08 ug/mL, 10.52±0.48 ug/mL and 12.30±0.55 ug/mL respectively.
**Conclusions** hTM-PAEC activate PC faster than hEPCR/hTM-PAEC, but cells genetically modified to coexpress human EPCR and TM activate PC for long time. Which condition *in vivo* is more useful in order to prevent xenograft rejection remains to clarify.

## OC079 THE VWF:ACTIVITY/VWF:ANTIGEN RATIO MEASURED BY A RAPID IMMUNO-TURBIDOMETRIC ASSAY AS MARKER OF ACTIVE THROMBOTIC THROMBOCYTOPENIC PURPURA

De Cristofaro R.[(1)], Lancellotti S.[(1)], Voso M.T.[(2)], Scaglione G.L.[(1)], Oggianu L.[(3)], Pagano L.[(2)], Leone G.[(2)]
*[(1)] Medicina Interna e Scienze Mediche Specialistiche Medicina Interna e Geriatria Roma; [(2)] Ematologia, Università Cattolica S. Cuore, Roma; [(3)] Section of Biology Applied to Human Health, Rome Tre University, Roma*

The inhibition of the ADAMTS-13 protease activity by autoantibody leads to an accumulation of ultra-large (UL) VWF multimers, highly reactive toward platelets and responsible for the occurrence of thrombotic thrombocytopenic purpura (TTP). However, a significant part of patients presenting the hematological and clinical abnormalities of TTP have a normal level of ADAMTS-13 activity. Moreover, the laboratory procedure to measure ADAMTS-13 activity is laborious and expensive. The VWF level, although generally higher than in normal subjects, is not a useful biomarker of the disease in terms of sensitivity and specificity. In this study, we studied 14 patients admitted to the A. Gemelli hospital of the Catholic University School of Medicine of Rome with the clinical signs and hematological markers of TTP. Four patients showed a moderate renal failure while in seven patients there were neurological abnormalities. All patients showed severe thrombocytopenia (<30,000/L). ADAMTS-13 activity was measured by a FRET method, while VWF:Activity and VWF:Ag (and their ratio R) levels were measured by rapid (<15 min) immunoturbidometric assays using an automatic instrument (IL Top) from Instrumentation Laboratory (Milano, Italy). The patients were studied in the acute phase and under clinical remission after appropriate therapy. The ADAMTS-13 values was divided in tertiles (<10%, 10-30%, >30%, class A, B and C, respectively). A multiple comparisons analysis (Bonferroni) showed that in all cases VWF:Ag and VWF:Act were >100% without specific associations, while the R value was significantly lower only in the group A (mean value=0.77) compared to group C (mean value=1, p=0.015). In a multivariate analysis (Anova), only the R value and platelet count were significantly and positively associated with ADAMTS-13 level (p=0.012 and <0.001, respectively). Thus, in the presence of VWF values >100%, a value of R<0.8 is associated with a peripheral consumption of UL-VWF and may be an additional biomarker of active TTP

## OC080 ANTIPHOSPHOLIPID ANTIBODIES PROFILE AND DIAGNOSTIC PROBABILITY FOR ANTI-PHOSPHOLIPID

Sciascia S.[(1)], Bertero M.T.[(2)], Cosseddu D.[(3)], Kuzenko A.[(2)], Sena L.M.[(4)], Bazzan M.[(5)], Roccatello D.[(1)]
*[(1)] Centro Multidisciplinare di Immunopatologia, Ospedale Giovanni Bosco, Torino; [(2)] Allergologia ed Immunologia Clinica, AO Mauriziano, Torino; [(3)] Laboratorio Analasi AO Mauriziano, Torino; [(4)] Dip. di Medicina ed Oncologia, Università di Torino, Torino; [(5)] Ematologia, Centro Multidisciplinare di Immunopatologia, Ospedale Giovanni Bosco, Torino*

**Background** The antiphospholipid syndrome (APS) is a clinical syndrome consisting of thrombosis and/or pregnancy morbidity in the presence of antiphospholipid antibodies (aPL): lupus anticoagulant (LAC), anticardiolipin antibodies (aCL), anti-β2glycoprotein-I (anti-β2GPI).
LAC and the positivity of multiple aPL seem to be closely involved in the diagnosis of APS, while the association of different tests used to detect LAC with the diagnosis of APS is still unknown.
**Purpose/Methods** To evaluate the correlation between aPL profile (considering the association of available tests for LAC, multiple positivity of aPL and their titer) and the diagnosis of APS according to Sydney Criteria, in a retrospective study involving 3,088 subjects in whom the presence of aPL was tested. Odd Ratio (OR) values for APS diagnosis were calculated for each test by logistic regression. A chart was developed and chosen factors for construction were aPL titer (0-10 kU/L neg, 10-30 kU/L low, 30-50 kU/L medium, >50 kU/L high) and methods used for research in LAC (DRVVT, KCT, SCT and STACLOT-LA).
**Results** In 200 subjects (6.5%) LAC was positive in 2 occasions, and of these, 72 (36%) patients were diagnosed with APS. In 425 subjects (13.8%) aPL (aCL and/or anti-β2GPI) medium to high titer were present in 2 occasions, in the absence of LAC and of these only 4 (0.9%) receiving the diagnosis of APS. The OR for the diagnosis of APS were calculated for each method LAC: STATCLOT-LA6.6, DRVVT5.7, SCT 0.93, KCT1.24. The OR for the diagnosis of APS calculated for each level of antibody titer (low, medium, high) of aCL and anti-β2GPI were: aCL-IgM1.66, aCL-

IgG2.68; antiβ2GPI-IgM2.33; antiβ2GPI-IgG2.43.
Risk cut-off values were estimated (Low risk OR<1, Medium 1-5, High >9).
**Conclusions** Among the aPL, LAC is more strongly associated with the diagnosis of APS, particularly if detected with STACLOT-LA and DRVVT. Moreover, multiple aPL positivity, particularly the association LAC, aCL and antiβ2GPI further increases the diagnostic probability of APS.

OC081 **PLASMA MATRIX METALLOPROTEINASE (MMP)-2 AS A MARKER OF ARTERIAL THROMBOSIS IN PATIENTS WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME**

Falcinelli E.[(1)], Pompili M.[(1)], Appolloni V.[(1)], Pengo V.[(2)], Gresele P.[(1)]
*[(1)] Division of Internal and Cardiovascular Medicine, Dept. of Internal Medicine, Perugia; [(2)] Clinical Cardiology Thrombosis Centre, University, Padova*

Primary antiphospholipid antibody syndrome (PAPS) is an autoimmune condition associated with venous or arterial thrombosis or pregnancy complications together with antiphospholipid antibodies, in the absence of other known autoimmune conditions.
In the last few years it has become increasingly evident that alterations in the structure and composition of the extracellular matrix (ECM) play a key role in atherosclerosis and in precipitating ischemic events and MMPs play a major role in the degradation of ECM. A recent study in ACS identified plasma MMP-2 as an independent predictor of all-cause mortality. Alterations of MMPs have been reported also in autoimmune inflammatory diseases (RA and SLE).
We measured plasma MMP-2 in samples from two previously reported case-series of well characterized patients with PAPS (Gresele, Thromb Res 2009, 123: 444; Pengo, JTH 2007, 5: 925).
In the first, 20 APS patients (11 females, age 42±4.0 years, 80% positive for 3 APL markers and 20% only for LAC) and 39 controls (22 females, age 41±2.9) were studied. In the second, 57 patients with APS (triple positivity), with a history of objectively proven venous or arterial thromboembolism (TE) (27 arterial and 30 venous), were studied.
MMP-2 was measured by zymography in citrated plasma samples stored at –80°C.
Plasma MMP-2 was significantly higher in patients with APS than in controls (970.8±115 vs. 624.5±45.1 ng/mL, $p<0.05$ by t-Test) in the first study.
In the second study plasma MMP-2 was significantly higher in patients with arterial TE (957±121.4 ng/mL) than in patients with venous TE (669.9±71.8 ng/mL, $p<0.05$ by t-Test).
Plasma MMP-2 is significantly elevated in patients with APS as compared with healthy controls, and within APS patients, those with arterial events have higher levels than those with VTE. MMP-2 seems to be a marker of arterial thrombosis in APS. Further, prospective studies to assess the prognostic value of plasmatic MMP-2 in APS are warranted.

OC082 **EFFECTIVENESS OF BIVALIRUDIN THERAPY IN PATIENTS WITH HIGH RESIDUAL PLATELET ACTIVITY AFTER CLOPIDOGREL LOADING DOSE IN PATIENTS UNDERGOING ELECTIVE PERCUTANOUS CORONARY INTERVENTION: SIX-MONTH RESULTS**

Marcucci R.[(1)], Parodi G.[(2)], Migliorini A.[(2)], Buonamici P.[(2)], Antonucci D.[(2)], Gensini G.F.[(1)], Said K.[(3)], Elfaramawy A.[(3)], Hassan M.[(3)], Abbate R.[(1)]
*[(1)] Area critica medico-chirurgica, Università di Firenze, Firenze; [(2)] Dipartimento del cuore e dei vasi, Ospedale di Careggi, Università di Firenze, Firenze; [(3)] Aswan Heart Center, Aswan, Egypt*

**Background** The ARNO trial randomized patients (pts) undergoing elective percutanous coronary intervention (PCI) for stable or unstable angina to bivalirudin (Biv) vs. unfractionated heparin followed by protamine at the end of the procedure (UFH+P). At 1 and 6 months Biv significantly reduced rates of major bleeding, composite ischemic events (death, MI, and target vessel revascularization), and net clinical outcome (composite ischemia or major bleeding). Whether these results are influenced by residual platelet activity (RPA) after clopidogrel loading is unknown.
**Methods** Of 850 randomized pts, RPA was assessed in 672 (79%) pts 12 to 18 hours after a 600 mg clopidogrel loading dose with light transmission aggregometry using 10-mol adenosine diphosphate as agonist. High RPA was defined as >70%. All pts received aspirin (325 mg daily) and clopidogrel (75 mg daily) for ≥1 year.
**Results** High RPA was detected in 126 pts (18.7%). Among these, pts randomized to Biv vs. UFH+P were well matched including TIMI risk score, abciximab use, and the use of drug eluting stents. Pts randomized to Biv, compared to UFH+P, had not significantly different major bleeding rates but had significantly better net clinical outcomes at 1 and 6 months mainly due to reduction of MI rates (table). In pts with RPA 70%, all outcomes rates were similar in both treatment arms.
**Conclusion** These data suggest that in pts undergoing elective PCI with high RPA after clopidogrel loading, treatment with Biv monotherapy improves net clinical outcomes compared with UFH.

OC083 **RESIDUAL EMBOLI AT LUNG PERFUSION SCAN OR MULTI DETECTOR COMPUTED TOMOGRAPHY AFTER A FIRST EPISODE OF PULMONARY EMBOLISM**

Cosmi B.[(1)], Nijkeuter M.[(2)], Valentino M.[(3)], Huisman M.V.[(2)], Barozzi L.[(3)]
*[(1)] Department of Angiology and Blood Coagulation M. Golinelli, Università di Bologna, Ospedale S. Orsola-Malpighi, Bologna; [(2)] Department of Internal Medicine, Leiden University Medical Centre, Leiden, The Netherlands; [(3)] Emergency Radiology Unit, S. Orsola-Malpighi, University Hospital, Bologna*

**Background/Aims** The rate of resolution of emboli after a first episode of pulmonary embolism (PE) is uncertain. A baseline test indicating any residual PE is pivotal in aiding a more accurate diagnosis of recurrent PE. The aim of this study was to assess the rate and risk factors of residual PE with lung perfusion scan (LPS) or Multidetector CT (MDCT) after a first episode of PE.
**Materials and Methods** In a cross-sectional study, consecutive patients with a first objectively documented episode of PE who had received at least three months of

anticoagulation and who were considered for possible VKA withdrawal. They underwent either LPS or MDCT to establish residual PE.
**Results** Residual PE was present in 28% (26/93; 95% CI: 19-38%) of patients undergoing LPS at a mean of 9 months after the initial PE. Age above 65 years and presence of known pulmonary disease were significantly associated with residual PE at LPS. Residual PE was present at the follow-up CT scan in 15% of subjects (12/80; 95% CI: 8-25%) who underwent MDCT at a mean of 9 months after the initial PE. No patient characteristic significantly influenced the presence of residual PE at helical CT.
**Conclusions** Follow-up LPS entails less irradiation but it is influenced by preexisting pulmonary disease and age. In younger patients without known coexisting pulmonary disease, LPS could be performed at the time of anticoagulation withdrawal for aiding in the diagnosis of suspected recurrent PE. In case of known pulmonary disease or abnormal follow-up lung perfusion MDCT should be preferred.

## *ATEROSCLEROSI: EPIDEMIOLOGIA E FATTORI DI RISCHIO*

### OC084 **A WHOLE BLOOD TEST IN THE LABORATORY EVALUATION OF CARDIOVASCULAR RISK: RESULTS FROM THE MOLI-FAMILY STUDY**

Napoleone E., Gianfagna F., Zurlo F., De Curtis A., Vohnout B., Iacoviello L., Donati M.B., Lorenzet R.
*Laboratori di Ricerca, Università Cattolica, Campobasso*

**Background/Aim** To test whole blood (WB) procoagulant activity (PCA) as a possible marker of cardiovascular risk in a general population.
**Material & Methods** The study population included 746 subjects (aged 43±18 SD; 45% men) from 51 extended pedigrees (range 4-29 subjects, 1-4 generations): 22 families with at least one CV familial event and 29 control families from the general population of a Southern Italian region (the Moli-Family study). On enrolment, WB was drawn and incubated for 2h at 37°C with or without bacterial endotoxin (LPS) or TNF-α. At the end of incubation, PCA of cells was assessed by a one-stage clotting time. The association between the variables was verified with a linear regression adjusted for age and sex using SOLAR software.
**Results** Basal, LPS- and TNF-α-stimulated WB PCA were significantly different in the subjects who had a familial history either of myocardial infarction or of stroke ($p<0.01$). A similar association was observed when, on a sample of 316 subjects (10 control and 12 family cases), plasma levels of P-selectin ($p<0.08$) and CD40L ($p<0.001$) were measured. Surprisingly, lower levels of cholesterol and LDL in stimulated WB were associated with higher PCA. Conversely, higher levels of white blood cells were associated with higher PCA, both in basal ($p=0.04$) and stimulated WB ($p<0.01$).
Subjects with stronger stimulated WB PCA tended to have increased levels of plasma CD40L, and IL-6 antigens ($p<0.05$ each). PCA following stimulation of WB with LPS was, for more than 50%, attributable to TF as shown by a monoclonal anti-TF antibody.
**Conclusion** In a large population sample, WB PCA was found positively associated with inflammatory cytokines and both parameters with a familial history of CV events. WB PCA may be developed as a simple and reliable test for large scale epidemiological studies.
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### OC085 **DISTRIBUTION OF 10-YEAR AND LIFETIME PREDICTED RISKS FOR CARDIOVASCULAR DISEASE IN ITALIAN ADULTS: FINDINGS FROM THE MOLI-SANI STUDY**

Di Castelnuovo A., Costanzo S., Olivieri M., de Curtis A., Persichillo M.R., Zito F., de Gaetano G., Donati M.B., Iacoviello L.
*Laboratori di Ricerca Università Cattolica del Sacro Cuore, Campobasso, on behalf of the Moli-sani Investigators*

**Background** A large proportion of adults, particularly women and younger men, are at low short-term (10 years) coronary heart disease (CHD) risk. This large low risk population comprises those who remain low risk as well those who become high risk across the lifespan. Guidelines for primary CHD prevention suggest consideration of lifetime in addition to short time risk, but it is currently unknown how many Italians would be identified as having low short-term but high lifetime risk.
**Methods** We analysed 9,775 (40% men) cardiovascular disease-free individuals aged 40 to 60 years, randomly recruited from the Italian general population, and included in the MOLI-SANI study. We stratified participants into 3 groups: low 10-year (≤10%)/low lifetime (<39%) risk, low 10-year (≤10%)/high lifetime (≥39%) risk, and high 10-year (≤10%) risk or diagnosed diabetes. Short-time risk was evaluated using the NCEP/ATPIII equations. Lifetime risk was evaluated from the Framingham cohort.
**Results** Ninety-five percent (N=9,309) of Italian adults aged 40-60 are at low short-term CHD risk. Among them, 933 women (17%) and 436 men (11%) are at low (≤39%) lifetime CHD risk; 83% of women and 89% of men are at high (≥39%) lifetime CHD risk. The addition of lifetime risk estimation identifies higher-risk women and younger men in particular. Unfavourable total cholesterol (≥200 mg/dL) was the most important risk factor in classifying high versus low lifetime risk, with a prevalence of 75%, followed by unfavourable blood pressure (49%) and smoking (42%).
**Conclusions** Whereas 95% of Italian adults aged 40-60 are at low short-term risk, 85% of this group are at high lifetime CHD risk. These results support the use of a stepwise stratification system aimed at improving risk communication, and they provide a baseline for public health efforts aimed at increasing the proportion of Italians with low short-term and low lifetime CHD risk.

### OC086 **PREVALENCE OF CARDIOVASCULAR DISEASE AND METABOLIC SYNDROME IN A LARGE ITALIAN POPULATION: RESULTS FROM THE MOLI-SANI PROJECT**

Persichillo M., Di Castelnuovo A., Costanzo S., Verna A., Rago L., Zito F., de Gaetano G., Donati M.B., Iacoviello L.
*Research Laboratories, Centre of High Technology Research, Campobasso*

**Background** Cardiovascular disease (CVD) is the main cause of mortality and morbidity in industrialized Countries. We evaluated the prevalence of CVD and metabolic syndrome (MS) in the Italian population from the MOLI-SANI Project.
**Methods** The Moli-sani Project is a cohort study in men and women, aged ≥35 years, randomly recruited from subjects included in the city-hall registries of Molise, a Southern Italian region. The rate of rejection was 30%. Exclusion criteria are pregnancy or impossibility or refusal to participate. From March 2005 to December 2009, 23,806 subjects were enrolled. 232 subjects (1%) with incomplete data concerning CVD or CVD risk factors were excluded. Personal history of CVD was assessed by the Roses questionnaire and events were validated. MS was defined using the ATPIII criteria.
**Results** We analyzed 23,574 subjects (11,306 men, mean age 56±12 and 12,268 women, mean age 55±12; age range 35-99). Prevalence of CVD in men and women were respectively: myocardial infarction (MI) 3.4% and 0.7%; coronary heart disease (CHD=MI or angina or revascularization procedure) 5.5% and 1.9%; cerebrovascular disease (TIA or stroke) 2.0% and 1.5%; peripheral artery disease (PAD) 0.5% and 0.1%; CVD (CHD+cerebro-vascular+PAD) 7.6% and 3.4%. MS was found in 3,181 men (28.3%) and 3,121 women (25.6%). Prevalence of antihypertensive, antihyperlipidemic and antidiabetic treatments were 28.1%, 8.2% and 6.2% in men, and 27.8%, 7.3% and 3.7% in women. Prevalence of CVD in MS patients was high (10.6% in men and 6.6% in women), and higher in subjects aged ≥65 years (22.0% in men and 12.4% in women) as compared to the general population.
**Conclusions** This study strengthens the data available from the Italian Cardiovascular Observatory and provides an extensive and up-to-date description of the CVD and MS burden in Italy.

## OC087 **EFFECT OF A PERSONALIZED PHYSICAL ACTIVITY PROGRAMME ON WEIGHT REDUCTION AND ENDOTHELIAL PROGENITOR CELLS IN OVERWEIGHT SUBJECTS**

Cesari F.[(1)], Sofi F.[(1)], Corsani I.[(2)], Gori A.M.[(1)], Pucci N.[(1)], Caporale R.[(3)], Abbate R.[(1)], Gensini G.F.[(1)], Casini A.[(1)]
*[(1)] Area Critica Medico-Chirurgica, Università degli Studi di Firenze, Firenze; [(2)] Agenzia Nutrizione, Università degli Studi di Firenze, Firenze; [(3)] Laboratorio Centrale, Azienda Ospedaliero-Universitaria, Firenze*

**Aim** To assess the effect of a personalized physical activity program on weight and on circulating (CPC) and endothelial progenitor cells (EPC) in healthy subjects.
**Material and Methods** Anthropometric measurements with body composition, cardiopulmonary test, a maximal stress exercise test with maximal oxygen uptake (VO2max), and a series of biochemical analyses were conducted before (T0) and after 3 months of physical activity (T1). CPC and EPC were determined by using flow cytometry and defined as CD34+, CD133+ and CD34+/CD133+ for CPC and CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+ for EPC.
**Results** A total of 80 healthy overweight and obese subjects completed the program. The exercise program consisted of sessions of 45 minutes of aerobic exercise 3 times per week tailored according to the individual anaerobic threshold. At the end of the program, we divided the population into 2 groups, responders (group A) and non-responders (group B) according to the increase of $VO_2$max. Group A reported significant reductions of weight by 3.1% (92.3±17.1 vs. 89.4±15.9 kg; p<0.0001) and fat mass by 4.4% (32.2±9.9 vs. 30.9±9.5 kg; p<0.0001) while group B showed a percentage of increase of fat mass by 1.5% at T1. In group A, a trend of increase at T1 for circulating levels of CPC and EPC was observed, reaching the statistical significance for all the three types of EPC. On the contrary, group B showed no significant increase in CPC and EPC. Furthermore, a significant correlation between decrease of fat mass and increase of CD133+/KDR+ EPC was reported in group A subjects (r=0.50; p=0.04).
**Conclusion** Three months of physical activity significantly improved anthropometric measurements. A beneficial effect was observed regarding the increased number of EPCs in the responders group, in relation to weight loss.

## OC088 **GENE-ENVIRONMENT INTERACTION: ROLE OF eNOS -786C/4A HAPLOTYPE IN INFLUENCING PREDISPOSITION TO PERIPHERAL ARTERIAL DISEASE**

Sticchi E.[(1)], Fatini C.[(1)], Sofi F.[(1)], Pratesi G.[(2)], Pulli R.[(2)], Romagnuolo I.[(1)], Cioni G.[(1)], Pratesi C.[(2)], Abbate R.[(1)], Gensini G.F.[(1)]
*[(1)] Medical and Surgical Critical Care, Thrombosis Center, Florence; [(2)] Medical and Surgical Critical Care, Unit of Vascular Surgery, University of Florence, Florence*

**Background** Peripheral arterial disease (PAD) is a common manifestation of systemic atherosclerosis. Apart from traditional cardiovascular risk factors, several biological mediators and genetic predisposing factors may contribute to the development of the atherosclerotic process.
eNOS gene, encoding for the endothelial nitric oxide synthase responsible for nitric oxide synthesis, has been proposed as a candidate gene in the predisposition to the disease. This study investigated the role of -786T>C, 894G>T and 4a/4b polymorphism in eNOS gene in modulating PAD susceptibility.
**Methods** We investigated 281 consecutive patients, 220 males and 61 females (median age 72 yrs), with PAD and 562 healthy controls, comparable for sex and age.
**Results** eNOS -786C allele frequency was significantly higher in PAD patients in comparison to controls (p=0.03). The eNOS -786C allele was significantly associated to the predisposition to PAD [OR=1.52, 95% CI 1.11-2.09; p=0.009] at univariate analysis, but not after adjustment for traditional risk factors. The eNOS 894G>T and 4a/4b polymorphisms did not influenced PAD susceptibility. We observed a higher eNOS -786C (0.49 vs. 0.43) and 4a (0.20 vs. 0.16) allele frequency in smokers in comparison to non-smokers. In the smokers group the eNOS -786C/4a haplotype significantly and independently influenced the predisposition to PAD [OR=2.71, 95% CI 1.38-5.30; p=0.004].
**Conclusions** Our results provide evidence of a gene-environment interaction in influencing PAD susceptibility.

## OC089 **ILIAC AND INFRAINGUINAL ENDOVASCULAR REVASCULARIZATION PROCEDURES IN PATIENTS**

**WITH PERIPHERAL ARTERIAL DISEASE: COMPLICATIONS AND LONG-TERM RESULTS**

Conti E.[(1)], Pili C.[(1)], Sartori M.[(1)], Losinno F.[(2)]
[(1)] *Angiologia, AOSP Policlinico S. Orsola-Malpighi, Bologna;* [(1)] *Radiologia Interventistica, AOSP Policlinico S. Orsola-Malpighi, Bologna*

**Aims** To compare iliac and femoro-popliteal percutaneous transluminal angioplasty (PTA) procedures in patients with peripheral arterial disease (PAD) with regards to rates of periprocedural and antithrombotic treatment-related complications, 3 years-restenosis and rethrombosis, need of further revascularization and leg amputations.
**Materials and Methods** Consecutive PAD patients undergoing PTA were prospectively followed-up at 1-6-12 months then yearly. Visits included color Doppler with Ankle-Brachial-Index (ABI) measurement. After PTA double antiplatelet therapy was administered for 30 days, then aspirin alone, for infrainguinal procedures LMWH (half therapeutic dosage) was added for a week. All patients received statins for at least six months.
**Results** 201 patients were enrolled. Mean age was 68.5 y and 60.2% were male, Mean follow-up was 36 months. Indications for PTA were claudication (58.2%), rest pain (10.9%), tissue loss (18.4%), and acute thrombosis (12.4%). In 220 procedures 396 lesions in 272 limbs were treated. Iliac procedures were 141 (51.8%), infrainguinal 99 (36.4%), and both sites 32 (11.8%). Stent was used in 79% of 200 iliac lesions and in 6% of 196 infrainguinal ones. Thirty-day mortality was 0.5%. Periprocedural and 30-day antithrombotic treatment-related haemorragic complication rates were 9.1% and 2.7% respectively. One-year and 3-year ≥75% restenosis or rethrombosis cumulative rates were 1.3% and 7.7% respectively in iliac-treated patients. Corresponding rates of infrainguinal restenoses were 25.3% and 41.6% (p<.0001). Among 18 late reocclusions, only 2 concerned iliac vessels. Baseline <0.70 ABI value was significantly related to recurrency. Endovascular or surgical reinterventions were performed in 29 and 14 patients respectively. Ten patients underwent major amputation, 5 within the first month after PTA.
**Conclusions** Iliac PTA has a long-term sustained better prognosis than infrainguinal procedures, the second strongest predictor of recurrent disease being baseline ABI. Periprocedural and 30-day antithrombotic treatment-related haemorragic complications are relevant.

OC090 **PROTEIN C AND PROTEIN S CHANGES IN GH-DEFICIENT ADULTS ON R-HGH REPLACEMENT THERAPY. CORRELATIONS WITH PAI-1 AND T-PA PLASMA LEVELS**

Di Minno M.N.D., Guida A., Russolillo A., Di Capua M., Cimino E., De Gregorio A.M., Arturo C., Ieranò P., Di Minno G.
*Department of Clinical and Experimental Medicine, "Federico II" University, Naples*

**Background** In the rat liver, growth hormone (GH) affects the synthesis of vitamin-K-dependent factors, including Protein C (prot.C) and protein S (prot.S), two natural anticoagulants that prevent hypercoagulable states. Adults with GH deficiency (GHD) are at risk of thrombotic events. High circulating levels of PAI-1 and t-PA, that reflect hypercoagulable states, may contribute to such risk. In GHD on replacement therapy with recombinant human GH (r-HGH), %ΔPAI-1 and %Δt-PA are related to %Δinsulin changes.
**Objectives** To evaluate changes in vitamin-K-dependent factors in GHD on r-HGH replacement.
**Methods** In 60 GHD adults, plasma levels of vitamin-K-dependent factors at baseline and following a 6-month (6-mo) replacement with r-HGH were related with those of PAI-1, t-PA and insulin.
**Results** After 6-mo r-HGH replacement, %Δinsulin enhancements occurred in 36/60 subjects. PAI-1, t-PA, Prot.C, Prot.S and FVII act did not change in them. In the 24/40 subjects that experienced %Δinsulin reductions, PAI-1 (p=0.019); t-PA antigen (p=0.009), Prot.C (p=0.025), Prot.S (p=0.031) and FVII act (p=0.049) decreased significantly. %ΔPAI-1 (β=0.436, p<0.01) was the strongest predictor of %Δprot.S. %Δt-PA (β=0.385, p<0.008) and %Δinsulin (β=0.429, p<0.004) were the strongest predictors of %Δprot.C (multivariate stepwise analysis).
**Conclusions** In GHD adults on r-HGH replacement, changes in vitamin-K-dependent factors may reflect a subtle adaptation of the natural anticoagulant system to a hypercoagulable state and argue for a link between changes in prot.C and prot.S with PAI-1 and t-PA changes, via the response of insulin to r-HGH.

## *DIAGNOSTICA DI LABORATORIO*

OC091 **A RAPID FLOW CYTOMETRIC ASSAY FOR DIRECT DEMONSTRATION OF ANTIBODY-MEDIATED PLATELET ACTIVATION: A REAL WORLD EXPERIENCE**

Degl'Innocenti G., Rogolino A., Marcucci R., Fedi S., Gori A.M., Cesari F., Cellai A.P., Alessandrello Liotta A., Gensini G.F., Abbate R.
*Dipartimento di Area Critica Medico-Chirurgica, Università di Firenze, Firenze*

Heparin-induced thrombocytopenia (HIT) is a prothrombotic disorder related to antibody-mediated platelet activation.
Aim of study was to assess sensitivity, specificity, positive and negative predictive value of a functional test, based on cytofluorimetric method, to detect anti heparin-PF4 antibodies.
We have enrolled 94 patients for whom anti heparin-PF4 antibodies were required to confirm diagnosis of HIT. An ELISA test (GTI Diagnostics) was performed to detect and quantify antibodies reactive with platelet factor 4 (Ab). HIPA and cytofluorimetric test were performed to evaluate the functional activity of these Ab.
19/94 (20.2%) patients had a positive ELISA test (≥0.4 O.D.): 3 with a low pretest probability (4T score: 0-3); 12 with moderate pretest probability (4T score: 4-5) and 4 with high pretest probability (4T score: 6-8). 2 patients (with 4T score: 7 and ELISA test: 3.56 O.D. and 2.15 O.D., respectively) had positive HIPA and cytofluorimetric tests. The remaining patients were all negative at both HIPA and cytofluorimetric tests. ROC analysis demonstrated that a value ≥2.00 O.D. at the ELISA test discriminates with the highest sensitivity and specificity patients with a positive functional test (AUC 0.99 (95% CI 0.98-1.00); p=0.017). At the ROC analysis we found

that the following values at the cytofluorimetric test had the highest sensitivity and specificity to detect patients positive at HIPA test: a mean fluorescence intensity of 1.179, platelets activated ratio between sample and negative controls of 4.58, IMFs ratio between sample and negative controls of 1,32, percentage of activated platelets in patients of 8.7.
In our population we found that: 1) a positivity of ELISA test (PF4 ENHANCED, GTI Diagnostics) defined as value ≥2.0 O.D. has the highest sensitivity and specificity to detect patients positive at HIPA test; 2) a rapid cytofluorimetric test has the same sensitivity and specificity of HIPA test.

OC092 **EVALUATION OF A NEW AUTOMATED PANEL OF ASSAYS FOR THE DETECTION OF ANTI-PF4/HEPARIN ANTIBODIES IN PATIENTS SUSPECTED OF HAVING HEPARIN-INDUCED THROMBOCYTOPENIA (HIT)**

Legnani C., Cini M., Pili C., Boggian O., Frascaro M., Lo Manto G., Poggi M., Palareti G.
*Dept. Angiology and Blood Coagulation, University Hospital S. Orsola-Malpighi, Bologna*

HIT is a life-threatening complication of heparin treatment; the prognosis depends on early diagnosis, and prompt start of alternative anticoagulants. Because of high sensitivity, the available immunologic assays are widely used, though not suited to be run on single samples and with a turnaround time of 2-3 hours. We evaluated two new, rapid, automated, semi-quantitative chemiluminescent immunoassays in HIT suspected patients: HemosIL AcuStar HIT-IgG(PF4-H) (specific for IgG) and HemosIL AcuStar HIT-Ab(PF4-H) (detecting IgG, IgM and IgA) (Instrumentation Laboratory).
HIT confirmation/exclusion was based on the flow chart proposed by Pouplard et al. (J Thromb Haemost 2007), that combines the results of the HIT pretest probability (PTP), estimated by the 4Ts clinical score, and of the ID-Heparin PF4 PaGIA, a rapid immunoassay.
In patients with positive ID-Heparin PF4 PaGIA test and in those with negative ID-Heparin PF4 PaGIA but with high PTP, a platelet aggregation assay was also performed. 102 patients with suspected HIT were included; HIT was diagnosed in 17 (16.7%). No false negative cases were observed using either the HemosIL AcuStar HIT-IgG(PF4-H) or the HIT-Ab(PF4-H) assay (sensitivity and negative predictive values = 100%; negative likelihood ratios <0.01). The specificity was higher for the HemosIL AcuStar HIT-IgG(PF4-H) in comparison with that of the HemosIL AcuStar HIT-Ab(PF4-H) (96.5% vs. 81.2%). Higher values of the HemosIL AcuStar HIT-IgG(PF4-H) were associated with increased HIT PTP. Patients with confirmed HIT and thrombotic complications had significantly higher levels of HemosIL AcuStar HIT-IgG(PF4-H) than those without thrombotic complications.
The HemosIL AcuStar HIT-IgG(PF4-H) and HIT-Ab(PF4-H) assays showed a very high sensitivity and therefore they can reliably be used to rule out HIT in suspected patients. The diagnostic specificity was greatly increased by using the HemosIL AcuStar HIT-IgG(PF4-H). The assays are reproducible (CVs<6%), rapid (30 min), automated, semi-quantitative, and can be run for single sample testing.

OC093 **HIGH-ON TREATMENT PLATELET REACTIVITY BY DIFFERENT STIMULI IS A DETERMINANT OF MORTALITY IN ACUTE CORONARY SYNDROME PATIENTS: DATA FROM AMI-FLORENCE 2 STUDY**

Marcucci R.[(1)], Gori A.M.[(1)], Paniccia R.[(1)], Cordisco A.[(1)], Balzi D.[(2)], Bagnoli L.[(3)], Santoni R.[(4)], Giusti B.[(1)], Abbate R.[(1)], Gensini G.F.[(1)]
*[(1)] Centro Trombosi, Dipartimento Area Critica Medico Chirurgica, Firenze; [(2)] Epidemiology Unit, Local Health Unit 10, Firenze; [(3)] Emergency Department, Nuovo San Giovanni di Dio Hospital, Firenze; [(4)] S.C. Analisi Chimico-Cliniche, Nuovo San Giovanni di Dio Hospital, Firenze*

High on treatment platelet reactivity by ADP has been associated with an increased risk of Stent thrombosis and cardiovascular death in patients. Scarce data are available on the possible role of high on treatment platelet reactivity by other stimuli, such as arachidonic acid (AA).
We sought to evaluate the role of a high-on treatment platelet reactivity by ADP and/or AA in the occurrence of adverse events at 6-month follow-up of patients with acute coronary syndrome undergoing PCI.
We have studied patients enrolled in the AMI-Florence 2 study which is a prospective, observational population-based study designed to evaluate the diagnostic and therapeutic approach to ACS. Platelet function was evaluated by platelet rich-plasma aggregation (PA) by 10 micromol ADP, 1 mmol/L AA within 24 hrs from the hospital admission for an ACS. All patients received 600 mg clopidogrel loading dose followed by 75 mg daily and aspirin 100 mg daily.
333 patients (228 M/105 F) were included in the analysis. All patients underwent PCI with stent implantation. 18 deaths were recorded at 6-month follow-up. ADP-PA and AA-PA mean values were respectively: 48.3±21.7% (48±21.6% in survivors vs. 53.8±23.8% in non survivors) and 16.5±12.9% (16.1±12.6% in survivors vs. 23.6±16.6% in non survivors). HPR by ADP and/or AA was detected in 97/333 (29.1%): HPR by ADP was diagnosed in 54/333 (16.2%) and HPR by AA in 74/333 (22.2%). At multivariate analysis adjusted for age, sex, cardiovascular risk factors, previous history of cardiovascular or cerebrovascular disease, renal failure, anemia, Killip class, the HPR was an independent risk factor for 6-month total mortality [HR=2.86, 95% CI 1.07-7.6, p<0.03].
We have found that a global HPR is an independent risk factor for 6-month total mortality. We have demonstrated that ACS patients with a worse prognosis are those with a global hyperactive platelet, independently of the responsiveness to a single antiplatelet drug.

OC094 **THROMBOELASTOMETRIC ASSAY FOR MONITORING HEMOSTASIS DURING EXTRA-CORPOREAL MEMBRANE OXYGENATION IN ACUTE RESPIRATORY DISTRESS SYNDROME PATIENTS**

Paniccia R.[(1)], Bonacchi M.[(1)], Ciapetti M.[(2)], Antonucci E.[(1)], Maggini N.[(1)], Miranda M.[(1)], Peris A.[(2)], Sani G.[(1)], Abbate R.[(1)], Prisco D.[(1)], Gensini G.F.[(1)]
*[(1)] Department of Medical and Surgical Critical Care, University of Florence, Florence; [(2)] Emergency Department, Azienda Ospedaliero-Universitaria Careggi, Florence*

Acute respiratory distress syndrome (ARDS) is a serious reaction to injuries of the lung. This condition is often fatal, usually requiring mechanical ventilation and admission to an intensive care unit. Extracorporeal membrane oxygenation (ECMO) is widely used as rescue therapy in the course of severe ARDS despite mechanical ventilation. The main problem in ECMO is the activation of haemostasis due to the foreign surface of the extracorporeal circuit. Bleeding and/or thrombosis are frequent complications in ECMO treatment.
This study was aimed to investigate the hemostatic system by thrombelastometry and aPTT point-of-care (POC) device, in 15 patients with ARDS undergoing venovenous ECMO: 7 patients with influence A(H1N1), 3 with lung trauma and 5 with bacterial infectious. The ECMO mean duration was 9.2±6.0 days.
Thromboelastometry was performed on native blood by ROTEM analyzer (Tem-International, Germany). The onset of coagulation (coagulation time, CT), kinetics of clot formation (CF) and maximum clot firmness (MCF) were measured by NaTEM assay (non-activated TEM). APTT was measured by POC coagulometer (Hemochron-ITC, USA). Anticoagulation with unfractionated heparin was titrated to an aPTT of 50-70 sec. NaTEM assay showed a shortening of CT (–39.7%, $p<0.001$), CFT(–21.5%, $p<0.01$) and an increase of MCF (+27.5%, $p<0.001$) in the second day in comparison to the first one. Throughout remaining period, CT and CFT prolonged, whereas MCF decreased, obtaining similar values to the first day. A marked shortening of CT, CFT (–28% and –8%, respectively, $p<0.01$) and a significant increase of MCF (+200%, $p<0.0001$) in comparison to previous values was observed, in the fourth day, in the patient in whom membrane oxygenator was changed due to the presence of clots.
These data indicate that thromboelastometry could be a useful tool to monitoring haemostasis in ARDS patients on ECMO and early identify complications.

## OC095 EFFICACY OF A QUALITY CONTROL SYSTEM FOR THE USE OF INR PORTABLE MONITORS

Paoletti O.[(1)], Bassi L.[(1)], Zimmermann A.[(1)], Alatri A.[(1)], Cancellieri E.[(1)], Delpero N.[(2)], Spotti E.[(2)], Testa S.[(1)]
*[(1)] Clinical Pathology Division, Haemostasis and Thrombosis Center, AO Istituti Ospitalieri Cremona, Cremona; [(2)] Clinical Pathology Division, AO Istituti Ospitalieri Cremona, Cremona*

**Introduction** Since 2002 the Hemostasis and Thrombosis Center of Cremona decentralized the management of Vitam K Antagonist patients in peripheral health units (PHU), through using portable monitors (PM).
**Objectives** We defined a quality control (QC) analytical system to ensure accuracy and efficacy.
**Methods** Central laboratory coagulometer (STA-R; Roche) was considered as reference system. In PHU were used Coaguchek XS (Roche). Based on current guidelines for laboratory QC, the following criteria were defined:

1) PM suitability: a) precision calculated on normal plasmas pool, repeated 10 times, acceptable if CV<5%; b) accuracy, evaluated on 10 pathological specimens with INR<4.0, acceptable if INR differences <0.5.
2) Intra-assay precision: each PHU elaborates monthly the Lewej-Jennings cards. Internal QC, provided by the company, was performed at the beginning of each session and every 20 samples. A CV between ±20% was considered acceptable.
3) Quarterly accuracy to assess the agreement between analytical instruments: 3 samples at different therapeutic range were analyzed in duplicate. Differences 0.5 INR was considered acceptable.
4) External quality assessment (NEQAS): it considers both laboratory data and clinical treatment, to assess the accuracy of the global therapeutic management.

**Results** In the 9 PHU 18 portable monitors were used to perform 18,210 test/year. Analytical precision was very good with a CV always <5%. Control system showed 2 cases out of range (on 360 total controls =0.55%), giving practical indication for immediate instrument replacement. The external QC was optimal.
**Conclusions** The adopted QC protocol makes an accurate and precise control of PM in use, ensuring the quality of analytical data and, by consequence, optimal patient therapeutic management. QC is a mean to ensure good results and we think that national authorities should guarantee the application of correct protocols to allow PM use.

## OC096 HYPERCOAGULABILITY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS DETECTED BY A THROMBIN GENERATION ASSAY

Chantarangkul V.[(1)], Tripodi A.[(1)], Branch A.[(2)], Cleric M.[(1)], Merati G.[(1)], Artoni A.[(1)], Mannucci P.M.[(1)]
*[(1)] Angelo Bianchi Bonomi Hemophilia Thrombosis Center, Department of Internal Medicine, Milano; [(2)] Department of Internal Medicine, IRCCS Ca' Granda Ospedale Maggiore, Milano*

**Background** Diabetes is a risk factor for the development of venous thromboembolism despite normal conventional coagulation tests.
**Aims** We investigated whether plasma from patients with type 2 diabetes has an imbalance of pro- vs. anti-coagulation resulting in hypercoagulability.
**Methods** We analyzed blood samples from 60 patients with type 2 diabetes and 60 gender- and age-matched healthy subjects (controls) for the levels of pro- and anti-coagulant factors, for thrombin generation and for the numbers of cell-derived circulating microparticles bearing such pro-coagulant triggers as tissue factor and negatively-charged phospholipids.
**Results** The levels of pro- or anti-coagulants as measured with conventional coagulation tests or single factor measurements were similar to those of the control population. On the contrary, the median (range) of the height of the thrombin peak (taken as an index of thrombin generation) was higher in patients [205 nM (126-352)] than controls [151 nM (41-289)], $p<0.001$. The median numbers of circulating microparticles were higher for patients [5,041/L (1,821-13,132)] than for controls [1,753/μL (554-13,308)], $p<0.001$ and their values were correlated with the height of the thrombin peak (rho=0.66, $p<0.001$).
**Conclusions** Plasma from patients with type 2 diabetes possesses an imbalance of pro- vs. anti-coagulation resulting in hypercoagulability that can be detected by thrombin generation tests, but not by the measurement of the single pro- or anti-coagulant factors. This hypercoagulability is associated

with increased numbers of circulating microparticles bearing endogenous pro-coagulant triggers. These findings might explain the relatively high risk of venous thromboembolism observed in these patients.

OC097 **DIRECT COMPARISON OF DIFFERENT PROCEDURES TO PREPARE PLATELET-RICH PLASMA FOR STUDIES AT LIGHT TRANSMISSION AGGREGOMETRY**

Femia E.A., Pugliano M., Podda G.M., Dosti K., Cattaneo M.
*Dipartimento di Medicina, Chirurgia e Odontoiatria, Azienda Ospedaliera San Paolo - Università degli Studi di Milano, Milano*

**Introduction** Light transmission aggregometry (LTA), the gold standard for the study of patients with defects of platelet function, is a poorly standardized technique. Therefore, guidelines on how LTA should be performed are in preparation. However, these guidelines will be largely based on consensus of experts, due to the absence of studies directly comparing different procedures. Therefore, ad hoc studies are needed to gather scientific evidence on how to choose the most appropriate procedures for LTA measurement. Aim of the study was to test the most appropriate conditions to prepare samples of platelet-rich plasma (PRP) from whole blood.
**Methods** Blood samples were collected in 10.9 mM sodium citrate from 32 healthy individuals. Samples were centrifuged at four different rates (150, 200, 250, and 300 *g*) at room temperature, without brake, for 10 min, to obtain PRP. The sequence of centrifugations was balanced in order to equilibrate the waiting time of each sample since blood collection. For each PRP sample, the following measurements were recorded: platelet, white blood cell (WBC) and red blood cell (RBC) counts, mean platelet volume (MPV), platelet aggregation induced by ADP (2 M) or collagen (2 µg/mL), platelet agglutination induced by ristocetin (1 mg/mL).
**Results** Contamination by RBC was significantly lower in PRP prepared at 250 or 300 *g*; no statistically significant differences in MPV, platelet and WBC counts were found among different PRP preparations. Although differences in mean platelet aggregation and agglutination were not statistically significant, individual responses tended to be more scattered in PRP prepared at 300 *g*.
**Conclusions** Centrifugation at 250 or 300 *g* for 10 min allowed the preparation of PRP with the lowest RBC contamination; 250 *g* is probably preferable to 300 *g*, because it allowed the preparation of PRP that was characterized by lower variability of platelet responsiveness to some agonists.

## *EMOFILIA E ALTRE SINDROMI EMORRAGICHE: EPIDEMIOLOGIA E CLINICA*

OC098 **MOLECULAR GENETICS AND VITAMIN K SUPPLEMENTATION IN COMBINED DEFICIENCY OF VITAMIN K-DEPENDENT COAGULATION FACTORS**

Lunghi B.[(1)], Marchetti G.[(1)], Redaelli R.[(2)], Caimi T.M.[(2)], Canella A.[(1)], Lapecorella M.[(3)], Mariani G.[(4)], Bernardi F.[(1)]
[(1)] *Dipartimento di Biochimica e Biologia Molecolare, Università di Ferrara, Ferrara;* [(2)] *Dipartimento di Ematologia, Ospedale Niguarda Ca' Granda, Milano;* [(3)] *Centro Emofilia e Trombosi, Ospedale San Salvatore, L'Aquila;* [(4)] *Dipartimento di Medicina Interna, Università di L'Aquila, L'Aquila*

Hereditary combined deficiency of vitamin K-dependent coagulation factors (VKCFD) is a rare autosomal recessive bleeding disorder associated with defects in either the gamma-glutamil carboxylase (GGCX) or the vitamin K epoxide reductase (VKORC1).
We report a male patient, characterised after traumatic hemarthrosis for prolonged PT and PTT (INR 2.7 and 2.4), and for reduced activity levels of factors II (18%), VII (32%), IX (44%), X (17%), protein C (43%) and protein S (61%).
Sequencing of the GGCX and VKORC1 genes detected three new mutations in the GGCX gene causing the P80L, M174R and I532T substitutions. Family analysis showed that the P80L and I532T changes were paternally transmitted, while the M174R substitution was transmitted by the mother. Noticeably, compound heterozygosity for three mutations in the GGCX gene has been previously associated to this rare deficiency. The P80, M174 and I532 residues are located in evolutionarily conserved GGCX regions. In particular, P80 and M174 are located in a potential propeptide binding region.
As also observed in other VKCFD patients, the baseline activity levels of procoagulant VKCFs, which might reflect the residual carboxylase activity, were the highest for FIX and the lowest for FX. The levels of FVII, FIX and FX, but not FII, inversely correlated with the reported affinity of the GGCX for the corresponding VKCF propeptides, which might contribute to the factor-specific residual activity level in VKCFD.
The oral/intravenous administration of vitamin K resulted in slightly modified or unchanged coagulation factor activities in patient plasma. By comparison, vitamin supplementation has previously improved coagulation parameters in patients doubly heterozygous for the W493C/R704X or R83W/Q374X GGCX changes. Moreover, we observed normalized clotting times and completely restored factor VII and IX levels in a patient homozygous for the VKORC1 R98W mutation. These data suggest relationships between vitamin K supplementation outcome and specific gene lesions.

OC099 **DOES FXI DEFICIENCY PREDISPOSE TO BLEED OR PROTECT FROM THROMBOSIS?**

Favuzzi G.[(1)], Tomaiuolo M.[(1)], Cappucci F.[(1)], Pisanelli D.[(1)], Sarno M.[(2)], Tiscia G.L.[(1)], Musto P.[(3)], Scaraggi F.A.[(4)], Margaglione M.[(5)], Grandone E.[(1)]
[(1)] *Emostasi e Trombosi I.R.C.C.S. "Casa Sollievo della Sofferenza", San Giovanni Rotondo;* [(2)] *Scienze Biomediche, Genetica Medica, OO.RR. Ospedali Riuniti, Foggia;* [(3)] *Unità di Ematologia e Trapianti, I.R.C.C.S. "Centro di Riferimento Oncologico", Rionero in Vulture;* [(4)] *Centro Emofilia, Clinica Medica II, Università degli Studi di Bari, Bari;* [(5)] *Scienze Biomediche, OO.RR. Ospedali Riuniti, Foggia*

FXI deficiency is a rare bleeding disorder, resulting in a wide range of bleeding manifestations, from asymptomatic bleeding to injury related bleeding.
To identify mutations in FXI deficient patient and to establish a possible relationship between clinical phenotype and

genotype, we studied 4 patients from Southern Italy with FXI deficiency (1 severe and 3 mild). They were identified by pre-surgical or routine laboratory screening. One of them suffered from cerebellar ischemia and another one from an idiopathic superficial vein thrombosis. None of them showed bleeding, except for the patient with the cerebellar ischemia, who showed epistaxis and spontaneous bruising.
Four different mutations were detected (Glu117Stop, Cys118Arg, Gly400Val, Trp497Gly); two of them were novel (Cys118Arg and Trp497Gly). In three patients (with mild FXI levels) the mutations were in heterozygosity (Glu117Stop, Gly400Val, Trp497Gly), and in one patient (with severe FXI levels) in compound heterozygosity (Glu117Stop with Cys118Arg). Two novel missense mutations (Trp497Gly, Cys118Arg) were highly conserved among different species.
The change Trp497Gly converts a slightly polar residue into a non-polar one in the region of catalytic serine protease domain. The catalytic serine protease domain is highly conserved and contains a catalytic triad of amino acids: His 413, Asp 462 and Ser 557.
Cys118 is located in the Ap 2 domain and is involved in the formation of a disulphide bridge (Cys 118-Cys147). In the Ap 2 domain the disulphide bonds are responsible for the correct folding of Ap 2 domain. Thus, this substitution could change the conformational stabilization of the Ap 2 domain.
In our patients bleeding tendency did not appear to be correlated with FXI levels or with a single mutation in heterozygosis. On the other hand, the compound heterozygosis or the homozygosis might explain low FXI levels, but it is not associated with bleeding.

## OC100 **THE COMPLETE IMPAIRMENT OF FACTOR VII GENE EXPRESSION BY THE IVS6+1G/T MUTATION IS COMPATIBLE WITH A SEVERE BUT NOT LETHAL BLEEDING DISORDER**

Cavallari N.[(1)], Balestra D.[(1)], Rizzotto L.[(1)], Chuansumrit A.[(2)], Mariani G.[(3)], Pagani F.[(4)], Bernardi F.[(1)], Pinotti M.[(1)]
*[(1)] Biochimica e Biologia Molecolare, Università degli Studi di Ferrara, Ferrara; [(2)] Department of Pediatrics, Ramathibodi Hospital, Mahidol, Bangkok, Thailand; [(3)] Medicina Interna e Salute Pubblica, Università de L'Aquila, L'Aquila; [(4)] Human Molecular Genetics International Centre for Genetics, Trieste*

The complete deficiency of Factor VII (FVII), the protease triggering blood coagulation, is potentially lethal, as suggested by the absence of homozygous large gene deletions in humans and knock-out experiments in mice.
We characterized the IVS6+1G>T mutation at the IVS6 donor splice site (5'ss) of FVII gene. The mutation was identified in homozygous condition in two severe FVII deficient patients from Thailand who experienced gastrointestinal bleeding in the first months of life and were subsequently kept under prophylaxis regimen.
Plasma FVII activity levels, measured by PT-based and fluorogenic functional assays (FXa and thrombin generation) were strikingly below the commercial FVII deficient/depleted plasma (negative controls).
The FVII mRNA was investigated in leukocyte mRNA from a heterozygous subject, which did not reveal any trace of normal transcripts deriving from the mutant IVS6+1T allele.
Through the expression of extended FVII minigenes in a human liver cell line (Hep3B), we demonstrated that the mutation primarily induced exon 6 skipping and, to a lower extent, total and partial IVS6 retention, thus producing frame-shifts incompatible with FVII biosynthesis and function.
The IVS6+1G>T is predicted to impair recognition of the donor splice site by the spliceosomal small nuclear ribonucleoprotein U1 (U1-snRNP), a crucial step in exon definition. To assess whether compensatory U1-snRNP could rescue FVII expression, we developed a modified U1-snRNA+1T complementary to the mutated 5'ss. However, co-transfection with this U1snRNA did not increase, to any appreciable extent, usage of the correct 5'ss.
Altogether these findings do not provide experimental evidence even for minimal expression of this FVII gene mutation, affecting the invariable GT dinucleotide of the 5ss and abolishing correct FVII mRNA processing. Intriguingly, our findings also suggest that very low FVII levels, below the detection threshold of recombinant methodology, cause a life-threatening disorder but can be compatible with life.

## OC101 **F8 mRNA STUDIES IN HEMOPHILIA A PATIENTS WITH DIFFERENT SPLICE SITE MUTATIONS**

Giacomelli S.H.[(1)], Sanna S.[(1)], Mancuso M.E.[(2)], Santagostino E.[(2)], Rodeghiero F.[(1)], Castaman G.[(1)]
*[(1)] Department of Cell Therapy and Hematology, Hemophilia and Thrombosis Center, San Bortolo Hospital, Vicenza; [(2)] Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Medicine and Medical Specialities, University of Milan, IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Milano*

Analysis of cDNA is a useful way of investigating splicing mutations and provides more information than using in silico analysis to understand disease pathogenesis better.
To understand how splicing mutations result in Hemophilia A (HA) of different severity in 4 index cases with HA and splice site mutations, we performed a detailed analysis of F8 lymphocyte mRNA using a nested-PCR approach. In silico analysis was made using three different software: BDGP (Berkeley Drosophila Genome Project), NetGene2 and HSF (Human Splicing Finder).
The first patient with mild HA presents the splice site mutation in intron 4 c.601 +5 G>A. At mRNA level this variation produces four different transcripts: wild-type, skipping exon 4, skipping exons 4 and 5 and skipping exons 4, 5 and 6, while in silico analysis predicts a low reduction of the splicing score. F8 mRNA of a c.1538 -18 G>A mutation in mild HA patient lacks the first 36 base pairs (c.1538-1573 Del36) of exon 11, resulting in a protein lacking the first 12 amino acids coded by exon 11, while in silico software predict the creation of a new acceptor splice site with the introduction of 16 bp of intron 10 within the coding region. In keeping with in silico prediction, a c.1443 +1 G>C variation in severe HA causes the insertion of the first 28 bp of intron 9 with the production of a truncated protein of only 465 amino acids and a c. 602 -1 G>A change in severe HA produces the skipping of exon 5.
We emphasize the importance of mRNA analysis to elucidate the pathophysiological mechanism associated with the presence of splicing mutations. In silico analysis may not be always reliable, especially when the variation is away from the GT/AG dinucleotides in the splice site region.

OC102 **CANCERS IN HEMOPHILIA PATIENTS: A SURVEY FROM THE ITALIAN ASSOCIATION OF HEMOPHILIA CENTERS**

Tagliaferri A.[(1)], Di Perna C.[(1)], Santoro C.[(1)], Schinco P.[(3)], Coppola A.[(4)], Santoro R.[(5)], Scaraggi F.A.[(6)], Rossetti G.[(7)], Valdrè L.[(8)], Macchi S.[(9)], Franchini M.[(10)]

*[(1)] Regional Reference Centre for Inherited Bleeding Disorders, University Hospital of Parma, Parma; [(2)] Haemophilia Centre, Department of Cellular Biotechnology and Haematology, La Sapienza University, Roma; [(3)] Haemostasis and Thrombosis Unit - Haemophilia Centre, University Hospital S. Giovanni Battista, Torino; [(4)] Regional Reference Centre for Coagulation Disorders, Federico II University of Napoli, Napoli; [(5)] Department of Haematology and Oncology, Haemophilia Center, 'Pugliese-Ciaccio' Hospital, Catanzaro; [(6)] Haemostasis and Thrombosis Centre, Department of Internal Medicine, University Hospital, Bari; [(7)] Haemostasis and Thrombosis Centre, Department of Internal Medicine, S. Chiara Hospital, Trento; [(8)] Haemophilia Centre, University Hospital S. Orsola-Malpighi, Bologna; [(9)] Transfusion and Hemophilia Centre, S. Maria delle Croci Hospital, Ravenna; [(10)] Immunohematology and Transfusion Centre, University Hospital, Parma*

Since the early 1970s there have been dramatic improvements in the availability and quality of treatment for persons with hemophilia. As a result of this progress, barring the consequences of HIV and HCV epidemics in the 1980s, the life span of hemophiliacs has progressively become similar to that of males in the general population, at least in more developed countries. Accordingly, a considerable number of hemophiliacs now reach old age and consequently develop medical and surgical diseases (e.g., cardiovascular diseases, cancers, renal disorders) not previously seen in this group. However, there is very little information in literature, mostly as anecdotal case reports, regarding these co-morbidity's in hemophiliacs. Thus, in order to elucidate the Epidemiology, clinical characteristics and management of cancers in the Italian hemophilia population we have conducted a survey on behalf the Italian Association of Hemophilia Centers (AICE).

A questionnaire including demographic, clinical and therapeutic data of hemophilia patients with cancers was sent to all Italian hemophilia centers. Sixteen of the 50 (32%) Italian hemophilia centers provided information on 76 hemophiliacs (37 alive and 39 deceased) with 79 cancers collected during the period 1981-2009. Of them, 18 (23%) were hematological cancers, 31 (39%) solid cancers and 30 (38%) hepatocellular carcinomas. The median age of patients was 56 years (range 13-75 years). Sixty-one patients had hemophilia A (31 severe, 8 moderate, 21 mild and 1 carrier) and 15 hemophilia B (7 severe, 4 moderate and 4 mild). Ten of the 79 cancers were metastasized. According to the different decades, 7 cancers were diagnosed during the period 1981-1990, 13 during the period 1991-2000 and 52 during the period 2001-2009.

The data collected by this survey document that an increasing number of cancers are recorded among hemophiliacs and their management represents a new challenge for physicians operating in hemophilia centers.

OC103 **A WEB-BASED CLINICAL RECORD FOR HAEMOPHILIA XL'EMOFILIA®: 3 YEARS OF USE BY PATIENTS AND DOCTORS**

Rivolta G.F.[(1)], Pattacini C.[(1)], Di Perna C.[(1)], D'incà M.[(2)], Biasoli C.[(3)], Rodorigo G.[(4)], Valdrè L.[(4)], Macchi S.[(5)], Serino M.L.[(6)], Arbasi M.C.[(7)], Pedrazzi P.[(8)], Tagliaferri A.[(1)]

*[(1)] Regional Reference Centre for Inherited Bleeding Disorders, University Hospital, Parma; [(2)] Haemophilia Centre, Santa Maria Nuova Hospital, Reggio Emilia; [(3)] Haemophilia Centre, Bufalini Hospital, Cesena; [(4)] Haemophilia Centre, Sant'Orsola University Hospital, Bologna; [(5)] Haemophilia Centre, S. Maria delle Croci Hospital, Ravenna; [(6)] Haemophilia Centre, University Hospital, Ferrara; [(7)] Haemophilia Centre, G. da Saliceto Hospital, Piacenza; [(8)] Haemophilia Centre, University Hospital, Modena*

The treatment of haemophilia in developed countries is based on home self-infusion of concentrates. Improving communication between Haemophilia Centres (HC) and patients is very important. Event and infusion reporting by means of electronic systems is proved to be superior to paper old methods. The Hub Centre (Parma) designed a new outpatients clinical record xl'Emofilia® as part of a project Web connections of the region's HC funded by Emilia-Romagna Health Authority. It's a web-based application which shares the databases of the regions HC, integrated with regional and national registries that can be accessed from anywhere.

With a web-identity (WI, a personal USB key for secure web access), patients can record bleeds and home infusion, consult their own data and allow access to their general practitioners or in Emergency Departments anywhere in the world (also in English).

In December 2006 the HC started to use xl'Emofilia® and 809 (83-carriers) clinical records are now active.

Training is on going for all patients: 373 (51%) have been trained and are successfully using the system (73% of severe HA and 86% of severe HB).

289 patients accessed their clinical record; 115 entered data on 15,746 infusions, 726 bleeding episodes and 63 traumas.

79% of patient in prophylaxis record their infusions on a regular basis.

Our new information technology improves the management of data and the communications between patients and the network of HC in the Region of Emilia-Romagna: the HC work is facilitated and the quality of care offered to patients is improved. Patients appreciate this technology, taking an active part in using it and consulting their own data thus becoming protagonists in the management of their disease. Furthermore, the electronic clinical record expands the possibility of being cured better anywhere in the world, in accordance to European principles of haemophilia care.

OC104 **INTRACRANIAL HAEMORRHAGE (ICH) IN HAEMOPHILIA A AND B: AN ITALIAN RETROSPECTIVE SURVEY**

Zanon E.[(1)], Santagostino E.[(2)], Rocino A.[(3)], Mazzucconi M.G.[(4)], Messina M.[(5)], Tagliaferri A.[(6)], Giuffrida A.C.[(7)], Coppola A.[(8)], Santoro R.[(9)], Iorio A.[(10)], Valdrè L.[(11)], Musso R.[(12)]

*[(1)] Centro Emofilia - Clinica Medica 2, Dipartimento di Scienze Cardiologiche, Vascolari e Toraciche, Università degli Studi di Padova, Padova; [(2)] Centro Emofilia e Trombosi "A. Bianchi Bonomi", IRCCS Ospedale Maggiore e Università di Milano, Milano; [(3)] Centro Emofilia e Trombosi, Ospedale S. Giovanni*

*Bosco, Napoli; [4] Divisione di Ematologia, Università La Sapienza, Roma; [5] Centro Emofilia - Servizio Trasfusionale, Ospedale Infantile Regina Margherita, Torino; [6] Centro di Riferimento Regionale per la Cura dell'Emofilia, Ospedale Maggiore, Parma; [7] Azienda Ospedaliera di Verona Servizio di Immunoematologia e Trasfusione Verona; [8] Centro di Riferimento Regionale per le Emocoagulopatie, Dip. Ass. Clinica Medica, Azienda Ospedaliera Universitaria Federico II, Napoli; [9] Centro Emofilia, Azienda Ospedaliera Pugliese di Catanzaro, Catanzaro; [10] Sezione di Medicina Interna e Cardiovascolare, Azienda Ospedaliera Universitaria di Perugia, Perugia; [11] UO Angiologia e Malattie della Coagulazione, Università di Bologna, Bologna; [12] Centro di Riferimento Regionale Emofilia, Ospedale Ferrarotto, Catania*

Intracranial haemorrhage (ICH) is known to be the most serious bleeding in haemophiliacs, with a high mortality and disabling sequels.
The Association of Italian Haemophilia Centres (AICE) performed a retrospective survey (1987-2009) of the ICH episodes occurred in Italian population with haemophilia A (HA) and B (HB) to define: a) the incidence, the location of bleeding, the death rate and the number of disabling sequels; b) the treatment used during the acute phase of bleeding episodes and the prophylaxis used for prevention of recurrences; c) the risk factors of cerebral events.
Results pertain 111 ICH episodes in 87 haemophiliacs (77 HA, 10 HB). The diagnosis was made by CT or MRI in 85% of cases and transcranial ultrasound was used in 16 cases. ICH was intraparenchimal in 44 cases and subdural in 29. Neurosurgery was performed in 39 cases. In 76 patients (68.4%) ICH was spontaneous. In the adult cohort hypertension was the most frequent concomitant disease (31.8%) and the presence of inhibitor was found in the 25% of recurrences. Mortality was 31% in adult patients while resolution with sequels was present in 41% of newborns. In 94% of cases, the patients were in on demand treatment before ICH. 27 subjects (31%) started secondary prophylaxis after ICH in order to prevent recurrences. The annualized incidence of ICH was 0.65x1,000 py (0.530.81) and was higher in HA (0.92x1,000 py, 0.7±1.1) than HB (0.35 py, 0.23±0.53). In patients with inhibitor annualized incidence of ICH was 2.64x1,000 py (1.7±4.9). In a multivariate Cox regression analysis HA patients and patients with inhibitor show a hazard ratio for ICH of 2.16 (1.28-3.65; p=0.004) and 3.34 (1.97-5.69; p=0.00) respectively.
Data here reported may be useful to suggest new strategy able to prevent these kinds of life threatening bleedings.

## *RISCHIO TROMBOTICO E RIPRODUTTIVO NELLA DONNA*

### OC105 **LOW MOLECULAR WEIGHT HEPARIN IMPROVES THE OUTCOME IN REPEATED ART IMPLANTATION FAILURES**

Di Micco P.[1], Lodigiani C.[2], Ferrazzi P.[2], Librè L.[2], Quaglia I.[2], Rota L.L.[2], Levi Setti P.[3]
*[1] Medicina, Ospedale FBF, Napoli; [2] Centro Trombosi, ICH, Rozzano; [3] Ginecologia, ICH, Rozzano*

**Introduction** Implantation failure in patients submitted to ART assisted reproduction techniques is since many years a matter of debate. Low molecular weight heparin (LMWH) role is emerging as a possible factor improving the early phases of implantation.
**Study design and population** Retrospective observational analysis of patients with at least two implantation ART failures, screened for the presence of thrombophilic factors and submitted to successive ART cycles with or without administration of LMWH.
**Aim** Main aim of the study was to evaluate the pregnancy rate in patients with or without heparin administration.
**Materials and Methods** 265 patients fulfilled the enrolling criteria. 149 (56%) were primary infertile and 116 (4%) were secondary infertile. Their mean age was 36.3±3.6 years and the basal FSH level was 7.73±3.03. 81/265 were positive for at least 1 thrombophilic mutation (G1691A FV, G20210A FII, homozygous C677T- MTHFR); 25/265 (9.4%) if we consider only G20210A FII e G1691AFV. They were submitted to 569 new ART cycles 512 (90%) non supported and 57 (10%) supported by heparin administration.
**Results** 105 clinical pregnancies were observed in 569 cycles (18.80%). Stratified by age group, in term of previous pregnancies, previous ART cycles, basal FSH, number of retrieved oocytes, embryo transferred, BMI and smoking were not significantly different between the group with or without heparin administration; 17% (88/512) was the pregnancy rate in patients not treated with heparin and 33% (19/57) in the heparin treated group (p=0.006).
**Discussion** A significant higher pregnancy rate in ART implantation failures was observed in patients supported by heparin administration. These interesting findings from our observational study should be confirmed by further randomized controlled trials before routine application of LMWH for ART cycles.

### OC106 **FACTOR V LEIDEN, PT-G20210A , MTHFR C677T MUTATIONS AND THE RISK OF IMPLANTATION FAILURE IN WOMEN UNDERGOING ASSISTED REPRODUCTIVE PROCEDURES (IVF OR ICSI)**

Tormene D., Falcone L., Viganò F., Andrisani A., Ambrosini G., Baldo V., Simioni P.
*Department of Cardiologic, Thoracic and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua, Padova*

**Background** Several studies in the literature since 1980's underline the relationship between inherited and acquired thrombophilia and obstetric complications. Yet, recent studies underline a possible relationship between thrombophilia and repeated *in vitro* fertilization failure.
**Aim of the study** The aim of the study was to evaluate the association between inherited thrombophilia and an increased risk of implantation failure in women undergoing assisted reproductive procedures.
**Materials and Methods** A prospective cohort study was carried out in women undergoing IVF or ICSI in our PMA Center of Padova University in the period between May 2007 and May 2009. 201 women between 23 and 45 years were screened for inherited thrombophilia. In these women 83 were negative for thrombophilia mutations. Sixteen were positive for Factor V Leiden (12 heterozygous ad 4 homozygous), six

were heterozygous carries for PT-G20210A mutation, 70 were positive for methylenetetrahydrofolate reductase gene mutation in heterozygosis, 26 in homozygosis. The median age was similar in the two groups of women as well as the number of previous pregnancy losses and pregnancy.
The patients were classified into different causes of infertility: male sterility, tubal sterility, poor responder, endometriosis, mixed causes and unexplained sterility.
**Results** According to multivariate analysis of the thrombophilic carrier status in relation with implantation, considering the different infertility causes for each women and their age, it result that the presence of FV Leiden, PT-G20210A or MTHFR C677 is not a risk factor for implantation failure after IVF or ICSI: odds ratio 0.8 (95% CI 0.5 to 1.4) [p=0.6].
**Conclusions** In conclusion, this study provides no evidence that thrombophilia due to FV Leiden, PT-G20210A and MTHFR gene mutations in heterozygosis and in homozygosis may influence implantation failure in women undergoing assisted reproductive procedures (IVF or ICSI).To confirm our data we need of a larger cases study.

## OC107 **PROSPECTIVE STUDY OF LOW MOLECULAR WEIGHT HEPARIN PROPHYLAXIS FOR REDUCING OBSTETRIC COMPLICATIONS IN HETEROZYGOUS CARRIERS OF FACTOR V LEIDEN AND PHROTROMBIN MUTATION DURING PREGNANCY**

Valdrè L., Legnani C., Cosmi B., Cini M., Guazzaloca G., Rodorigo G., Palareti G.
*Angiologia e Malattie della Coagulazione "Marino Golinelli", Policlinico Ospedaliero-Universitario S. Orsola-Malpighi, Bologna*

**Background** No clear evidence is available yet about the role of low-molecular-weight heparin (LWMH) prophylaxis in thrombophilic women during pregnancy to reduce obstetric complications.
**Methods and Patients** We prospectively followed 154 consecutive pregnant women who were either heterozygous carriers of Factor V Leiden or Prothrombin Mutation. The decision to prescribe LWMH (enoxaparin or nadroparin in prophylactic doses) during pregnancy and six weeks post-partum was based on the presence of at least a previous pregnancy complication (such as intrauterine foetal death, preeclampsia, HELLP syndrome, intrauterine growth restriction, placental abruption and premature delivery) or at least two unexplained miscarriages (39 women; symptomatic group). Patients at their first pregnancy (98) or with at least a previously uncomplicated pregnancy (17) received prophylaxis (115 women; asymptomatic group) only for six weeks *post-partum*.
**Results** Among women of the symptomatic group who completed pregnancy, 30 received prophylaxis. One hundred and thirty five pregnancies occurred in the asymptomatic group of which 85 completed pregnancy and received post-partum prophylaxis. Results: Five (17%) adverse pregnancy outcomes occurred in the symptomatic group; nine (11%) adverse pregnancy outcomes occurred in the asymptomatic group (Fisher exact test p=0.516). Among asymptomatic patients who suffered an adverse pregnancy outcome 6 were Factor V Leiden carriers and 3 were Prothrombin Mutation carriers. All events occurred in patients at their first pregnancy. One deep vein thrombosis of the lower limbs occurred in an asymptomatic woman during pregnancy.
**Conclusions** LMWH seems to be effective in lowering the rate of adverse pregnancy outcome in previous symptomatic patients. The number of adverse pregnancy outcomes in asymptomatic patients seems to be higher than expected. A better analysis on possible risk factors in asymptomatic group could be useful to avoid prescribing prophylaxis in all thrombophilic women who become pregnant.

## OC108 **HEMORHEOLOGICAL PROFILE IN HEALTHY WOMEN UNDERGOING OVARIAN STIMULATION PROCEDURES**

Fatini C.[(1)], Mannini L.[(1)], Sticchi E.[(1)], Cioni G.[(1)], Alessandrello Liotta A.[(1)], Milanini M.N.[(2)], Noci I.[(2)], Abbate R.[(1)], Gensini G.F.[(1)]
*[(1)] Thrombosis Center, Dep. of Medical and Surgical Critical Care, Florence; [(2)] Dep. of Gynecology, Perinatology Center of Human Reproduction Pathology, Florence*

**Background** Women undergoing ovarian stimulation for *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedures may incur an altered hemostasis, which in turn may be responsible for the occurrence of arterial or venous thrombosis. This altered hemostasis may be influenced by blood hyperviscosity, which has been demonstrated to be associated with an increased risk of vascular diseases. In this study we investigated the hemorheological profile in women undergoing ovarian stimulation.
**Methods** 110 healthy women undergoing IVF or ICSI procedures have been investigated. Whole blood viscosity [WBV 0.512 s-1(mPa *s) and WBVat 94.5 s-1(mPa *s)], plasma viscosity (PV), deformability index (DI) and aggregation index (AI) throughout the stimulation cycle (T0-T3) have been analysed.
**Results** At T0 rheological parameters were within the normal range of laboratory values. A progressive increase in WBV 94.5 mPa*s at T1 and T2 was observed. No significant increase in plasma viscosity was found throughout the procedure. DI significantly decreased from the baseline to T1, thereafter remaining unchanged throughout the next days; AI values progressively and significantly decreased from baseline to T2, thus remaining unchanged from T2 to T3. Alterations in WBV 94.5 mPa*s and DI were more evident in smokers and in women with a BMI>25 kg/m$^2$.
Fibrinogen values were constantly lower than baseline concentrations, total cholesterol levels significantly increased throughout stimulation cycle.
**Conclusions** This study provides the novel evidence that women undergoing ovarian stimulation procedure exhibit mild alterations of the rheological profile, in particular smokers and with BMI>25 kg/m$^2$.

## OC109 **LOW-MOLECULAR-WEIGHT HEPARIN FOR PREVENTION OF OBSTETRIC COMPLICATIONS IN CARRIERS OF FACTOR V LEIDEN OR PTG20210A MUTATION**

Tormene D.[(1)], Grandone E.[(2)], De Stefano V.[(3)], Palareti G.[(4)], Castaman G.[(5)], Margaglione M.[(6)], Rossi E.[(3)], Legnani C.[(4)],

Tosetto A.[5], Simioni P.[1]
*[1] Department of Cardiologic, Thoracic and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua, Padova; [2] Atherosclerosis and Thrombosis Unit, I.R.C.C.S. Casa Sollievo della Sofferenza, San Giovanni Rotondo; [3] Institute of Hematology, Catholic University, Rome; [4] Department of Medicine, University of Bologna, Bologna; [5] Department of Hematology, S. Bortolo Hospital, Vicenza; [6] Istituto di Genetica Medica, Università di Foggia, Foggia*

**Background** Whether the administration of low-molecular-weight heparin (LMWH) during pregnancy is effective for prevention of obstetric complications (early or late foetal loss, pre-eclampsia, fetal growth restriction) in women who are carriers of factor V Leiden (FVL) and/or prothrombin variant G20210A (PTM) is controversial.
**Methods and Results** During a 7-year period, 508 pregnancies occurring in 422 women (age: 18 to 45) who were carriers of either mutation were monitored up to delivery at five Italian centres. 183 women had experienced one or more previous obstetric complications. The decision as to administer LMWH with either enoxaparin or nadroparin in prophylactic doses during the whole period of pregnancy was left to discretion of attending physicians. 278 women received the LMWH treatment, while the remaining 230 did not. 46 obstetric complications occurred in the former group and 59 in the latter, leading to a relative risk (RR) of 0.6 (95% CI, 0.5 to 0.9). When the analysis was confined to the only women with previous adverse obstetric events, the reduction in the RR became even stronger: 0.4 (95% CI, 0.2 to 0.8) in women with one event, and 0.5 (95% CI, 0.3 to 0.9) in those with more than one. No side effects were reported in women who had received the LMWH prophylaxis.
**Conclusions** LMWH prophylaxis safely reduces the risk of obstetric complications in carriers of FVL and/or PT mutation. The benefit is greater in those with previous obstetric events.

## OC110 **HEALTH CARE PROGRAM FOR THROMBO-EMBOLISM PREVENTION IN PREGNANCY**

Testa S.[1], Paoletti O.[1], Zimmermann A.[1],Alatri A.[1], Cancellieri E.[1], Ronca E.[2], Rigolli A.[3], Mammoliti D.[3], Riccardi A.[3]
*[1] Haemostasis and Thrombosis Center, Clinical Pathology Division, AO Istituti Ospitalieri, Cremona; [2] Obstetrics Health Care Unit, ASL Cremona, Cremona; [3] Obstetrics and Gynaecology Division, AO Istituti Ospitalieri, Cremona*

**Background** VTE is the major cause of mortality and morbidity in pregnant women in western countries. Even if the rates are low and knowledge has increased, mortality didn't decrease during the last decade.
**Aim of the programme**
1) Inform all new pregnant women about VTE risk and give them practical recommendations on VTE and lifestyle.
2) Recognize all new pregnant women at risk for VTE and apply different prophylactic/therapeutic regimen, through a risk assessment stratification model.

**Material and Method** The program started in 2007. We performed courses on VTE and pregnancy for hospital's staff, obstetricians, specialists and general practitioners to uniform clinical approach and management. A risk assessment dynamic model, with four different prophylactic/therapeutic approaches, was elaborated. VTE risk was evaluated during the three trimesters, at delivery and during puerperium. All new pregnant women received an information brochure, translated into 8 different languages.
**Results** From January 1st 2009 until December 31st 2009 Cremona Hospital reported a total of 1,290 deliveries, 327 of whom were caesarean sections (22 in emergency) and 963 vaginal deliveries. 553 out of 1,290 were included into the preventive program (group A) and followed up for VTE risk. 104 of them started a prophylactic treatment (70 mechanical, 24 pharmacological prophylaxes and 5 therapeutical treatments).
Among the 737 pregnant women (group B), who were not managed in the program, we observed 4 thromboembolic events: 1 DVT, 1 EP and 2 superficial thrombophlebitis (complication rate=0.54%). In group A we observed 1 DVT (complication rate=0.18%).
**Conclusion** Our program in 2009 reached the 42.8% of the new pregnant woman and showed a significant reduction in major thromboembolic complication, through a dynamic individual risk assessment. The integration among different specialists, through a common approach, is the base for a standardized pregnancy VTE risk assessment.

## OC111 **LOW MOLECULAR WEIGHT HEPARIN IN PREGNANT WOMEN WITH PREVIOUS OBSTETRICAL COMPLICATIONS. A MULTICENTER, RANDOMIZED TRIAL**

Martinelli I.
*Dipartimento di Medicina Interna, Centro Emofilia e Trombosi A. Bianchi Bonomi, Milano, on behalf of the Study HAPPY Investigators*

**Background** Low-molecular-weight heparin is frequently prescribed in pregnant women with previous unexplained obstetrical complications to improve the live-birth rate, although only one randomized, controlled trial is available.
**Aim** To assess whether or not treatment with heparin in women with previous obstetrical complications [pre-eclampsia, eclampsia, HELLP syndrome, abruptio placentae, fetal growth restriction (<10th percentile for gestational age) or spontaneous pregnancy loss >15th gestational week] and no evidence of anatomical, endocrine, chromosomal, immunological abnormalities or antiphospholipid antibodies result in a 40% reduction in the rate of expected complications in the index pregnancy (estimated as 40%). Data will also be collected on the immediate tolerance and safety of heparin therapy in pregnancy. The study was supported by the National Agenzia Italiana del Farmaco.
**Methods** Women were randomly assigned to receive nadroparin 40 mg od subcutaneously (started as soon as pregnancy was confirmed and no later than 12 gestational week) and intense surveillance (monthly clinical evaluation and ultrasound according to clinical protocol for pregnancies at risk) or intense surveillance alone. The primary outcome was the live-birth rate. Secondary outcomes were maternal and fetal adverse events. The calculated sample size was of 266 women, 133 in each group. An interim analysis was planned at half recruitment.
**Results** From Apr 2007 through Mar 2010, 133 women entered the study and the interim analysis was performed on

102 women (51 in each group) who ended the index pregnancy. Live-birth rate was 82.4% in the group receiving LMWH and 86.3% in the group with no treatment. The absolute difference in live-birth rate 3.9%, 95% CI −10.5 to 18.3. The rate of maternal and fetal adverse events was similar in the two groups. The study was discontinued because of futility.
**Conclusions** Nadroparin in pregnant women with previous unexplained obstetrical complications does not improve the live-birth rate (Eudract 2006-004205-26).

## *TEV: STORIA NATURALE*

### OC112 **PREVALENCE OF ARTERIAL AND VENOUS THROMBOEMBOLIC EVENTS IN DIABETIC PATIENTS WITH AND WITHOUT THE METABOLIC SYNDROME: A CROSS SECTIONAL STUDY**

Dentali F.[(1)], Squizzato A.[(1)], Caprioli M.[(1)], Fiore V.[(2)], Bernasconi M.[(2)], Paganini E.[(2)], Grandi A.M.[(1)], Venco A.[(1)], Ageno W.[(1)]
*[(1)] Dipartimento di Medicina Clinica, Università dell'Insubria, Varese; [(2)] Medicina Clinica, Ospedale di Cittiglio, Cittiglio*

**Background** Metabolic syndrome (MS) is associated with an increased risk of cardiovascular events. Recent studies have found a higher prevalence of the MS in patients with idiopathic venous thromboembolic events (VTE) compared to controls suggesting a role of the MS in the pathogenesis of VTE.
The presence of MS was shown to further increase the risk of arterial cardiovascular diseases (CVD) in diabetic patients. Conversely, there are no studies that have compared the risk of VTE in diabetic patients with and without the MS.
**Methods** a cross sectional study comparing the prevalence of arterial cardiovascular events and VTE in diabetic patients with and without the MS was conducted
**Results** Nine hundred and fifty three patients were included in the study; 85.7% of patients had MS.
Patients with the MS had an increased prevalence of CVD as compared with those without (23.4% vs. 11.8%; OR 2.28, 95% CI 1.33, 3.95%; p=0.0024) and the MS was an independent predictor of CVD in diabetic patients (OR 3.16, 95% CI 1.78, 5.59) after multiple logistic regression analysis. The prevalence of VTE was higher in patients with the MS in comparison to patients without the MS, but this association was not statistically significant (3.43% vs. 1.47%; OR 2.38, 95% CI 0.56, 10.10%).
**Conclusion** our study confirms the role of MS as an adjunctive cardiovascular risk factor in patients with diabetes and suggests that MS could be considered an adjunctive risk factor for VTE in these patients. Further studies are necessary to confirm these preliminary findings.

### OC113 **THYROID DISEASE, ANTITHYROID OR THYREOMIMETIC AGENTS, AND THE RISK OF PULMONARY EMBOLISM**

Squizzato A.[(1)], van Zaane B.[(2)], Biere-Rafi S.[(2)], Souverein P.C.[(3)], Brandjes D.P.M.[(4)], Buller Harry R.[(2)], Gerdes V.E.A.[(4)], de Boer A.[(3)]
*[(1)] Medicina Clinica, Università dell'Insubria, Varese; [(2)] Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands; [(3)] Pharmacoepidemiology and Pharmacotherapy, Utrecht University, Utrecht, The Netherlands; [(4)] Internal Medicine, Slotervaart Hospital, Amsterdam, The Netherlands*

**Introduction** There are indications that thyroid disease is associated with the risk of venous thromboembolism. We therefore aimed to evaluate the association between pulmonary embolism (PE) and the start of treatment for thyroid disease.
**Methods** A nested case-control study was conducted using the PHARMO Record Linkage System, a Dutch population-based pharmacy registry. Cases were patients hospitalised for PE and the date of hospitalisation was set as index date. Controls were gender- and age-matched subjects without a history of PE prior to this index date. Odds ratios (ORs) and their 95% confidence intervals (95% CI) were estimated for new use of antithyroid or thyreomimetic agents, or hospitalisation for thyroid disease from 12 months before to 12 months after the index date.
**Results** The study population consisted of 3,479 cases and 11,830 controls. New use of antithyroid agents or hospitalisation for thyrotoxicosis within 6 months after the index date (i.e. untreated hyperthyroidism at the index date) was significantly associated with PE (adjusted OR 3.22; 95% CI 1.12-9.22), whereas a relation between thyreomimetic agents and PE was observed for new use before the index date, especially within the first 3 months after treatment onset (adjusted OR 4.58; 95% CI 1.28-16.43). No association was found for new use of thyreomimetic agents after the index date or for new use of antithyroid agents before the index date.
**Conclusions** Our findings suggest that both patients with untreated hyperthyroidism and patients who have recently started with thyreomimetic agents for hypothyroidism are at an increased risk for PE.

### OC114 **CLINICAL PREDICTION OF VTE RECURRENCE IN PATIENTS WITH PREVIOUS UNPROVOKED VENOUS THROMBOEMBOLISM. RESULTS FROM AN INDIVIDUAL-LEVEL META-ANALYSIS**

Tosetto A.[(1)], Iorio A.[(2)], Marcucci M.[(2)], Baglin T.[(3)], Cushman M.[(4)], Eichinger S.[(5)], Palareti G.[(6)], Poli D.[(7)], Tait R.C.[(8)], Douketis J.[(9)]
*[(1)] Department of Hematology, S. Bortolo Hospital, Vicenza; [(2)] Department of Medicine, University of Perugia, Perugia; [(3)] Department of Hematology, Addenbrookes Hospital, Cambridge, United Kingdom; [(4)] Department of Medicine, University of Vermont, Colchester, USA; [(5)] Department of Medicine, University of Vienna, Vienna, Austria; [(6)] Department of Medicine, University of Bologna, Bologna; [(7)] Thrombosis Center, Azienda Ospedaliero-Universitaria Careggi, Florence; [(8)] Department of Haematology, Royal Infirmary, Glasgow, United Kingdom; [(9)] Department of Medicine, McMaster University, Hamilton, Canada*

**Background/Aim** Several patient characteristics, including D-Dimer after stopping anticoagulants, gender or thrombophilia, have been associated with recurrence of VTE after a first episode of unprovoked VTE. Very few data exist about their

joint effect on the prediction of recurrences, mainly because the sample size of individual studies does not allow such evaluate. We aimed to develop a model that could optimize the prediction of disease recurrence, while retaining sufficient simplicity for clinical use.
**Methods** We obtained individual patient data of 7 prospective studies. We selected all cases with unprovoked, proximal VTE as the primary event. A cross-sectional cohort was formed at 18 months of follow-up, by selecting all patients that had a VTE recurrence before 18 months (cases) together with all those patients without a VTE recurrence before 18 months of follow up (controls). Using parametric and non-parametric methods we developed two models for the identification of subjects at low risk for VTE recurrence.
**Results** Out of 1,862 patients with a first unprovoked proximal VTE, 1,406 subjects were analyzed, 1,218 being defined controls and 188 that were defined as cases. D-dimer levels, gender and use of estroprogestin at time of first VTE were associated with recurrence, whereas age, BMI, and presence of thrombophilia were not. Two independent prediction rules were able to identify patients with an annual risk of recurrence below 4%: the first one based on D-dimer testing in females suggested VKA suspension in females having normal D-dimer; the second one based on D-dimer testing in all patients suggested VKA suspension with normal D-dimer or estroprogestin-related VTE.
**Conclusions** A prediction rule based on the combination of D-dimer and gender (or estroprogestin use) may identify patients at low risk of recurrence, potentially sparing VKA therapy in at least one-fourth of subjects having a first episode of unprovoked VTE.

## OC115 VENOUS THROMBOEMBOLISM RECURRENCE AFTER A FIRST EPISODE OF PROVOKED VENOUS THROMBOSIS DUE TO A TRANSIENT RISK FACTOR. A SYSTEMATIC REVIEW OF THE LITERATURE

Filippucci E.[1], Kearon C.[2], Marcucci M.[3], Pengo V.[4], Siragusa S.[5], Palareti G.[6], Iorio A.[1,2]
*[1] Diagnostica per immagini, Ospedale S. G. Battista, Servizio di Angiologia, ASL3, Foligno; [2] Department Internal Medicine, McMaster University, Hamilton, Canada; [3] Department of Internal Medicine/Stroke Unit, University of Perugia, Perugia; [4] Internal Medicine, Department of Medicine and Surgery, University of Padua, Padua; [5] Ematologia, Dipartimento di Medicina Interna, Malattie Cardiovascolari e Nefrourologiche, Università degli Studi di Palermo, Palermo; [6] Angiology and Coagulation Disorders Unit, S. Orsola-Malpighi University Hospital, Bologna*

**Background** Venous thromboembolism (VTE) provoked by a transient risk factor is associated with a low risk of recurrence and it is usually treated with three months of anticoagulation. The rate of recurrence after provoked VTE could be taken as a cut-off value to stop anticoagulation in unprovoked VTE.
**Aim** Systematically appraise the risk of recurrence for VTE provoked by different transient risk factors.
**Materials and Methods** Data Sources: MedLine, EMBase and Cochrane Central up to June 2008. Study Eligibility Criteria. Cohort and randomized studies which included patients with a first episode of VTE provoked by a transient risk factor, treated for at least 3 months and prospectively followed after stopping anticoagulant therapy.
**Study Appraisal and Synthesis Methods** Number of patients, patient-years of follow-up, and recurrent VTE in each subgroup during the 0-12 and 0-24 month intervals after stopping therapies; study design and risk factor characteristics. Annualized recurrence rates in individual studies were combined to obtain pooled estimates.
**Results** In the 24 months after stopping therapy, the rate of recurrence was 3.3% per patient-year (95% CI 2.8% to 3.9%; 11 studies, 2,268 patients) for any transient risk factor, 0.7% (95% C.I. 0 to 1.5%; 3 studies, 248 patients) for a surgical factor, and 4.2% (95% CI 2.8 to 5.6; 3 studies, 509 patients) for a non-surgical factors. The rate ratio for a non-surgical compared with a surgical factor was 3.0 (95% CI 1.1-8.1). The rate ratio for unprovoked thrombosis was 2.3 (95% CI 1.9 to 2.8) when compared with any transient risk factor, and 1.8 (95% CI 1.2 to 2.5) when compared with a non-surgical factor.
**Conclusions** The risk of recurrence after stopping anticoagulant therapy is lower for VTE provoked by surgery than by a non-surgical transient risk factor. Both groups have a lower risk than unprovoked VTE.

## OC116 LONG-TERM CLINICAL OUTCOMES IN PATIENTS WITH VENOUS THROMBOEMBOLISM: FINDINGS FROM THE MASTER REGISTRY

Verso M.[1], Agnelli G.[1], Ageno W.[2], Imberti D.[3], Moia M.[4], Palareti G.[5], Pistelli R.[6]
*[1] Università di Perugia Medicina Interna e Vascolare-Stroke Unit, Perugia; [2] Divisione di Medicina Clinica, Università dell'Insubria, Varese; [3] Divisione di Medicina, Ospedale di Piacenza, Piacenza; [4] Fondazione IRCCS Ca' Granda, Milano; [5] Unità di Angiologia, Università di Bologna, Bologna; [6] Divisione di Pneumologia, Università Cattolica, Roma*

**Background and Aim** The long-term clinical outcomes in patients with acute venous thromboembolism (VTE) are less defined in cohorts of unselected patients. The aim of this study was to prospectively evaluate the long-term clinical outcomes in patients with acute VTE included in a multicenter registry.
**Materials and Methods** Consecutive patients with symptomatic, objectively confirmed, acute VTE were included in the MASTER registry in 25 Italian centers. Patients were followed-up at 6, 12, and 24-months for major clinical outcomes. A Cox regression analysis was used to assess major determinants of outcomes.
**Results** 2,119 patients with VTE (27.5% with pulmonary embolism and 72.5% with isolated deep vein thrombosis) were included in the registry. 908 patients (42.8%) had an idiopathic or unprovoked VTE. Long-term follow up data were obtained in 2,021 patients for mortality and in 1,988 patients for VTE recurrence. The 2-year cumulative incidence of mortality was 8.3% (167/2,021). The major determinants of mortality were: cancer (HR:7.16; 95 CI% 4.76-10.78), age (HR:1.02 per year; 95CI% 1.01-1.03), heparin treatment (HR:2.49; 95 CI% 1.78-3.47), in-hospital management of VTE (HR:1.96; 95CI% 1.28-2.99), extension of venous thrombosis to ileo-caval veins (HR:1.73; 95 CI% 1.23-2.44), while the prescription of compressive elastic stockings was protective (HR: 0.61; 95 CI% 0.44-0.84). The 2-year cumulative incidence of recurrent VTE was 6.2% (124/1988), 8.1% in idiopathic VTE and 4.8% in VTE associated with risk factors.

The major determinants of recurrent VTE were male gender (HR: 1.66; 95 CI% 1.15-2.41), in-hospital management of VTE (HR: 1.83; 95 CI% 1.16-2.87), while the presence of any temporary risk factor was protective (HR: 0.42; 95 CI% 0.27-0.64). The cumulative incidence of bleedings was 2.5% (48/1,883).
**Conclusions** In the MASTER registry, in addition to cancer, age and proximal extension of deep vein thrombosis are associated with increased mortality. Male gender was associated with an increased risk of recurrence.

### OC117 **TWO YEAR OUTCOME OF VENOUS THROMBO-EMBOLISM IN ELDERLY PATIENTS: FINDINGS FROM THE MASTER REGISTRY**

Ageno W.[(1)], Agnelli G.[(2)], Verso M.[(2)], Imberti D.[(3)], Moia M.[(4)], Palareti G.[(5)], Pistelli R.[(6)]
*[(1)] Medicina Clinica, Università dell'Insubria, Varese; [(2)] Medicina Vascolare, Università di Perugia, Perugia; [(3)] Medicina Interna, Ospedale di Ferrara, Ferrara; [(4)] Ematologia, IRCCS Ca' Granda, Milano; [(5)] Angiologia, Università di Bologna, Bologna; [(6)] Pneumologia, Università Cattolica, Roma*

**Background** Little information exists on the long-term clinical outcome of venous thromboembolism (VTE) in elderly patients. We aimed to prospectively compare the long-term clinical outcome of VTE in a cohort of elderly patients aged >75 years and in a cohort of patients aged ≤75 years enrolled in a large, multicenter registry and to identify independent predictors of clinical outcomes in the elderly.
**Patients and Methods** Consecutive patients with symptomatic, objectively confirmed, acute VTE were included in the MASTER registry in 25 Italian centers. Patients were followed-up for 24-months. Major clinical outcomes were death, recurrence of VTE and major bleeding. Cox regression analysis was used to assess major determinants of outcomes.
**Results** A total of 2,119 patients (49.8% males) were enrolled in the study, of whom 440 (20.8%) were >75 years and 1,679 (79.2%) ≤75 years. Information on mortality at 2 years was available for 2,021 patients (413 >75 years and 1,608 ≤75 years) and information on VTE recurrence and bleeding events was available for 1,988 patients (404 >75 years and 1584 ≤75 years). The 2-year cumulative incidence of mortality was 13.1% in patients >75 and 7.0% in patients ≤75, hazard ratio (HR) 1.52, 95% CI 1.09-2.13. Cancer (HR 3.44, 95% CI 1.94-6.09) was the only independent predictor of mortality in the elderly. The 2-year cumulative incidence of recurrent VTE was 6.4% in patients >75 and 6.2% in patients ≤75 (HR 1.05; 95% CI 0.67-1.63). The 2-year cumulative incidence of bleeding was 4.0% in patients >75 and 2.2% in patients ≤75, Odds Ratio 1.84; 95% CI 0.97-3.50.
**Conclusions** As expected, long term mortality rates after acute VTE are significantly higher in patients >75 years than in younger patients. Rates of recurrent thrombotic events were similar between the two groups, whereas bleeding events were nearly twice more frequent in the elderly.

### OC118 **VTE AND ELDERLY PATIENTS: DATA FROM RIETE REGISTRY**

Di Micco P.[(1)], Niglio A.[(2)], Ferrazzi P.[(3)], Enea I.[(4)], Palareti G.[(5)], Monreal M.[(6)] Vasco B.[(7)], Prandoni P.[(8)], Dalla Valle F.[(8)], Ciammaichella M.M.[(9)]
*[(1)] Medicina, FBF, Napoli; [(2)] Medicina Interna, SUN, Napoli; [(3)] Centro Trombosi, ICH, Rozzano; [(4)] Medicina Interna, Ospedale Civile di Caserta, Caserta; [(5)]Angiologia, Università di Bologna, Bologna; [(6)] Medicina Interna, Hospital Tria y Pujol, Barcelona, Spain; [(7)] Hematologia, Hospital Virxe da Xunqueira, Coruña, Spain; [(8)] Clinica Medica, Università di Padova, Padova; [(9)] Medicina Interna per l'Urgenza, ACO S. Giovanni, Roma*

**Background** The balance between the efficacy and safety of anticoagulant therapy in patients aged 90 years with venous thromboembolism (VTE) is uncertain.
**Patients and Methods** RIETE is an ongoing, prospective registry of consecutive patients with acute, objectively confirmed, symptomatic VTE. We evaluated the efficacy and safety of anticoagulant therapy during the first 3 months in all patients aged ≥90 years. In addition, we tried to identify those at a higher risk for VTE.
**Results** Out of 21,873 patients enrolled from March 2001 to February 2008, 610 (2.8%) were aged ≥90 years. Of these, 307 (50%) presented with pulmonary embolism (PE), 240 (39%) 271 (44%) had abnormal creatinine levels. During the first 3 months of therapy, 140 patients aged ≥90 years (23%) died. Of these, 45 (32%) died of PE (34 of the initial episode, 11 of recurrent PE), 18 (13%) had fatal bleeding. Recent immobility 4 days was the most common risk factor for VTE (240 of 610 patients, 39%), but only 54 of them (22%) had received thromboprophylaxis. The most frequent causes for immobility were senile dementia, acute infection, trauma or decompensated heart failure. The duration of immobility was <4 weeks in 126 patients (52%), and most of them were bedridden at home.
**Conclusions** One in every 4 VTE patients aged ≥90 years died during the first 3 months of therapy. Of these, one in every 3 died of PE, one in every 8 had fatal bleeding. Identifying at-risk patients may help to prevent some of these deaths.

## *TROMBOSI E TUMORI 1*

### OC119 **AUTOLOGOUS PERIPHERAL HSC INFUSION ENHANCE THE PRESENCE OF ENDOTHELIAL CELLS (CEC) IN BLOODSTREAM**

Pogliani E.M.[(1)], Speranza T.[(1)], Terruzzi E.[(2)], Kariqi A.[(1)], Parma M.[(2)]
*[(1)] Medicina Clinica, Università di Milano-Bicocca, Monza; [(2)] Ematologia-Centro Trapianto di Midollo, AO San Gerardo, Monza*

CEC concentration in bloodstream, ensuing from endothelial damage, is an indirect estimate of a prothrombotic environment. We assessed the reliability of this parameter as a measure of the distress caused by the high dose treatment and the infusion of thawed DMSO containing suspensions of peripheral blood stem cells. We measured the CEC levels in myeloma patients receiving Melphalan 200 mg/m2 and autologous PBSC 24 hour after the chemotherapy.
We evaluated 19 myeloma patients (13 males and 6 females). From each patient we obtained 5 samples: (1) before chemotherapy, (2) 24 hours after therapy before the cells

infusion, (3) 48 hours, (4) 7 days and (5) 14 days from the chemotherapy.
As control we evaluated 21 samples from healthy donors.
CECs measurement was performed using a combination of pre-enrichment of CD146+ circulating cells and multiparameter flow cytometry (FCM). CD146+ cells were isolated using CD146-coated magnetic nanoparticles and labelled with CD45-fluorescein isothiocyanate and CD146-PE or isotype control antibody and propidium iodide before FCM. CECs are defined as CD146+/CD45- nucleated cells. Results have been treated as non-parametric variables and analysed by matched-pairs Wilcoxon test.
Comparing for each patient the CEC levels at each selected time with the level before chemotherapy, we found a significant increase immediately after the PBSC reinfusion: p(t2-t0)=0.0156, while their values progressively reverted to basal levels near the 14$^{th}$ day after the treatment: p (t4-t0)=0.6359.
This data show that CEC concentration has a typical trend in relation to the main events of the treatment: chemotherapy and infusion of staminal cells. The increase of CEC level, being related to the endothelial damage could disclose a prothrombotic potential of these procedures.

## OC120 INHERITED THROMBOPHILIA AND THROMBOTIC EVENTS IN BCR-ABL NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: A RETROSPECTIVE STUDY IN 148 PATIENTS

Sacchi E., Carraro M.C., Birindelli S., Lattuada A., Ferrari S., Cassin R., Vanelli C., Arrigoni L., Russo U., Federici A.B.
*Division of Haematology and Transfusion Medicine, Luigi Sacco University Hospital, Milan*

**Background** Subjects with BCR-ABL negative Myeloproliferative Neoplasms (MPN), Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF) have increased risk of venous (VTE) and arterial (ATE) thromboembolism.
**Aims** To investigate the possible association between inherited thrombophilia (FV/G1691A and FII/G20210A) as well as fasting homocysteine (HCY) and ATE/VTE in MPN.
**Patients and Methods** A cohort of 148 patients with MPN followed-up at the Division of Haematology, L. Sacco University Hospital were enrolled in the study, diagnosed according to 2008 WHO criteria: PV=44 (median age 67, SE 2.3); ET=87 (median age 59, SE 1.97); and PMF=17 (median age 68, SE 4.2). ATE/VTE were managed according to CHEST recommendations. Mutation analysis of JAK2 V617F/G1849T, FV/G1691A, and FII/G20210A, and HCY were tested. Statistics were performed by SPSS-11.5.
**Results** The JAK2V617F, FV/G1691A, and FII/G20210A mutations were identified respectively in: PV=36 (81,8%), 1 (2.3%), 2 (4.5%); ET=54 (62.1%), 0 (0%), 5 (5.7%); PMF=10 (58.8%), 3 (17.6%), 1 (5.9%) PMF. ATE/VTE events were found respectively in: PV=8/9 in 14 (31.8%); ET=16/14 in 25 (28.7%); PMF=4/8 in 12 (70.5%). ATE (28 events/28 patients) were localized at the following sites: 5 TIA, 10 AMI, 7 stroke, 3 peripheral, 1 bowel ischemia and 1 placental infarction. VTE (31 events/ 23 patients) were identified by CUS or CT, occurred at the time of MPD diagnosis (23 events) or during follow up (8 events) and were localized at the following sites: 4 portal, 2 splenic, 9 DVT, 2 retinal, 1 cerebral sinus, 1 jugular, 9 thrombophlebitis. Linear regression analyses of MPN showed significant (p=0.031) results in PV only taking into account FV/G1691A (p=0.006), FII/G20210A (p=0.006), HCY (p=0.008).
**Conclusions** Based on these observations, we can confirm that inherited thrombophilia (FV/G1691A and FII/G20210A) and HCY levels enhance the risk of ATE/VTE of MPN patients.

## OC121 EVALUATION OF MICROPARTICLES (MP)-ASSOCIATED PROCOAGULANT ACTIVITY (PCA) AND THROMBIN GENERATION (TG) IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET)

Leuzzi A.[(1)], Marchetti M.[(1)], Panova-Noeva M.[(1)], Russo L.[(1)], Tartari C.J.[(1)], Rambaldi A.[(2)], Finazzi G.[(2)], Barbui T.[(3)], Woodhams B.[(4)], Falanga A.[(1)]
*[(1)] Division of Immunohematology and Transfusion Medicine & Hemostasis and Thrombosis Center, Department of Oncology-Hematology, Ospedali Riuniti di Bergamo, Bergamo; [(2)] Division of Hematology, Department of Oncology-Hematology, Ospedali Riuniti di Bergamo, Bergamo; [(3)] Direzione Scientifica, Fondazione Ricerca Ospedale Maggiore, Bergamo; [(4)] Stago R&D, Genneviliers, France*

Recently a role of circulating MP in the pathogenesis of cancer-associated-hypercoagulability has been suggested ET is a chronic myeloproliferative neoplasm characterized by a high thrombotic risk. In this study we used the calibrated automated thrombogram (CAT) and the P-PPL/1 assay (Stago R&D) to determine the MP-associated-PCA and TG in plasma from 23 ET (13 M/10 F) and 22 control subjects (9 M/14 F).
Platelet Free Plasma (PFP) was obtained by two serial centrifugations (4,000 rpm for 15 min, 11,000 rpm for 10 min) and MP-free plasma (MP-FP) by re-centrifuging PFP at 14,000 rpm for 30 min. MP were isolated from the pellet. For CAT assay, 80 µL of P-FP or MP-FP and 20 µL of buffer were mixed, and TG started adding $CaCl_2$. The results were expressed as LagTime, Peak, area under the curve (ETP), and Time-to-Peak (ttPeak). For P-PPL/1 assay, 100 µL of P-FP or MP-FP were mixed with 50 µL of phospholipid-depleted plasma. Clotting was started by adding FXa and CaCl2 and results expressed in seconds.
PFP from ET patients produced significantly ($p<0.05$) higher TG (LagTime: 20.04±3.9 min) and PCA (76.02±8.8sec) than controls (LagTime: 24.87±8.1 min; PCA: 86.56±9.7sec). This increase was due to the presence of MP, as no TG and significantly prolonged PCA were observed in MP-FP from both patients and controls. The addition of isolated MP to autologous MP-FP restored the TG and PCA of the samples, which was similar to the original values of PFP for both assays. There was a significant correlation between PCA by the P-PPL/1 assay and the different parameters of TG assay [LagTime ($R^2$=0.794), Peak ($R^2$=–0.528), ETP ($R^2$=–0.523) and TTPeak ($R^2$=–0.525)].
Our results show an increased and MP-associated PCA and TG in ET patients which possibly contribute to the hypercoagulable state associated to this disease. Prospective studies to evaluate whether MP levels and procoagulant activity can predict for thrombosis in these patients are warranted.

OC122 **PLATELET ACTIVATION AND VEGF RELEASE IN HEPATOCELLULAR CANCER**

Ferroni P., Spila A., D'Alessandro R., Martini F., Alessandroni J., Ettorre G.M., Vennarecci G., Santoro R., Guadagni F.
*Dept. Laboratory Medicine & Advanced Biotechnologies, IRCCS San Raffaele, Rome*

This study was designed to investigate the behaviour of vascular endothelial growth factor (VEGF) with respect to platelet activation [by soluble (s)P-selectin] in 70 patients with hepatocellular carcinoma (HCC), 45 patients with cirrhosis and 70 controls.
Given that platelets act as scavengers of VEGF into the vasculature, serum [s]VEGF measurement was also performed and normalized by platelet counts (plt-VEGF-load) to provide an estimate of total VEGF content. HCV infection was detected in approximately 70% of either HCC or cirrhotic patients. Mean platelet counts were significantly lower in both groups compared to control subjects ($p<0.0001$). Accordingly, both plasma (p)VEGF and sP-selectin levels were normalized by platelet counts. Median pVEGF levels were higher in HCC or cirrhotic patients compared to controls ($p=0.002$). A similar behaviour was observed for sP-selectin ($p<0.0001$).
Of interest, sP-selectin was the only independent variable predictive of pVEGF levels at multivariate analysis (coefficient 0.94, $p<0.0001$) thus suggesting the platelet origin of pVEGF in HCC patients. On the other hand, plt-VEGF-load correlated with tumour diameter (Rho=0.32, $p<0.05$) but not sP-selectin. Analysis of these variables in samples obtained from tumour draining vein compared to systemic circulation showed a consistent overlap for sP-selectin concentrations ($p=0.21$). Conversely, systemic plt-VEGF-load was lower than that measured in samples obtained from the tumour draining vein. ($p<0.0001$). Multivariate analysis including age, etiology, type of treatment, tumour diameter, AFP and plt-VEGF-load showed that the latter was an independent predictor for survival (Cox-Mantel test 2.6, $p=0.01$) over a median follow-up of 2 years.
In conclusion, the presence of a high plt-VEGF-load and its correlation to tumour diameter is indicative of tumour VEGF production. The latter would be quickly captured by circulating platelets and subsequently released at critical sites as a consequence of platelet activation.
*Partially supported by Grant RFPS-2006-7-342220 by the Italian Ministry of Health.*

OC123 **JAK2 V617F MUTATIONAL LOAD AFFECTS MONOCYTE HEMOSTATIC PROFILE IN ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV) PATIENTS**

Vignoli A.[(1)], Marchetti M.[(1)], Russo L.[(1)],Panova-Noeva M.[(1)], Salmoiraghi S.[(2)], Barbui T.[(3)], Rambaldi A.[(2)], ten Cate H.[(4)], Falanga A.[(1)]
*[(1)] Division of Immunohematology and Transfusion Medicine & Hemostasis and Thrombosis Center, Department of Oncology-Hematology, Ospedali Riuniti di Bergamo, Bergamo; [(2)] Division of Hematology, Department of Oncology-Hematology, Ospedali Riuniti di Bergamo, Bergamo; [(3)] Direzione Scientifica, Fondazione Ricerca Ospedale Maggiore, Bergamo; [(4)] Department of Internal Medicine and Cardiovascular Research Institute, Medical Center Maastricht, Maastricht University, The Netherlands*

In ET and PV patients, the V617F mutation of JAK2 gene has been found associated with an increased activation of the hemostatic system, particularly of platelet and neutrophil components, when compared to JAK2 wild-type (wt) patients. Monocytes (MNC), an important source of circulating tissue factor (TF), significantly contribute to blood coagulation activation. Therefore, we aimed to characterize the impact of JAK2V617F mutational load on MNC procoagulant status, in 37 ET patients (17 JAK2wt; 20 JAK2V617F), 41 PV patients (all JAK2V617F), and 23 healthy controls (C). TF was measured on MNC surface by flow cytometry, in basal condition and after *in vitro* MNC stimulation by bacterial lypopolysaccharide (LPS), as well as in cell lysates by ELISA. MNC endogenous thrombin potential (ETP), elicited in normal human platelet-poor plasma pool, was evaluated by the Calibrated Automated Thrombogram. In basal condition, significantly ($p<0.05$) higher surface TF expression was measured on MNC from either ET (16.5±2.9% positive cells) and PV (22.8±3.3% pos. cells) patients compared to C (8.61.9% pos. cells). A gradient of increased TF expression started from JAK2wt patients (12.1±2.4%), to patients with <50% JAK2V617F allele burden (19.1±2.9%), to PV patients with >50% allele burden (25.4±3.7%). LPS significantly ($p<0.05$) increased MNC-TF expression in all study subjects compared to their respective basal levels. LPS-induced TF was higher in JAK2V617F as compared with JAK2wt patients, with the highest expression in JAK2V617F PV with >50% mutated allele load. Similar results were obtained with MNC total TF antigen levels measured by ELISA. Thrombin generation (ETP) induced by MNC from ET (1,107±39 nM*min) and PV (1,193±54 nM*min) was significantly ($p<0.05$) increased compared to C (996±38 nM*min). PV subjects with >50% mutated allele burden had the highest ETP. In summary, these results provide an additional link between JAK2 genetic dysfunction and hemostatic cellular alterations in patients with myeloproliferative neoplasms.

OC124 **INCREASED RISK OF SPLANCHNIC OR CEREBRAL VENOUS THROMBOSIS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA CARRYING THE JAK2 V617F MUTATION**

De Stefano V., Rossi E., Za T., Ciminello A., Betti S., Luzzi C., De Ritis D., Chiusolo P., Leone G.
*Institute of Hematology, Catholic University, Rome*

In essential thrombocythemia (ET) the risk of thrombosis is increased in the patients carrying the JAK2 V617F mutation. The mutation is present also in a relevant portion of patients with splanchnic vein thrombosis (SVT) but without an overt chronic myeloproliferative neoplasm.
We recruited 224 ET patients (M/F 75/149, median age at diagnosis 55 years, range 20-92): 156 were asymptomatic, 39 (17.4%) had had arterial thrombosis, 13 (5.8%) venous thrombosis in common sites, 13 (5.8%) SVT, and three (1.3%) cerebral venous thrombosis (CVT). All the patients were tested for the presence of the JAK2 V617F mutation and inherited thrombophilia.
The mutation was present in 144 patients (64.2%), 41 of them with arterial thrombosis or venous thrombosis in common

sites, 11 with SVT, and three with CVT. The relative risk (RR) of thrombosis in unusual sites (SVT or CVT) associated with the mutation was 1.56 (95% CI 1.24-1.97) in respect to the asymptomatic patients, namely 1.51 (95% CI 1.15-1.98) for SVT, and 1.79 (95% CI 1.55-2.06) for CVT. All the patients with SVT or CVT were aged <60 years: within this age range the RR associated with the mutation was 1.81 (95% CI 1.36-2.41) for overall thromboses in unusual sites, 1.75 (95% CI 1.27-2.40) for SVT, and 2.07 (95% CI 1.66-2.57) for CVT. The RR did not substantially change after exclusion of the patients with inherited thrombophilia: one with SVT, one with CVT, and five without thrombosis. Notably, in the patient cohort the mutation did not increase the risk of venous thrombosis in common sites (RR 1.24, 95% CI 0.84-1.83).
In conclusion, in ET the risk for venous thrombosis associated with the JAK2 V617F mutation preferentially targets splanchnic or cerebral veins in young patients, and is independent of inherited thrombophilia.

OC125 **RESIDUAL VEIN THROMBOSIS FOR ASSESSING THE OPTIMAL MANAGEMENT OF DEEP VEIN THROMBOSIS IN CANCER PATIENTS: AN INTERIM ANALYSIS OF THE CANCER DACUS STUDY**

Malato A., Saccullo G., Lo Coco L.
*U.O.S. Interdipartimentale di Emostasi, Policlinico Universitario di Palermo, Palermo*

Type and duration of anticoagulation is still matter of debate in cancer patients with acute Deep Vein Thrombosis (DVT) of the lower limbs. In the present study we evaluate the role of a RVT-based management of anticoagulation with Low-Molecular Weight Heparin in cancer patients with acute DVT.
**Materials and Methods** Cancer patients with a first episode of DVT were treated with LMWH at therapeutic dosage for 1 month followed by dose reduction of 25% in the next 5 months. At this time, they were managed according to RVT findings: those with RVT were randomized to continue anticoagulants for 6 additional months (Group A1) or to stop (Group A2), while patients without RVT stopped LMWH (Group B).
**Results** Over a period of 18 months, 134 patients were evaluated across 12 centers in Italy. RVT was detected in 92 (68.6%) patients; recurrent events occurred in 23.4% of those who discontinued and 15.5% of those who continued LMWH. The adjusted Hazard Ratio (HR) for age and sex (Group A2 vs. A1) was 1.58 (95% confidence interval [CI], 0.85±2.93; P=.145). Of the 42 (31.3%) patients without RVT, one had a recurrence (2.3%). The adjusted HR (B vs. A1) was 4.54 (CI 2.3±6.66; P=.028). One major bleeding event occurred in each group of patients who stopped (Group A2 and B) and 2 in those who continued anticoagulation. Overall, 31 (23.1%) patients died due to cancer progression after a median follow-up of 13.2 months after randomization.
**Conclusions** The Cancer DACUS is the first study evaluating an individual marker for assessing duration of anticoagulation in active cancer population. This interim analysis shows that absence of RVT identifies a group of patients at low risk for recurrent thrombosis who can safely stop LMWH after 6 months.

## *TROMBOSI: FISIOPATOLOGIA E CLINICA 2*

OC126 **PERFORMANCE EVALUATION OF A NEW RAPID LATEX IMMUNOASSAY FOR THE DETECTION OF ANTI PF4/HEPARIN COMPLEX ANTIBODIES**

Rogolino A.[(1)], Degl'Innocenti G.[(2)], Marcucci R.[(2)], Fedi S.[(2)], Gori A.M.[(2)], Cesari F.[(2)], Cellai A.P.[(2)], Alessandrello Liotta A.[(1)], Gensini G.F.[(2)],Abbate R.[(2)]
*[(1)] Heart and Vessels, Atherothrombotic Disease Unit, Az. Ospedaliero Universitaria Careggi, Florence; [(2)] Medical and Surgical Critical Care, University of Florence, Florence*

Heparin-induced thrombocytopenia (HIT) is characterized by platelet-activating antibodies directed against complexes of heparin with platelet factor 4(anti-PF4/Hep). The antigen-based immunoassays are characterized by high sensitivity and low specificity. It is now available an automated latex enhanced immunoassay for the determination of total antibodies anti-PF4/Hep.
We evaluated the performance of a new rapid immunoassay in detecting anti-PF4/Hep antibodies. In 57 patients investigated for suspected HIT we performed: 1-ELISA assay (GTI Diagnostic), 2-rapid automated latex enhanced immunoassay (Instrumentation Laboratory) and 3-functional assay heparin-induced platelet aggregation (HIPA).
23/57 (40.4%) patients had a positive test with ELISA method (≥0.4 O.D.) and 15/57 (26.3%) with latex immunoassay (≥0.7 U/mL). Ten patients were positive at both tests, and 18 patients had discordant results: 5 patients had a positive latex immunoassay but negative ELISA and 13 had positive ELISA test but negative latex immunoassay.
2/23 patients, positive for ELISA test, had a low pretest probability (4T-score 0-3), 14/23 had a moderate pretest probability (4T-score 4-5) and 7 had a high pretest probability (4T-score 6-8). Among patients positive for the latex immunoassay 2/15 patients had a low pretest probability, 9/15 had a moderate and 4 had a high pretest probability.
By using HIPA test as gold standard, ROC curve analysis showed that both tests had a 100% sensitivity, whereas specificity was 63% (50-76%, C.I. 95%) for the ELISA method and 78% (67-89%, C.I. 95%) for the latex immunoassay. The optimal cut-off values for identifying patients positive at HIPA test was 2.0 O.D. for the ELISA test and 2.7 U/mL for the latex immunoassay test. Our results demonstrate high sensitivity of both ELISA and latex immunoassay test, with a higher specificity for the rapid latex immunoassay, suggesting that this rapid and easy to perform assay may be a useful tool for laboratories to detect anti-PF4/Hep antibodies.

OC127 **FONDAPARINUX EFFICACY AND SAFETY IN THE MANAGEMENT OF HEPARIN-INDUCED THROMBOCYTOPENIA (HIT): CRUCIAL ROLE OF EARLY DIAGNOSIS**

Barco S.[(1)], Barone M.[(1)], Beltrametti C.[(1)], De Amici M.[(2)], Piovella F.[(1)]
*[(1)] S.C. Angiologia - Malattie Tromboemboliche, Fondazione IRCCS Policlinico San Matteo, Pavia; [(2)] S.C. Pediatria, Fondazione IRCCS Policlinico San Matteo, Pavia*

**Introduction** HIT is a rare immune-mediated adverse reaction caused by platelet-activating IgG antibodies recognizing platelet factor 4 bound to heparin. Fondaparinux is a selective inhibitor of factor Xa which does not react with HIT antibodies in *in vitro* testing.
**Methods** From 2005 to 2009 we treated 52 patients with strong suspect of isolated HIT (20 patients) or HIT and thrombosis, HITT (32 patients). In the HITT group, we applied therapeutic dosages of fondaparinux, i.e. 7.5 mg QD or lower, according with bleeding risk. Twenty patients with isolated HIT were given prophylactic dosages of fondaparinux, i.e. 2.5 mg QD. The mean platelet count nadir was $36.7\pm26.5x10^9$/L.
**Results** Seven patients (13.5%) had thromboembolic events during fondaparinux administration, of which 6/32 with HITT (18.7%) and 1/20 with HIT (5%). In 2 patients out of 7 (one with isolated HIT and one with HITT), increasing the dose of fondaparinux (to 7.5 mg/day) allowed resolution of the thromboembolic event. Of the 7 patients with thromboembolic complications, 6 had a delayed diagnosis of HIT. Three episodes of major bleeding (5.8%) were recorded. Of these, 2 had mild or severe renal insufficiency (2/16, 12.5%). Minor bleeding was recorded in 4 patients (7.7%). All cause mortality was 28.8% (n=15/52). No patient died due to HIT solely. Delay in assessing the diagnosis was pivotal element for the mortality rate. Excluding deaths due to co-morbid conditions and restricting the analysis to HITT deaths only (n=11) one could point out a significant correlation with the diagnostic delay). The ROC curve analysis, considering a 5 days delay cut-off, showed a sensitivity and specificity of 91% and 83%, respectively, in predicting death.
**Conclusions** This report provides evidence supporting the role of fondaparinux in the management of strongly suspected HIT and underlines the key role of early diagnosis in reducing mortality.

## OC128 ALTERATIONS IN COAGULATION AND FIBRINOLYSIS AFTER LEVOTHYROXINE EXPOSURE IN HEALTHY VOLUNTEERS: A CONTROLLED RANDOMISED CROSSOVER STUDY

Squizzato A.[(1)], van Zaane B.[(2)], Debeij J.[(3)], Dekkers O.M.[(4)], Meijers J.C.M.[(5)], van Zanten A.P.[(6)], Buller H.R.[(2)], Gerdes V.E.A.[(7)], Cannegieter S.C.[(3)], Brandjes D.P.M.[(7)]
*[(1)] Medicina Clinica, Università dell'Insubria, Varese; [(2)] Vascular Medicine, Academic Medical Center, Amsterdam; [(3)] Clinical Epidemiology, Leiden University Medical Centre, Leiden, The Netherlands; [(4)] Endocrinology and Metabolic Diseases, Leiden University Medical Centre, Leiden, The Netherlands; [(5)] Experimental Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands; [(6)] Clinical Biochemistry, Slotervaart Hospital, Amsterdam, The Netherlands; [(7)] Internal Medicine, Slotervaart Hospital, Amsterdam, The Netherlands*

**Introduction** Several haemostatic abnormalities have been reported in hyperthyroidism, but the overall effect of thyroid hormone excess on coagulation and fibrinolysis is unclear. Our aim was to assess whether the use of supraphysiological doses of levothyroxine leads to coagulation activation and inhibition of fibrinolysis.
**Methods** Healthy volunteers were randomised to receive levothyroxine or no medication for 14 days with a wash-out period of at least 28 days in a crossover design. To study the effects of different degrees of thyroid hormone excess, 16 participants received levothyroxine in a dose of 0.3 mg per day, and 12 received levothyroxine 0.45 or 0.6 mg per day depending on body weight.
**Results** Levels of von Willebrand factor antigen (VWF:Ag) and activity (VWF:RiCo), factor (F) VIII, plasminogen activator inhibitor-1 (PAI-1) and clotlysis time were slightly higher after levothyroxine 0.3 mg per day than after the control situation, but only levels of VWF were significantly increased compared to baseline values. After levothyroxine 0.45 or 0.6 mg per day, levels of fibrinogen increased by 17%, VWF antigen by 26%, VWF activity by 24%, factor VIII by 19%, factor IX by 14%, factor X by 7%, PAI-1 by 116%, clot-lysis time by 14%, and activated partial thromboplastin time decreased by 3%; all significant changes compared to after the control situation. We did not observe clear evidence of coagulation activation.
**Conclusions** Our data suggest that thyroid hormone excess increases coagulation factor levels and inhibits fibrinolysis, in a dose-dependent fashion. This implies an increased risk of venous thrombosis during hyperthyroidism.

## OC129 ASSOCIATION OF THE HOMOZYGOUS NONSENSE MUTATION R402X IN COAGULATION FACTOR VII WITH ASYMPTOMATIC PHENOTYPE

Branchini A.[(1)], Rizzotto L.[(1)], Giansily-Blaizot M.[(2)], Canella A.[(1)], Mari R.[(3)], Lapecorella M.[(4)], Napolitano M.[(4)], Mariani G.[(4)], Pinotti M.[(1)], Bernardi F.[(1)],
*[(1)] Biochimica e Biologia Molecolare, Università degli Studi di Ferrara, Ferrara; [(2)] Laboratory of Haematology, Hospital Saint Eloi, Montpellier, France; [(3)] Centro Emostasi e Trombosi, Università degli Studi di Ferrara, Ferrara; [(4)] Centro Emofilia e Trombosi, Università de L'Aquila, L'Aquila*

Factor VII (FVII) is the serine protease triggering blood coagulation. The deficiency of FVII is associated to variable bleeding tendency and its complete absence is virtually lethal.
The mutational pattern of FVII is heterogeneous and mainly characterized by missense changes. The few nonsense mutations so far described in homozygous state, C72Stop and K316Stop, are associated to moderate or life-threatening symptoms, respectively.
We investigated the R402Stop nonsense mutation, identified in the homozygous condition in two asymptomatic patients. FVII antigen and coagulant levels in patients plasma were 0.8% and 5% of normal, respectively, thus suggesting the presence of a truncated molecule (FVII-R402Stop, wild type stop codon at the 407 position), poorly secreted but with improved procoagulant activity. Functional FXa and Thrombin generation assays confirmed these observations in plasma.
The deleted residues are located in the carboxyl-terminal region, which has been shown to be crucial for secretion of the highly homologous coagulation serine proteases PC and FIX. To investigate this issue, we expressed the naturally truncated FVII-402Stop and the FVII variants 403-406Stop. Similarly to the 402Stop, the rFVII-403Stop and rFVII-402Stop variants were poorly secreted (~1%). We found an inverse relationship between secreted protein levels in medium and the extent of

the deletion for the rFVII-406Stop (50-60% of WT), rFVII-405Stop (15-20%) and rFVII-404Stop (9-12%). FXa generation as well as PT-based assays revealed a normal specific activity for the rFVII-406Stop, rFVII-405Stop, rFVII-404Stop variants. Intriguingly, upon concentration of conditioned media, the specific activity of the rFVII-402Stop appeared to be 2-3 fold higher than that of rFVII-wt, thus contributing to explain its association to an asymptomatic phenotype.
Altogether these findings demonstrate the importance of the carboxyl-terminal region for FVII biosynthesis/secretion, and also support its participation in FVII function.

OC130 **OXIDATION OF VON WILLEBRAND FACTOR BY POLYMORPHONUCLEAR CELLS ACCELERATES ITS HYDROLYSIS BY LEUKOCYTE SERINE PROTEASES**

Lancellotti S.[1], De Filippis V.[2], Pozzi N.[2], Oggianu L.[3], Rutella S.[4,5], Scaglione G.L.[1], Peyvandi F.[6], Mannucci P.M.[6], De Cristofaro R.[1]
*[1] Medicina Interna e Scienze Mediche Specialistiche, Medicina Interna e Geriatria-Università Cattolica S. Cuore, Roma; [2] Department of Pharmaceutical Sciences, University of Padua, Padova; [3] Section of Biology Applied to Human Health, Rome Tre University, Rome; [4] Department of Hematology, Catholic University School of Medicine, Rome; [5] IRCCS San Raffaele Pisana, Rome; [6] A. Bianchi Bonomi Hemophilia and Thrombosis Center, University of Milan and Department of Medicine and Medical Specialties, IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Luigi Villa, Milano*

It was recently shown that the serine proteases elastase, proteinase 3 and cathepsin G of polymorphonuclear cells (PMNs) cleave von Willebrand factor (VWF) in the A2 domain, near or at the same cleavage site of ADAMTS-13, the zinc protease that specifically controls the proteolytic processing of VWF. On the other hand, recent studies showed also that oxidation of VWF severely impairs its proteolysis by ADAMTS-13.
In this study we investigated whether or not oxidation of VWF by activated PMNs affects its cleavage by elastase, proteinase 3 and cathepsin G.
These enzymes cleave both VWF multimers and a VWF peptide of 74 amino acids (VWF74) with kcat/Km values similar to those of ADAMTS-13. VWF74 containing a sulfoxy-methionine residue at position 1606 was cleaved with higher kcat/Km values by cathepsin G and proteinase 3 in comparison with VWF74, whereas no difference was observed for elastase. A similar positive effect was also observed monitoring the multimeric pattern of VWF oxidized by HClO or by superoxide anions produced by activated leukocytes and hydrolyzed by the leukocyte serine proteases. The biochemical effects of reactive oxygen species (ROS) on VWF structure cause conformational transitions that were observed by spectrometric analysis of intrinsic protein fluorescence and circular dichroism. These conformational changes would promote the exposure of the peptide bonds cleaved by the leukocyte serine proteases.
These findings suggest that oxidation by leukocytes ROS improves cleavage by leukocyte proteases, with a resulting positive effect on VWF proteolysis under conditions where high concentrations of ROS would impede the activity of ADAMTS-13.

OC131 **CYTOSKELETAL ARCHITECTURE REGULATES CYCLOOXYGENASE-2 EXPRESSION IN HUMAN ENDOTHELIAL CELLS**

Eligini S.[1], Cavalca V.[2], Songia P.[1], Crisci M.[1], Tremoli E.[3], Colli S.[4]
*[1] Centro di Ricerche Farmacologiche per lo Studio e la Prevenzione delle Malattie Cardiovascolari, Centro Cardiologico Monzino I.R.C.C.S, Università degli Studi di Milano, Milano; [2] Dipartimento di Scienze Cardiovascolari Cardiologico Monzino I.R.C.C.S, Università degli Studi di Milano, Milano; [3] Dipartimento di Scienze Farmacologiche, Centro Cardiologico Monzino I.R.C.C.S, Università degli Studi di Milano, Milano; [4] Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Milano*

Endothelium is a highly dynamic tissue that regulates numerous physiological and pathological functions to preserve permeability, vasodilation and anti-thrombotic properties of the vessel wall. This unique feature requires a constant adaptive rearrangement of endothelial cells, either in terms of shape or function that cannot set aside the role of cytoskeleton. Here, we show that nocodazole, a microtubule disrupting agent, strongly up-regulates cyclooxygenase-2 (Cox-2) expression in human endothelial cells, in close association with gap formation. In parallel, the levels of acetylated α-tubulin, marker of microtubule stability, were reduced. All these effects were prevented by the microtubule stabilizer paclitaxel, relating them to the disruption of the tubular network. In addition, nocodazole diminished the intracellular ratio of reduced to oxidized glutathione (GSH/GSSG) and increased the extent of glutathionylated actin with concomitant dissolution of F-actin cortical ring and stress fiber formation. Either the antioxidant N- acetylcysteine (NAC) or exogenous GSH reduced Cox-2 levels and preserved the integrity of the endothelial monolayer. Similarly, the Cox-2 metabolite prostacyclin (PGI2) down-regulated Cox-2 levels through a receptor-mediated mechanism, and restored cell monolayer. In a quest for signalling molecules that connect microtubule disruption to Cox-2 induction, we identified Src family kinase activity, serine/threonine phosphatase 2A inhibition, and phosphorylation of mitogen activated protein kinase p38 (p38 MAPK) as essential.
Overall, data link alterations in microtubule and actin cytoskeleton to Cox-2 expression in human endothelial cells and provide a molecular basis for the observation that Cox-2 is up-regulated by mechanical stress to promote an adaptive response for the maintenance of the vasodilatory and anti-thrombotic properties of the vessel wall.

OC132 **CULTURED MEGAKARYOCYTES OF SEVERE FV DEFICIENCY SUBJECTS ENDOCYTOSE FV**

Radu C.M., Spiezia L., Tognin G., Bulato C., Gavasso S., Rossetto V., Simioni P.
*Department of Cardiologic, Thoracic and Vascular Sciences, 2nd Chair of Internal Medicine; University of Padua, Padova*

**Introduction** Factor V (FV) is an essential clotting factor with

both pro- and anticoagulant functions. Severe FV deficiency (parahaemophilia) is an autosomal recessive disorder that confers a bleeding phenotype ranging from mild to moderate. Platelet FV is stored in a partially proteolysed (activated) form that makes it more procoagulant than its plasma counterpart. The origin of platelets FV has been partly identified. Whether megakaryocytes (MKs) synthesize or endocytose FV from plasma, and which of the two mechanisms is more relevant, is still a matter of debate.

**Aim of the study** To demonstrate unequivocally the mechanism of FV endocytosis by MKs using the peculiar and unique model of MKs cultures derived from three patients with homozygous FV deficiency (severe parahemophilia patients).

**Materials and Methods** Three parahemophilia patients with plasma and platelets FV antigen of less than 1% were selected. Haematopoietic stem cells from peripheral blood were isolated and grown in a serum free medium with thrombopoietin and IL-3. FV was purified from plasma of healthy donors and added to MKs medium.

**Results** Cultured MKs were characterized by multilobated cells with cytoplasmatic elongations. FV was not detectable in MKs from parahemophilia patients while it was in normal subjects. Adding purified plasma FV to the culture medium, MKs stained positive for FV due to the uptake of the exogenous FV.

**Conclusions** Normal MKs synthesize FV. MKs from homozygous FV deficient patients cannot; synthesize even though they preserve the ability to endocytose purified FV from the culture medium. This mechanism may account for the small amount of FV detectable in platelets from severe parahemophilia patients which has been shown to influence thrombin generation and, possibly, bleeding manifestations in these patients. Furthermore it could be considered a rescue mechanism of intraplatelet FV levels after administration of normal plasma or FV concentrates (still not available).

## *CARDIOPATIA ISCHEMICA*

### OC133 **GENETIC VARIANTS BESIDES CYP2C19*2 POLYMORPHISM ARE ASSOCIATED WITH MAJOR ADVERSE CARDIOVASCULAR EVENTS IN HIGH RISK VASCULAR PATIENTS ON DUAL ANTI-PLATELET THERAPY**

Giusti B.[(1)], Saracini C.[(1)], Vestrini A.[(1)], Magi A.[(1)], Marcucci R.[(1)], Rossi L.[(1)], Cordisco A.[(1)], Nanna C.[(1)], Gensini G.F.[(1)], Abbate R.[(1)]

*[(1)] Dpt. Medical and Surgical Critical Care - Atherothrombotic Diseases Unit, AOU Careggi, University of Florence, Florence; [(2)] Dpt. Medical and Surgical Critical Care - Dpt. Heart and Vessel, AOU Careggi, University of Florence, Florence*

Persistent platelet reactivity despite antiplatelet treatment confers an increased risk of major adverse cardiovascular events (MACE) in patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI) with stent implantation. Genetic and nongenetic mechanisms are involved in high on-treatment platelet reactivity. Recently, the CYP2C19*2 polymorphism has been demonstrated an independent determinant of MACE occurrence in patients on antiplatelet therapy. In addition to polymorphisms linked to clopidogrel metabolism, genetic variants in different genes coding for platelet receptors and enzymes have been investigated with discordant results.

In 922 consecutive ACS patients undergoing PCI with stent implantation on dual antiplatelet therapy followed up for 6 months, aim of our study was to assess the relation to the risk of MACE of 41 allelic variants in 14 genes coding for prostaglandin-endoperoxide synthase-1 (PTGS1 or COX1), -2 (PTGS2 or COX2), von Willebrand factor (VWF), integrin α 2b (ITGA2B), glycoprotein Ib (GP1BA), glycoprotein VI (GP6), selectin P (SELP), integrin α2 (ITGA2), fibrinogen β chain (FGB), purinergic receptor P2Y12 (P2RY12), ATP-binding cassette sub-family B member 1 (ABCB1), different isoforms of CYP450 (-3A4, -2C9, and -2C19). Genotyping was performed with the specific allelic discrimination Taqman assays.

We confirmed the independent association of the CYP2C19*2 polymorphism with the occurrence of MACE. Moreover, patients with MACE showed a higher prevalence of carriers of the rs3842788 COX1 polymorphism and of homozygotes for the rs7969672 VWF polymorphism, and a lower prevalence of carriers of the rs5911 ITGA2B polymorphism (18.5% vs. 7.9%, p=0.007; 7.4% vs. 2.3%; p = 0.022 and 46.3% vs. 61.7%, p=0.023, respectively); at multivariate regression analysis only COX1 polymorphism remained significantly associated to MACE [OR=3.4 (1.45-8.02), p=0.005].

Our data indicated that, besides the well known CYP2C19*2, other polymorphisms in genes involved in platelet function play a crucial role in the outcome of high risk vascular patients.

### OC134 **VARIABLE EFFECT OF P2Y12 INHIBITION ON PLATELET THROMBUS VOLUME IN FLOWING BLOOD**

Mendolicchio G.L.[(1)], Zavalloni D.[(2)], Bacci M.[(1)], Corrada E.[(3)], Marconi M.[(3)], Lodigiani C.[(1)], Presbitero P.[(2)], Rota L.[(1)], Ruggeri Z.M.[(4)]

*[(1)] Centro Trombosi, Istituto Clinico Humanitas IRCCS, Rozzano; [(2)] Invasive Cardiology Unit, Istituto Clinico Humanitas IRCCS, Rozzano; [(3)] Unità Coronarica, Istituto Clinico Humanitas, Rozzano; [(4)] Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, USA*

**Background/Aims** Patients treated by percutaneous coronary intervention (PCI) receive aspirin and P2Y12 ADP receptor inhibitors to reduce thrombotic complications. We evaluated how the decrease in P2Y12 function influences platelet aggregate (thrombus) size measured ex vivo.

**Materials and Methods** Blood from 21 controls and 31 patients receiving aspirin and clopidogrel (19) or ticlopidine (12) was drawn from an antecubital vein with informed consent. Vasodilator-stimulated phosphoprotein (VASP) platelet reactivity index (PRI) was measured with a fluorescein-labelled monoclonal antibody by flow cytometry; thrombus formation in flowing blood perfused over fibrillar collagen by quantitative confocal videomicroscopy; plasma von Willebrand factor (VWF) antigen by ELISA.

**Results** The volume of thrombi was smaller in the group of patients than controls, but 61.3% of individual patient values

were above the lower normal limit. In the group of patients, thrombus formation was normal during the initial 3 minutes; 71% of individual volume values were within the normal range. In 45.2% of patients, but never in controls, disaggregation occurred thereafter resulting in a significant thrombus size difference between the two groups at later times. Vasodilator-stimulated phosphoprotein (VASP) phosphorylation, which reflects P2Y12 inhibition, was also decreased in the patient group and only 22.6% of individual values were above the lower normal limit. Plasma VWF, which supports platelet aggregation in flowing blood, remained elevated in patients tested >90 days after PCI. We found no correlation between platelet thrombus volume and levels of P2Y12 inhibition or plasma VWF, suggesting that the effect of anti-platelet drugs on platelet function is modulated by multiple, individually variable factors.
**Conclusions** Measuring platelet thrombus formation in flowing blood reflects the consequences of anti-platelet therapy in a manner that is not proportional to P2Y12 inhibition. The combined results of both assays may be relevant for assessing residual thrombotic risk in patients receiving anti-platelet drugs.

## OC135 **PROTEOMICS INVESTIGATION OF PLATELETS AFTER INHIBITION AND CARDIOVASCULAR EVENTS (APICE PROJECT)**

Boccardi C.[(1)], Giusti B.[(2)], Gori A.M.[(2)], Marcucci R.[(2)], Rocchiccioli S.[(1)], Romagnuolo I.[(1)], Antoniucci D.[(3)], Parodi O.[(1)], Citti L.[(1)], Abbate R.[(2)]
*[(1)] Institute of Clinical Physiology, CNR, Pisa; [(2)] Athero-thrombotic Diseases Unit, Dpt. Medical and Surgical Critical Care, AOU Careggi, University of Florence, Florence; [(3)] Department of Heart and Vessels, AOU Careggi, Florence*

Dual antiplatelet therapy (aspirin and clopidogrel), aimed to inhibit platelet reactivity, is the recommended standard of care for reducing the occurrence of major adverse cardiovascular events (MACE) in patients with acute coronary syndromes (ACS) undergoing percutaneous coronary intervention (PCI) with stent implantation. A large body of evidence exists demonstrating that non-responsiveness to clopidogrel and/or aspirin is associated with increased risk of adverse clinical events.
In the framework of the "Activity of Platelets after Inhibition and Cardiovascular Events" (APICE) project, we performed the proteomic analysis of platelet protein profiles from ACS patients undergoing PCI with stent implantation on dual antiplatelet treatment. Total protein extracts obtained from platelets of patients with or without residual platelet reactivity [RPR - by 10 M ADP or 1 mM arachidonic acid (AA)-induced platelet-rich-plasma aggregation] were analyzed at the time of the acute event. Protein content and platelet reactivity have been assessed within 24 hours from the clopidogrel loading.
We have identified more than 1,000 plasma proteins. Some differentially modulated proteins among patients with or without RPR by ADP (>70%) or AA (>20%) have been observed. Interestingly, significant differences were found between patient with or without RPR and between patients with AA-RPR or ADP-RPR. Many of the differentially expressed proteins are directly involved in adherence and activation of platelet aggregation (such as platelet/endothelial cell adhesion molecule, von Willebrand factor). Other proteins are involved in the platelet cytoskeleton organization (such as filamin A, tubulin β 1).
In conclusion, our preliminary proteomic profiling of platelets in ACS patients on dual antiplatelet treatment identifies differences among patients with or without RPR by ADP or AA identifying different actors that might play a role in the pathological mechanisms underling AA-RPR and ADP-RPR.

## OC136 **EFFECTS OF CIGARETTE SMOKING ON HUMAN PLATELET PROTEOME**

Della Corte A., Tamburrelli C., Crescente M., Barisciano M., D'Imperio M., Di Michele M., Donati M.B., de Gaetano G., Rotilio D., Cerletti C.
*Laboratori di Ricerca, Università Cattolica, Campobasso*

**Background/Aims** Cigarette smoking is one of the major risk factors of coronary artery disease, peripheral vascular disease and stroke. Platelets from smoker and non-smoker healthy volunteers were studied by a proteomic approach, in parallel to some platelet function parameters.
**Materials and Methods** PFA-100 collagen/ADP closure time and markers of platelet (P-selectin, PAC-1) and leukocyte (CD11b and Mac-1) activation and interaction (mixed aggregates) were evaluated in whole blood from smokers and non smoker subjects (n=16 per group), in basal conditions and after *in vitro* stimulation. Platelet proteome from 8 smoker and 8 non-smoker samples were resolved by 2-DE DIGE, compared by Decyder software and identified by MALDI-TOF MS and LC-MS/MS. Regulated proteins were further analysed by Ingenuity Pathway Analysis which builds hypothetical networks from identified proteins and other proteins, on the basis of a regularly updated database.
**Results** No significant difference in either the PFA-100 closure time and cell markers of activation and interaction was evident between the two groups. In-gel analysis by Decyder software of platelet proteins from smokers and non-smokers revealed 5 significantly different protein spots ($p<0.05$): three (Factor XIII precursor, platelet glycoprotein IIb and β actin) were significantly higher in smokers, while WDR1 protein and chaperonine HSP60 were down-regulated. These proteins were used for Ingenuity Pathway Analysis which identified one high ranking network (score 14). The five proteins identified by DIGE-based proteomic, together with 29 recognized to be related, belong to the cluster "Cellular Development, Lipid Metabolism, Small Molecule Biochemistry".
**Discussion and conclusion** An effect of cigarette smoking on platelet proteome is shown here for the first time: the proteins identified are mainly involved in disease-associated inflammatory responses. Proteomics may help clarify smoke-associated disease mechanisms and identify candidate biomarkers for risk prevention, such as Factor XIII.

## OC137 **FXIII LEVELS IN MYOCARDIAL INFARCTION: A POTENTIAL NOVEL PROGNOSTIC BIOMARKER?**

Gemmati D.[(1)], Zeri G.[(1)], Mari R.[(1)], Orioli E.[(1)], Bovara M.[(2)], Moratelli S.[(1)], Bernardi F.[(3)], Pinotti M.[(3)], Fiore T.[(4)], Ferrari R.[(4)], Ansani L.[(4)], Serino M.L.[(1)]

*(1) Biomedical Sciences & Advanced Therapies - University of Ferrara, Ctr. Hemostasis & Thrombosis, Ferrara; (2) IL-Instrumentation Laboratory, Milano; (3) Biochemistry & Molecular Biology Section, University of Ferrara, Ferrara; (4) Cardiology Section, Clinical & Experimental Medicine, University of Ferrara, Ferrara*

Despite excellent therapy for myocardial infarction (MI), progression of the infracted area and adverse cardiac events are still critical points influencing survival. Experimental evidences suggest that FXIII plays a key role in myocardial healing, and its low values led to cardiac rupture and post-MI death. Normal-high FXIII levels are crucial in scar stability, scar thickness, MI expansion and left ventricle dilatation.
We recently recognized an acute FXIII phase reduction in the first week post-MI, but not all cases had drastic diminishing and this was also FXIII-genotype dependent. Our objective is to recognize if FXIII depression and/or late recovery could be a key marker for poor prognosis.
146 documented MI patients were enrolled from the Coronary-Care-Unit of the Hospital-University of Ferrara and FXIII was assessed (FXIII, IL) at the recruitment (t0) and every 24-hour for five additional days (t1-5) post-MI. Control samples were drawn at 30 day (t30) to have steady state FXIII levels. All patients were genotyped for FXIIIV34L. Globally, t0 had mean FXIII levels of 96.7±30.7%; the lowest value was at t4-5 (81.7±27.6%) and t30 was 103.3±26.2%. Stratifying data by genotype, L-carriers had the highest fall when compared to VV-genotype.

| Days-post-MI | FXIII (%) VV34 (n=94) | FXIII (%) L34-carriers (n=52) | P |
|---|---|---|---|
| $t_0$ | 101.4±29.9 | 88.1±30.6 | 0.005 |
| $t_{3-4}$ | 84.7±23.3 | 75.1±26.8 | 0.04 |
| $t_{30}$ | 106.7±29.9 | 97.6±24.4 | NS |

The low FXIII mean level observed in the carriers is explained by the lower activation threshold of FXIII-L34. After 30-days post-MI no significant differences were observed, according to the hypothesis that the early activation of L34 at the injured site could rapidly and better promote myocardial wound healing and have positive effects on prognosis. Understanding whether FXIII level monitoring may be useful to select cases with poor MI outcome could pave the way to utilize FXIII molecule as a tailored treatment to improve myocardial healing, recovery of functions and survival.

## *EMBOLIA POLMONARE*

### OC138 MULTIDETECTOR COMPUTED TOMOGRAPHY FOR ACUTE PULMONARY EMBOLISM: ROLE OF THE EMBOLIC BURDEN

Vedovati M.C.(1), Becattini C.(1), Agnelli G.(1), Pruszczyk P.(2), Casazza F.(3), Grifoni S.(4), Salvi A.(5), Bianchi M.(6), Douma R.(7), Konstantinides S.(8)
*(1) Medicina Interna e Cardiovascolare-Stroke Unit, Università di Perugia, Perugia; (2) Internal Medicine and Cardiology, Medical University, Warsaw, Poland; (3) Cardiologia, Ospedale San Carlo Borromeo, Milano; (4) Medicina d'Urgenza, Azienda Ospedaliero-Universitaria Careggi, Firenze; (5) Medicina d'Urgenza, Azienda Ospedaliera-Universitaria, Ancona; (6) Medicina, Ospedale Valduce, Como; (7) Academic Medical Center Vascular Medicine Amsterdam, The Netherlands; (8) Cardiology, Democritus University of Thrace, Greece*

In patients with acute pulmonary embolism (PE) the prognostic role of the embolic burden is still undefined.
**Methods** Patients with acute PE diagnosed by multidetector computed tomographic (MDCT) angiography were included in this international study. The embolic burden was centrally assessed at MDCT by a panel unaware of clinical patient data and was calculated according to the scoring system of Qanadli. The score was calculated as (n.d) (n, value of the proximal clot site, equal to the number of segmental branches arising distally; d, degree of obstruction scored as partial [value=1] or total [value=2]). The highest possible score is 40 (thrombus completely obstructing the main pulmonary artery), which corresponds to a 100% obstruction index (OI). Student's t-test was used for comparisons between groups and the logistic regression analysis to identify predictors for in-hospital death or clinical deterioration.
**Results** Overall, 389 patients were included in the analysis. The mean value of OI was 35±17% (range 2 to 93%). Higher OIs were found in patients with dyspnoea or tachycardia at clinical presentation while no association was observed between OI and chest pain, haemoptysis, or hypotension. The OI was higher in obese patients while no association was observed between OI and deep venous thrombosis, cancer, chronic obstructive pulmonary disease, recent surgery, type II diabetes mellitus, or hypertension. The OI was higher in patients with right ventricle dysfunction, either assessed by echocardiography (41±16% vs. 27±15%, $p<0.0001$) or by MDCT (40±16% vs. 26±14%, $p<0.0001$) and in patients with high troponin levels (40±17% vs. 32±17%, $p<0.0001$). RVD, either assessed by echocardiography or by MDCT, but not the OI was an independent risk factor for death or clinical deterioration at multivariable analysis.
**Conclusions** In patients with acute pulmonary embolism MDCT-detected RVD but not MDCT-detected embolic burden is a predictor of death or clinical deterioration.

### OC139 THE VALUE OF 64-DETECTOR ROW COMPUTED TOMOGRAPHY FOR THE DIAGNOSIS OF PULMONARY EMBOLISM

Pesavento R.(1), De Conti G.(2), Minotto I.(3), Maurizi F.(2),Mongiat M.(2), Pagnan A.(1), Prandoni P.(1)
*(1) Department of Cardiothoracic and Vascular Science, 2nd Chair of Internal Medicine, Padua; (2) Radiology Unit, Department of Radiology, Padua; (3) Thromboembolic Unit, Department of Cardiothoracic and Vascular Science, Padua*

**Background** A diagnostic management strategy using a clinical decision rule, D-dimer testing, and either single-detector or multidetector-row spiral computed tomography (CT) was recently found to be effective in the evaluation and management of patients with clinically suspected pulmonary embolism (PE)[1]. However, the rate of thromboembolic complications in the 3-month follow-up of patients with negative CT was still substantial (1.7%) and included fatal events.
**Methods and Results** 542 consecutive patients with clinical suspicion of the first episode of PE and either a high pre-test

probability of PE (using the simplified Wells score) or a low pre-test probability in combination with a positive D-dimer underwent a 64-detector row spiral CT, which was not applied or gave uninterpretable findings in 7 patients (1.3%), confirmed the clinical suspicion in 168 (31.0%), and excluded it in the remaining 367 (67.7%). Patients with negative CT underwent a prospective 3-month follow-up. In the 367 patients with negative CT scan the 3-month follow-up, which was uneventful in all but one, who experienced a distal DVT. The incidence of overall thromboembolic events was 0.27% (95% CI, 0 to 1.51).

**Conclusions** We conclude that the 64-detector row spiral CT has the potential to safely exclude the presence of pulmonary embolism in all patients presenting with the clinical suspicion of this clinical disorder.

1) Christopher Study Investigators. Effectiveness of managing suspected pulmonary embolism using an algorithm combining clinical probability, D-dimer testing, and computed tomography. JAMA 2006; 295: 172-179.

## OC140 CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION (CTEPH): ADEQUATE ANTI-COAGULANT TREATMENT IS MANDATORY BEFORE PLANNING ENDARTERECTOMY

Piovella F., Barone M., Beltrametti C.
*S.C. Angiologia - Malattie Tromboemboliche, Fondazione IRCCS Policlinico San Matteo, Pavia*

Chronic thromboembolic pulmonary hypertension (CTEPH) results from obstruction of the pulmonary arteries by organized pulmonary emboli which have become incorporated into the pulmonary artery wall, eventually causing elevated pulmonary vascular resistances. Pulmonary endarterectomy (PEA) is the treatment of choice. Without intervention, CTEPH is a progressive, lethal disease with no effective medical therapy. Careful pre- and post-operative management is essential for a successful outcome.

In 1994 in Pavia was started a programme in which members of a multidisciplinary team work in close interaction. From April 1994 to April 2010, 290 PEAs were performed at our Center. The operability rate rose from 74% (year 2004) to 89% (year 2009). The mean age at PEA was 5616 (11-84), and 26.1% of patients were older than 70 years. Patients were preoperatively in WHO functional class II (6.0%), III (45.9%) or IV (48.1%). Most CTEPH patients require elective surgery. Therefore there is margin for a safe planning of the procedure and of the related pre-operative tests. Complete anatomic resolution of acute pulmonary embolism (PE) is not common.

Perfusion scan studies performed months after the embolic event show in 15%-25% of cases only partial resolution. Complete thromboembolic resolution may represent the exception rather than the rule. The value of residual thrombus detected during CT scan is limited. The simple detection of incomplete resolution of pulmonary embolism should not drive the decision to plan endarterectomy. Patients should have completed at least six months of adequate anticoagulant treatment. The mortality rate during the first postoperative year is reduced to 5%, but this figure is very high in compared to the outcome of a well-managed anticoagulant treatment for venous thromboembolism. Patients with CTEPH diagnosed early after the embolic episode should therefore be referred to experienced thrombosis centers, in order to verify the real need for immediate surgery.

## OC141 INCIDENCE, HOSPITAL BURDEN AND DIAGNOSTIC APPROACH OF ACUTE PULMONARY EMBOLISM (PE) IN A TUSCAN PROVINCE

Masotti L.[(1)], Pampana A.[(1)],Pocobelli B.[(2)], Landini G.[(3)]
*[(1)] UO Medicina Interna, Ospedale di Cecina, Cecina; [(2)] UO Sistemi Informatici, ASL 6, Cecina; [(3)] UO Medicina Interna, Ospedale Santa Maria Nuova, Firenze*

**Background and Aim** Acute PE remains a medical emergency in hospital practice. Diagnostic and therapeutic guidelines have had wide diffusion, but little is known about hospital burden and diagnostic approach to acute PE in real practice.

**Materials and Methods** Data of DRGs and schedules of patients recovered from 2006 to 2009 in four hospitals of a Tuscan province (Livorno) and discharged with diagnosis of acute PE.

**Results** In the analysed period 794 patients (189 in 2006, 206 in 2009) were discharged with PE as one of the first six diagnoses (74.5% of them as first diagnosis). Based on our analysis, considering population of Livorno, incidence of PE in population could range between 60 and 65 cases/100,000 inhabitants/year. Diagnosis of PE accounted for 0.48% of total hospital admissions. Mean age was 74.4±13.1 years. Mortality rate was 14.8%. 33.9% died within 24 hours, 60% within 5 days, 82% within 14 days. Mortality rate increased with increasing age (11.7% in patients under 64 years, 18% in over 85) and remained similar along years. Mortality for acute PE accounted for 1.8% of entire hospital mortality. DVT was associated to PE in 31.6%, cancer in 27.7%. Around 60% of patients were recovered in Internal Medicine, while 9.1% developed PE in surgical wards. Diagnosis was objective in 77.9% (37% by lung scan, 32.3% by angio-CT, 8.6% by lung scan+CT, 3.5% by pulmonary angiography), while was indirect in 11.9% and not objective in 10.2%. Echocardiogram was performed only in 34.2%, legs ultrasonography in 46.4%, chest x-ray in 39.2%.

**Conclusion** Acute PE represents one of the leading causes of mortality and morbidity in hospital practice. Our study, focused in a Tuscan province, could contribute to the knowledge of hospital burden and diagnostic approach of it. Prognostic assessment of PE based on echocardiography should be implemented.

## OC142 PULMONARY EMBOLISM IN CRITICALLY ILL PATIENTS: REASONS FOR SUSPICION, FREQUENCY AND OUTCOMES

Donadini M.P.[(1)], Cook D.[(2)], Zaffaroni R.[(3)], Dentali F.[(1)], Severgnini P.[(3)], Pelosi P.[(3)], Ageno W.[(1)]
*[(1)] Medicina Clinica, Università dell'Insubria, Varese; [(2)] Department of Medicine, McMaster University, Hamilton, Canada; [(3)] Ambiente, Salute e Sicurezza, Università dell'Insubria, Varese*

**Background** Pulmonary embolism (PE) is a challenging diagnosis in critically ill patients and has potentially serious complications; however, few studies have been performed in

the intensive care unit (ICU).
**Objectives** To explore the clinical features leading to computed tomography pulmonary angiography (CTPA) to diagnose PE, to evaluate the findings of CTPA, and to compare the outcomes of patients with PE vs. without PE.
**Methods** Retrospective study of clinical and radiographic records of consecutive ICU patients undergoing a CTPA for suspected PE during their ICU stay at 2 hospitals in Canada and Italy. Data were collected on baseline characteristics, features leading to a suspicion of PE, CTPA findings and hospital mortality.
**Results** Among 97 patients, PE was confirmed in 16 out of 103 (15.5%) CTPAs. Most CTPAs (71 of 103, 68.9%) were performed to evaluate the reason for ICU admission. Overall, the only clinical features distinguishing patients with PE vs. without PE was hypotension (68.8% vs. 47.1%, respectively, p=.011) and decreasing O2 saturation in ICU (18.8% vs. 3.4%, respectively, p=.047). Among the 31,1% of patients who had a suspected PE during their ICU stay, the only clinical feature distinguishing patients with PE vs. those without PE was the suspicion or presence of deep vein thrombosis (50% vs. 3.8%, respectively, p=.015). Regarding other CTPA findings, pneumonia were significantly less frequent in patient with PE vs. without PE (18.8% vs. 51.7%, p=.027). Patients with PE were more likely to die in hospital than those without PE (50% vs. 33.3%, p=not significant).
**Conclusions** In ICU patients, PE was more frequently suspected as a cause of ICU admission than as a result of clinical events developing during the ICU stay. In the latter setting, no particular clinical features were associated with the diagnosis of PE. Further research is urgently required in this challenging field.

## *FIBRILLAZIONE ATRIALE E CARDIOEMBOLISMO*

### OC143 **STROKE RISK STRATIFICATION IN A REAL-WORLD ELDERLY ANTICOAGULATED ATRIAL FIBRILLATION POPULATION**

Poli D.[(1)], Lane D.A.[(2)], Antonucci E.[(3)], Grifoni E.[(3)], Lip G.Y.H.[(2)]
*[(1)] Thrombosis Centre, Dept of Heart and Vessels, Azienda Ospedaliero-Universitaria Careggi, Firenze; [(2)] Centre for Cardiovascular Sciences, University of Birmingham, Birmingham, UK; [(3)] Department of Medical and Surgical Critical Care, University of Florence, Firenze*

Appropriate stroke risk stratification is essential to ensure suitable tailoring of antithrombotic therapy. However, many available stroke risk schemas place the majority of people in the moderate-risk category where guidelines recommends either aspirin or warfarin, creating ambiguity over the most appropriate antithrombotic therapy.
660 elderly AF patients [mean (SD) age 74 (7.7) years; 54.4% female] from the Thrombosis Center of Florence, Italy were included and followed for a mean 3.57±2.73 years for the incidence of thromboembolic (TE) events. The ability of the CHA2DS2-VASc schema to predict TE was compared with six other stroke risk schema [AFI, CHADS2 classical & revised, NICE 2006, ACC/AHA/ESC 2006, ACCP 2008] in a large real-world cohort of anticoagulated AF patients, by determining the c-statistic.
Univariate predictors of thromboembolic events were female gender [odds ratio 1.9; 95% confidence intervals 1.01-3.70] and previous stroke/TIA/TE [OR 5.6; 95% CI 2.70-11.45], although after adjustment only previous stroke/TIA/TE was an independent predictor of TE [OR 5.5; 95% CI 2.68-11.31; p=0.000.l]. All stroke risk schema had modest discriminating ability, with c-statistics ranging from 0.54 (AFI) to 0.72 (CHA2DS2-VASc). The proportion of patients assigned to individual risk categories varied widely across the schema, with those categorised as moderate-risk ranging from 5.3% (CHA2DS2-VASc) to 49.2% (CHADS2-classical). Patients classified as low-risk by all-risk schema were truly low risk, with no TE events recorded.
Current published risk schemas have modest predictive ability. CHA2DS2-VASc minimises the classification of AF patients into the rmoderate-risk category and clearly discriminates those at truly low risk. This refined schema may assist in stroke risk stratification and antithrombotic therapy decision-making in AF patients.

### OC144 **RISK FACTORS ASSOCIATED WITH BLEEDING IN VERY OLD ATRIAL FIBRILLATION PATIENTS ON VKA TREATMENT: RESULTS FROM A PROSPECTIVE COLLABORATIVE STUDY. ON BEHALF OF THE AD HOC STUDY GROUP OF FCSA**

Poli D.[(1)], Antonucci E.[(2)], Grifoni E.[(2)], Carini U.[(3)], Ciampa A.[(4)], Da Col P.[(5)], Galbo L.[(6)], Insana A.[(7)], Nante G.[(8)], Restifo D.[(9)], Testa S.[(10)]
*[(1)] Dept of Heart and Vessels Thrombosis Centre, Azienda Ospedaliero-Universitaria Careggi, Florence; [(2)] Department of Medical and Surgical Critical Care, University of Florence, Florence; [(3)] Cardiovascular Medicine, Grosseto Misericordia Hospital, Grosseto; [(4)] UOSS "Thrombosis Centre", AORN SG Moscati, Avellino; [(5)] Thrombosis Centre, Sanitary Service, Regione Friuli Venezia Giulia, Trieste; [(6)] Laboratory Service, Cervello Hospital, Palermo; [(7)] Dept Clinical Pathology, S. Croce Hospital, Moncalieri; [(8)] Dept. of Medical and Surgical Science, University of Padova, Padova; [(9)] Thrombosis Centre, AO Desio-Vimercate, Vimercate; [(10)] Haemostasis and Thrombosis Centre, A.O. Istituti Ospitalieri of Cremona, Cremona*

The increasing number of very old patients with atrial fibrillation (AF) on treatment with vitamin K antagonists (VKAs) requires a better knowledge of the risks associated with this treatment in elderly.
We performed a prospective collaborative study among Centres affiliated to FCSA to assess the adverse events of VKAs in patients who started treatment after 80 years of age. Patients ≥80 years were prospectively followed-up from the start of treatment. Quality of anticoagulation and adverse events occurring during follow-up were recorded. The total number of patients recruited was 4,067, 3,015 patients for AF (males 45%; 7,620 patient/years; mean time of follow-up 2.52 years). The total quality of anticoagulation measured as time spent within, above and below the international normalized ratio therapeutic range was 63%, 14% and 23%, respectively [IQR for time in therapeutic range (TTR) 50-75]. During follow-up 133 major bleeding events (1.75x100 patient/years) were recorded. The univariate and multivariate analysis for

risk factors associated with bleeding risk are reported in the Table.

| | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | p value | OR | 95% CI | p value |
| Age | 1.0 | 0.9-1.0 | 1.0 | | | |
| Male gender | 1.6 | 1.1-2.3 | 0.008 | 1.4 | 0.9-2.2 | 0.15 |
| Previous stroke/TIA | 1.4 | 1.0-2.0 | 0.03 | 1.2 | 0.7-1.9 | 0.46 |
| Hypertension | 1.3 | 0.8-2.1 | 0.2 | | | |
| Diabetes Mellitus | 1.4 | 0.9-2.1 | 0.1 | | | |
| Heart failure | 1.1 | 0.7-1.6 | 0.6 | | | |
| CAD/AOP | 1.5 | 1.1-2.1 | 0.009 | 1.4 | 1.0-2.1 | 0.07 |
| Previous bleed | 7.1 | 4.1-12.3 | 0.000 | 7.0 | 3.7-13.4 | 0.000 |
| Antiplatelet therapy | 1.2 | 0.9-1.7 | 0.2 | | | |
| Active Cancer | 3.7 | 2.2-6.2 | 0.000 | 3.8 | 2.0-7.4 | 0.000 |
| History of falls | 4.6 | 2.6-8.0 | 0.000 | 4.7 | 2.3-9.5 | 0.000 |
| TTR | 1.0 | 1.0-1.1 | 0.9 | | | |
| Renal failure | 1.9 | 1.1-3.1 | 0.01 | 1.4 | 0.8-2.3 | 0.30 |

In conclusion, among very old AF patients on VKA treatment history of falls, active cancer and history of previous bleed are independently associated with bleeding risk.

## OC145 RISK FACTORS FOR CEREBRAL ISCHEMIC AND HEMORRHAGIC EVENTS IN PATIENTS WITH ATRIAL FIBRILLATION ON WARFARIN FOR STROKE PREVENTION

Paciaroni M.[(1)], Manina .[(1)], Agnelli G.[(1)], Ageno W.[(2)], Caso V.[(1)], Corea F.[(3)], Lanari A.[(4)], Previdi P.[(4)], Silvestrelli G.[(4)]
*[(1)] Internal Medicine Stroke Unit, Division of Cardiovascular Medicine, University of Perugia, Perugia; [(2)] Department of Clinical Medicine, University of Insubria, Varese; [(3)] UO Gravi Cerebrolesioni, S. Giovanni Battista Hospital, Foligno; [(4)] Stroke Unit, Division of Neurology, Carlo Poma Hospital, Mantova*

The aim of this study was to evaluate the risk factors for cerebral ischemic or hemorrhagic events in warfarin-treated atrial fibrillation (AF) patients with an International Normalized Ratios (INR) above 1.8 on admission.
In a case control study, cases were consecutive patients with AF who were on warfarin and who were admitted to four Italian hospitals after an acute cerebrovascular event (ischemic or hemorrhagic stroke, subarachnoid hemorrhage or transient ischemic attack) with an INR above 1.8. Controls were selected from a single anticoagulation clinic and were patients with AF on adequate warfarin treatment who did not suffer cerebrovascular events.
Cases were identified among 5,684 consecutive patients; 4,785 with an ischemic event and 899 with a hemorrhagic event. One-hundred-forty-eight cases out of the patients with ischemic events (3.1%, 21 with transient ischemic events and 127 with ischemic strokes) and 36 cases out of the patients with hemorrhagic events (4.0%) had AF and were taking warfarin with an INR above 1.8 on admission. On multivariate analysis, diabetes (OR 3.8; 95% CI 1.09-13.82, p=0.025), hyperlipidemia (OR 4.5; 95% CI 1.11-18.23, p=0.035) and carotid/vertebral atherosclerosis on ultrasound (OR 3.0; 95% CI 1.13-8.41, p=0.028) were independent predictors for ischemic cerebral events. The use of statins was inversely correlated with an ischemic event (OR 0.1; 95% CI 0.06-0.47. p=0.001). Hypertension (OR 2.8; 95% CI 0.99-8.0, p=0.052) and high INR values on admission (OR 1.9; 95% CI 0.99-3.89, p=0.051) were associated with hemorrhagic events. Hyperlipidemia was inversely correlated with a cerebral bleeding complication (OR 0.05; 95% CI 0.01-0.50. p=0.01).
Carotid/vertebral atherosclerosis, diabetes and hyperlipidemia are associated with an increased risk for ischemic events in patients with AF on adequate warfarin treatment. Statins significantly reduce the risk of ischemic events. Hypertension and excessive anticoagulation are associated with intracranial bleeding events while hyperlipidemia is inversely correlated with them.

## OC146 USE OF STATINS AND RECURRENCE OF ATRIAL FIBRILLATION AFTER CATHETER ABLATION OR ELECTRICAL CARDIOVERSION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Dentali F.[(1)], Gianni M.[(2)], Squizzato A.[(1)], Guasti L.[(1)], Castiglioni L.[(1)], Hylek E.[(3)], Grandi A.M.[(1)], Cazzani E.[(2)], Venco A.[(1)], Ageno W.[(1)]
*[(1)] Medicina Clinica, Università dell'Insubria, Varese; [(2)] Cardiologia, Ospedale di Tradate, Tradate; [(3)] Department of Medicine, Research, Boston University, Boston, USA*

**Background** Statins have important pleiotropic effects and have been shown to reduce vascular inflammation. Some evidence suggests that statins may have a role in the primary prevention of atrial fibrillation (AF), whereas little is know on the role of statins in patients with existing AF. We performed a meta-analysis of the literature to assess the effect of statins on the recurrence of AF after electrical cardioversion or ablation.
**Methods** MedLine and Embase databases were searched up to January 2010. Relative risks (RR) and 95% confidence intervals (CIs) were then calculated and pooled using a random-effects model. Statistical heterogeneity was evaluated through the use of I2 statistics.
**Results** Sixteen studies were included in our systematic review. Statins did not reduce the risk of AF recurrence after ablation (4 studies including 750 patients; RR, 1.04; 95% CI, 0.85-1.28, p=0.71; I2=34%). Conversely, the use of statins was associated with a significantly reduced risk of AF recurrence after electrical cardioversion (12 studies including 1,790 patients; RR, 0.78; 95% CI, 0.67-0.90, p=0.0003; I2=34%). This reduction was not statistically significant when the analysis was restricted to randomized controlled trials (RCTs) only (5 studies, 458 patients, RR, 0.76; 95% CI, 0.48-1.20).
**Conclusion** Statins may lower the risk of AF recurrence after electrical cardioversion, but not ablation. However, this finding should be considered with caution, and larger RCTs are warranted to confirm our preliminary results.

## OC147 RISK OF RECURRENT CEREBROVASCULAR EVENTS IN PATIENTS WITH CRYPTOGENIC STROKE OR TRANSIENT ISCHEMIC ATTACK AND PATENT FORAMEN OVALE. THE FORI (FORAMEN OVALE REGISTRO ITALIANO) STUDY

Paciaroni M.[(1)], Ageno W.[(2)], Agnelli G.[(1)], *for the 13 Italian Investigators Centers*
*[(1)] Medicina Vascolare, Università di Perugia, Perugia; [(2)] Medicina Clinica, Università dell'Insubria, Varese*

**Background** The optimal management of patients with cryptogenic stroke found to have a patent foramen ovale (PFO) remains unclear. Aims of this observational multicentre study were to evaluate the risk of recurrent cerebrovascular events in patients with cryptogenic stroke or TIA and PFO who underwent either percutaneous PFO closure or medical treatment; the risk factors associated to recurrent events.
**Methods** Consecutive patients (aged 55 years or less) with PFO and first-ever cryptogenic minor ischemic stroke or TIA were recruited in 13 Italian hospitals between January 2006 and September 2007 and followed-up for two years.
**Results** 238 patients were enrolled (mean age 42.2±10.0 years; 118 males); 117 (49.2%) received antithrombotic therapy while percutaneous PFO closure was performed in 121 (50.8%). Presence of ASA and the evidence of 20 bubbles or more on TCD were significantly associated with PFO closure (p=0.002 and p=0.02). Eight patients (6.6%) experienced a complication during PFO closure. At 2 year follow-up, 17 recurrent events (TIA or stroke) (3.6% per year) were observed. Seven recurrent events (2.9% per year) were observed in the percutaneous PFO closure group while 10 recurrent events (4.2% per year) were observed in the medical treatment group. The risk of recurrent stroke was 0.4% per year in patients who underwent percutaneous closure (1 event) in comparison to 3.4% per year in patients receiving medical treatment (8 events). On multivariate analysis, percutaneous closure was not superior to medical therapy in preventing recurrent TIA or stroke (OR 0.1, 95% CI 0.02-1.5, p=0.1) while it was barely superior to medical therapy in preventing recurrent stroke (OR 0.1, 95% CI 0.0-1.0, p=0.053).
**Conclusions** In this observational study, PFO closure was superior to medical therapy in the prevention of recurrent stroke. Complication during PFO closure was the trade-off for this clinical benefit.

## *NUTRIZIONE E DISMETABOLISMO*

### OC148 **UPDATED META-ANALYSIS ON ADHERENCE TO MEDITERRANEAN DIET AND HEALTH STATUS**

Sofi F.[(1)], Gori A.M.[(1)], Abbate R.[(1)], Gensini G.F.[(1)], Casini A.[(2)]
*[(1)] Area Critica Medico-Chirurgica, Università degli Studi di Firenze, Firenze; [(2)] Agenzia Nutrizione, Università degli Studi di Firenze, Firenze*

**Objective** Recently we have demonstrated that a greater adherence to Mediterranean diet is able to confer a significant protection versus mortality, occurrence of cardiovascular diseases and major chronic degenerative diseases. Aim of this study was to update the evidences reported in our previous meta-analysis with recent studies published in the literature, in order to investigate whether the protective role of Mediterranean diet remained to be statistically significant as the number of studies and subjects increased.
**Methods** We conducted an electronic literature search through MedLine, Embase, Google Scholar, Web of Science, The Cochrane Library, and bibliographies of retrieved articles up to April, 2010. Studies were included if they analysed prospectively the association between adherence to Mediterranean diet and chronic degenerative diseases.
**Results** The updated review process evidenced 7 prospective studies published in the last 2 years and not included in the previous meta-analysis (1 for overall mortality, 3 for cardiovascular incidence and/or mortality, 1 for cancer incidence and/or mortality, and 2 for neurodegenerative diseases). Of note, these recent studies included 2 health outcomes not previously investigated (i.e. mild cognitive impairment and stroke). The meta-analysis for all the studies under a random-effects model, conducted after the inclusion of these recent studies, showed that a 2-point increase of adherence to Mediterranean diet remained to be associated with a significant reduction of overall mortality (RR 0.92, 95% CI 0.90-0.94), cardiovascular incidence and/or mortality (RR 0.90, 95% CI 0.87-0.93), cancer incidence and/or mortality (RR: 0.94, 95% CI 0.92-0.96) as well as neurodegenerative diseases (RR: 0.87, 95% CI 0.81-0.94). Meta-regression analysis showed that sample size is the most significant contributor of the model, by significantly influencing estimate of association for overall mortality.
**Conclusion** This updated meta-analysis confirms, in a higher number of subjects and studies, the significant and consistent protection for adherence to Mediterranean diet versus occurrence of major chronic degenerative diseases.

### OC149 **DELPHINIDIN-3-GLUCOSIDE, AN ANTHOCYANIN FROM WILD BLUEBERRIES, PROTECTS ENDOTHELIAL CELLS BY PROANGIOGENIC AND PROTHROMBOTIC STIMULI**

Bonacina G.[(1)], Marchetti M.[(1)], Vignoli A.[(1)], Diani E.[(1)], Panova-Noeva M.[(1)], Riso P.[(2)], Porrini M.[(2)], Falanga A.[(1)]
*[(1)] Division of Immunohematology and Transfusion Medicine, Ospedali Riuniti di Bergamo, Bergamo; [(2)] Dipartimento di Scienze e Tecnologia Farmaceutica , Università degli Studi di Milano, Milano*

Anthocyanins (ACNs) are natural pigments that provide colours from dark blue to purple to fruit and vegetables, such as edible berries, and belong to the flavonoid class. Since these compounds are ingested regularly with the diet and their intake had been estimated to exceed 200 mg/day, they may have a relevant long-term effect. Studies investigating the health effects of ACNs have observed anti-inflammatory and anticarcinogenic properties. Delphinidin-3-glucoside (Dp-3-g) is one of the ACNs contained in wild blueberries (*Vaccinium angustifolium*) able to inhibit tumour cell proliferation. It is not known whether Dp-3-g can also act on proangiogenic and prothrombotic properties of endothelial cells.
We designed an *in vitro* study to investigate the effect of Dp-3-g on the angiogenic and the procoagulant activities of human microvascular endothelial cells (HMEC-1). HMEC-1 were incubated for up to 24h with culture media Dp-3-g (0.1, 1, 10, 100 microM) alone or in combination with purified proangiogenic factors (VEGF, FGF-2), or bacterial endotoxin (LPS, 10 microg/mL). After incubation, angiogenesis was evaluated by capillary-like tube formation in Matrigel and wound healing assay, while procoagulant activity was tested by the thrombin generation assay (TG).
The results show that Dp-3-g inhibited capillary tube formation, both alone and in combination with proangiogenic factors, reaching significant reduction at 100 microM compared to control cells ($p<0.05$). The same concentration of Dp-3-g significantly reduced the migration of HMEC-1. In the

TG assay, Dp-3-g significantly counteracted the prothrombotic stimulus of LPS starting from 10 μM.
In conclusion, the data show that Dp-3-g can affect angiogenic, migratory and procoagulant properties of endothelial cells. These findings, together with the known capacity of Dp-3-g to affect tumour cell proliferation, make this compound a potential cancer chemopreventive agent. Our results support health promotion strategies that favour coloured fruit and vegetables consumption.

## OC150 **EFFECTS OF GRAPE SKIN EXTRACTS ON TF EXPRESSION AND TF-DEPENDENT INHIBITION OF FIBRINOLYSIS**

Milella R.A.[(1)], Incampo F.[(2)], Crupi P.[(1)], Antonacci D.[(1)], Semeraro N.[(2)], Colucci M.[(2)]
[(1)] *CRA UTV, Turi;* [(2)] *Dept. Biomedical Sciences, Sect. General and Experimental Medicine, Bari*

The antithrombotic effect of wine and some of its constituents has been amply demonstrated. However, little is known about the effect of fresh grape. We investigated the *in vitro* effect of three different grape varieties (Italia b., Palieri n. and Summer Royal n.) on monocyte tissue factor (TF) expression. Grape extracts were prepared by overnight incubation of grape skins in 30% ethanol-1% hydrochloric acid. Total phenolic content was measured by a colorimetric assay using Folin-Ciocalteus phenol reagent and expressed as catechin equivalents (pg/mL). Preincubation (30 min) of human blood mononuclear cells (MNCs) with grape extracts (1-20 g/mL) prior to stimulation with LPS (1 pg/mL) resulted in a concentration-dependent inhibition of TF induction as assessed by functional (clotting) and immunological assays ($p<0.001$). Maximal inhibition amounted to 90% and was observed at concentration of 5 g/mL (Italia b.) to 20 pg/mL (Palieri n.). Grape extracts did not influence TF expression when added 30-60 min after LPS stimulation or just before TF activity determination ruling out a toxic effect or an interference with the clotting assay. Inhibition of LPS-induced TF expression was also observed when adherent monocytes were used instead of MNC suggesting a direct effect of grape extracts on TF-producing cells. Moreover, a plasma clot generated on top of LPS-stimulated adherent monocytes displayed a greater sensitivity (>50%) to t-PA-induced lysis if cells were pre-treated with 5-10 g/mL grape extracts ($p<0.001$). The cell-mediated profibrinolytic activity of grape extract disappeared in the presence of a neutralizing TF antibody or a TAFIa inhibitor (PTCI).
These data indicate that grape skin extracts inhibit LPS-stimulated TF synthesis in monocytes, which in turn may promote fibrinolysis by reducing TAFI activation. The combined effect on TF-dependent fibrin formation and fibrinolysis might represent an important antithrombotic mechanism associated with grape consumption.

## OC151 **ELEVATED SENSITIVITY AND SPECIFICITY OF SOME ANTI HIGH DENSITY LIPOPROTEIN FRACTIONS FOR ATHEROSCLEROSIS RELATED EVENTS**

Ames P.R.J.[(1)], Batuca J.R.[(2)], Ciampa A.[(3)], Brancaccio V.[(4)], Delgado A.J.[(2)]
[(1)] *Haematology/Rheumatology, Airedale DGH, Steeton, UK;* [(2)] *Pharmacology, New University of Lisbon, Lisbon, Portugal;* [(3)] *Coagulation Unit, Moscati Hospital, Avellino;* [(4)] *Ex Coagulation Unit, Ex Ospedale Cardarelli, Naples*

Antibodies against high density lipoprotein (HDL) fractions have been described in systemic lupus erythematosus and antiphospholipid syndrome but not outside the autoimmune setting. In a cross sectional study we investigated antibodies against HDL, apolipoprotein AI (ApoAI), ApoAII , apolipoprotein B (ApoB), apolipoprotein C (ApoC), paraoxonase antigen (PON) in patients with atherosclerosis related events namely ischaemic stroke (IS, n=40, 20 M, 20 F, age 68±12 years) and myocardial infarction (MI, n=40, 20 M, 20 F, age 78±6 years), deep vein thrombosis (DVT, n=26, 9 M, 17 F, age 48±12 years) due to inherited thrombophilia and normal control subjects (CTR, age 67±12 years). IgG anti-HDL (% positive control) was 189±162 in MI, 178±158 in IS, 118±136 in DVT and 89±48 in CTR ($p<0.0001$); IgG anti-ApoAI (mg/L) was 1.18±1.4 in MI, 1.20±1.18 in IS, 0.4±0.26 in DVT and 0.42±0.36 in CTR ($p<0.0001$); IgG anti-PON (% positive control) was 212±183 in MI, 176±148 in IS, 98±104 in DVT and 86±54 in CTR ($p<0.0001$); IgG anti-ApoAII, anti-ApoB, anti-ApoC were not different across groups. The specificity of IgG anti-HDL was 38%, that of IgG anti-ApoI was 84% and that of IgG anti-PON was 75%; sensitivities with regards to IS was 65% for IgG anti-HDL, 45% for IgG anti-ApoAI and 40% for IgG anti-PON; sensitivities with regards to IS was 80% for IgG anti-HDL, 40% for IgG anti-ApoAI and 70% for IgG anti-PON. Given its high specificity and sensitivity IgG anti-PON may represent a novel marker for ischaemic heart disease.

## OC152 **OXIDATIVE STRESS MARKERS AND PLATELET FUNCTION IN PATIENTS WITH LIVER CIRRHOSIS**

Raparelli V.[(1)], Nigro C.[(2)], Pastori D.[(2)], Pignataro F.S.[(2)], Riggio O.[(3)], Merli M.[(1)], Del Ben M.[(2)], Pignatelli P.[(2)], Basili S.[(1)], Violi F.[(2)]
[(1)] *Dipartimento di Clinica e Terapia Medica, Prima Clinica Medica, Roma;* [(2)] *Dipartimento di Medicina Sperimentale prima Clinica Medica Roma;* [(3)] *Dipartimento di Medicina Clinica II Gastroenterologia Roma*

**Background and Aims** *In vitro* and experimental models, it has previously been demonstrated that NADPH oxidase complex play a critical role as mediator of liver fibrosis and chronic liver diseases progression. However, its role in humans is still unclear and data *in vivo* are lacking.
**Methods** To investigate oxidative stress in cirrhotic patients, we performed a cross-sectional study in 50 cirrhotic patients with different degree of liver disease defined as low (class A), moderate (class B), or severe (class C) according to Child-Pugh (CP) criteria. Serum soluble-gp91phox, the catalytic core of NADPH oxidase, urinary excretion of isoprostanes, as markers of oxidative stress as well as sCD40L plasma levels, were measured and compared with control group.
**Results** Compared to controls, cirrhotic patients had higher serum soluble-gp91phox [Median (IQR): 30.8 (7.2-72.8) vs. 8.2 (2.5-52.4) pg/mL, $p<0.001$], urinary isoprostanes [Median (IQR): 311.2 (42.2-899.6) vs. 58.2 (82.5-312.4) pg/mg creatinine, $p<0.001$] and sCD40L (5.63.3 vs. 2.3±0.7 ng/mL, $p<0.0001$).

Patients with higher levels of urinary isoprostanes, sgp91phox and sCD40L were more likely to have moderate or severe than mild liver failure ($p<0.05$).
Serum gp91phox levels as well as urinary isoprostanes excretion significantly correlated with albumin levels ($Rs=-0.32$, $p<0.001$; $Rs=-0.30$, $p<0.001$, respectively), CP score ($Rs=0.47$, $p<0.001$; $Rs=0.43$, $p<0.001$, respectively] and sCD40L ($Rs=0.70$, $p<0.0001$ and $Rs=0.66$, $p<0.001$, respectively).
**Conclusions** In liver cirrhosis sgp91phox is up-regulated and correlated with increase of isoprostanes and sCD40L. This up-regulation is associated with liver damage. Further study is needed to see if such changes are epiphenomenon of liver disease or concur in perpetuating liver damage and/or in haemostatic dysfunction that complicates the clinical course of liver cirrhosis.

## *TEV: PROFILASSI*

### OC153 **LONG-TERM INCIDENCE OF VENOUS THROMBOEMBOLISM IN PATIENTS WITH ACUTE SPINE INJURY: A PROSPECTIVE STUDY**

Giorgi Pierfranceschi M.[(1)], Imberti D.[(2)], Dentali F.[(3)], Marazzi M.[(4)], Bocchi R.[(4)], Lotta S.[(4)], Prisco D.[(5)], Ageno W.[(3)]
*[(1)] Thrombosis and Haemostasis Center, Emergency Departement, Hospital of Piacenza, Piacenza; [(2)] Internal Medicine, University Hospital of Ferrara, Ferrara; [(3)] Internal Medicine, University of Insubria, Varese; [(4)] Spinal Unit, Hospital of Piacenza, Piacenza; [(5)] University of Florence, Thrombosis Center, Firenze*

**Background** Venous thromboembolism (VTE) is a frequent complication in patients with acute spine injury. However, scarce data are available on the duration of the risk of VTE in this clinical setting. Aim of the study: To prospectively evaluate the long term risk of VTE in a cohort of patients with acute spine injury during their rehabilitation and post-rehabilitation follow-up.
**Patients and Methods** Consecutive adult patients with recent spine injury hospitalized for rehabilitation course in the Spinal Unit of Piacenza Hospital. Patients were followed-up every six months after discharge for symptomatic VTE or death. For the outcome VTE we performed survival analysis using Kaplan-Meier and Cox regression statistics.
**Results** From January 2003 to November 2007, 94 patients (male 81, 86%; mean age 40.3 years) were recruited; 40 tetraplegic (42.5%) and 54 paraplegic (47.5%). All the patients underwent thromboprophylaxis during hospitalization (range 1-19 months; mean 5.8 months) with low molecular weight heparin and compressive stoking. Mean duration of follow-up was 34.3 months (range 1-80 months). The cumulative incidence of VTE was 22.3% (21/94). DVT was diagnosed in 20/21 of the cases (95%) and isolated pulmonary embolism (EP) in 1/21 (5%). The majority of VTE events were recorded during the first three months of follow-up (78.2 vs. 11.2 VTE events/1,000 patients/year); the major determinant of VTE was age over 45 years (HR 3.7; 95% CI 1.4-9.2). Sex, severity of disability and hypertone were not related to the development of VTE complications.
**Conclusions** The long term risk of VTE in patients suffering from acute spinal injury is high. In addition, VTE complications are more common during the first three months after trauma and in patients over 45 years old.

### OC154 **NADROPARIN FOR THE PREVENTION OF THROMBOEMBOLIC EVENTS IN AMBULATORY PATIENTS WITH LUNG CANCER RECEIVING CHEMOTHERAPY: A TREATMENT-BASED SUB-GROUP ANALYSIS OF THE PROTECHT STUDY**

Verso M.[(1)], Mandalà M.[(2)], Barni S.[(3)], Cavanna L.[(4)], Gasparini G.[(5)], Gussoni G.[(6)], Labianca R.[(2)], Passalacqua R.[(7)], Perrone T.[(8)], Agnelli G.[(1)]
*[(1)] Medicina Interna e Vascolare- Stroke Unit, Università di Perugia, Perugia; [(2)] Dip. di Oncologia ed Ematologia, Oncologia Medica, Bergamo; [(3)] Oncologia Medica, Treviglio; [(4)] Oncologia ed Ematologia, Oncologia Medica, Piacenza; [(5)] Oncologia, Roma; [(6)] FADOI, Milano; [(7)] Oncologia Medica, Cremona; [(8)] Italfarmaco, Milano*

The PROTECHT study (NCT 00951574) showed that nadroparin was associated with a reduction in thrombotic events (TE) in ambulatory patients with metastatic or locally advanced cancer who were receiving chemotherapy. About 50% of the thromboembolic events occurred in lung cancer patients (n=279, 24.3% of the overall study population). The aim of this exploratory sub-group analysis was to investigate the effect of nadroparin on lung cancer patients included in the PROTECHT study according to the administered chemotherapy regimens.
**Methods** Ambulatory patients were randomly assigned in a double-blind manner to receive subcutaneous injections of nadroparin (3,800 IU anti-Xa once a day) or placebo, in a 2:1 ratio. The primary endpoint was symptomatic TE, as adjudicated by an independent committee. TE incidence was assessed according to the type of chemotherapy regimen (including platinum compounds or its combination with gemcitabine).
**Results** NSCLC and SCLC were 79.9% and 21.6%, respectively. Two of 199 patients in the nadroparin group and no patients in the placebo group had a major bleeding event (two-sided $p=0.368$). The results of this analysis shown that in lung cancer patients receiving regimens containing Platinum compounds the incidence of TE was 2.5% (2/80) in nadroparin group and 7.4% (2/27) in placebo group, (RRR=66%, NNT=20). In lung cancer patients receiving regimens containing Platinum compounds plus gemcitabine the incidence of TE was 2.5% (2/79) and 11.4% (5/44) respectively in nadroparin and placebo group (RRR=78%, NNT=11).
**Conclusion** In this exploratory subgroup analysis, nadroparin significantly reduces the TE risk in lung cancer patients treated with regimens containing platinum compounds and in patients treated with the association platinum plus gemcitabine.

### OC155 **EXTENDED PROPHYLAXIS WITH BEMIPARIN FOR PREVENTION OF LATE VENOUS THROMBO-EMBOLISM AFTER ABDOMINAL OR PELVIC SURGERY FOR CANCER: A DOUBLE-BLIND, RANDOMISED, PLACEBO-CONTROLLED TRIAL**

Prandoni P.[(1)], Kakkar V.V.[(2)], Balibrea J.L.[(3)], Martinez-González J.[(4)],
[(1)] *Scienze Cardiotoraciche e Vascolari, Università di Padova, Padova;* [(2)] *Thrombosis Research Institute, University of London, London, UK;* [(3)] *Surgery Department, Complutense University, Madrid, Spain;* [(4)] *Medical Department 3, Laboratorios Farmaceuticos, Madrid, Spain*

**Background** Low-molecular weight heparins are currently recommended for routine thromboprophylaxis in patients undergoing major cancer surgery, but there is not enough clinical evidence for making a strong recommendation on the optimal duration of such prophylaxis.
**Methods** CANBESURE is a multicenter, randomised, double-blind clinical trial. Patients admitted to undergo an elective, open, abdominal or pelvic surgery for cancer were enrolled. Before randomization, they received once daily subcutaneous injections of Bemiparin 3,500 IU for 8 days, and then randomised to receive either Bemiparin or Placebo for 20 additional days. Bilateral venography was performed after 20 days and blinded evaluated. Patients were followed up to 3 months. The primary efficacy endpoint was the combined incidence of symptomatic and asymptomatic deep vein thrombosis, non-fatal pulmonary embolism and all-cause mortality at the end of double-blind period. Major venous thromboembolism (proximal-vein thrombosis, non-fatal pulmonary embolism and venous thromboembolism-related deaths) was also evaluated. The primary safety endpoint was the incidence of major haemorrhage at the end of double-blind period.
**Findings** 625 were included in the safety and 488 in the main efficacy analyses. The primary efficacy endpoint occurred in 25 of 248 patients (10.1%) in the bemiparin group and 32 of 240 (13.3%) in the placebo group [relative risk reduction 24.4%; 95% CI: –23.7% to 53.8%; p=0.263]. At the end of double-blind period, major venous thromboembolism occurred in 2 (0.8%) and 11 (4.6%) patients respectively [relative risk reduction 82.4%; 95% CI: 21.5% to 96.1%; p=0.010]. No significant differences were found between the two groups in major bleedings.
**Interpretation** Four compared to one week of prophylaxis with bemiparin after abdominal or pelvic surgery for cancer did not significantly reduce the primary efficacy endpoint, but decreased major venous thromboembolisms, without increasing hemorrhagic complications.

## OC156 **THOMBOPROPHYLAXIS FOLLOWING CESAREAN SECTION: A ONE SITE PROSPECTIVE PILOT STUDY TO EVALUATE THE APPLICATION OF A RISK SCORE MODEL**

Cavazza S.[(1)], Rainaldi M.P.[(2)], Bonazzi F.[(2)], Palareti G.[(1)]
[(1)] *Angiologia e Malattie della Coagulazione, Az. Ospedaliero-Universitaria, Bologna;* [(2)] *Anestesia e Rianimazione, Azienda Ospedaliera S. Orsola-Malpighi, Bologna*

**Background** Venous thromboembolism (VTE) remains an important cause of maternal mortality and morbidity. Caesarean section (CS) is a known risk factor for VTE, with an incidence of 0.5% in most recent series. Data from clinical trials of thromboprophylaxis following CS are lacking and current guidelines are based on experts' opinion. A risk score model to determine the need for pharmacological thromboprophylaxis has been established at our institution following a consensus reached among obstetricians, anesthesiologists and vascular doctors. Our aim was to assess the efficacy of this model in preventing CS-related VTE.
**Materials and Methods** Before undergoing CS women received the risk score evaluation, which is based on age, weight, history of VTE, thrombophilia, immobility, parity, varicose vein, and invasive procedure in post-partum. Women with moderate-high risk were given pharmacological prophylaxis (LMWH, 2000 or 4,000 UI od, starting 6-8 h after delivery and at least for 7d); all women wore antithrombotic stockings. They had a visit before discharge and were advised to come back for visit if required. All received a follow-up phone call after three months.
**Results** 501 consecutive women were included in the study; 239 (47.7%), classified as at low risk, had no pharmacological prophylaxis; only one of them developed deep vein thrombosis of a leg on the 16th day following delivery; 262 (52.2%), at moderate-high risk, received LMWH and none of them developed VTE. The incidence of DVT was 0.2% (1/501). Bleeding complications occurred in 2 (0.76%) and in 1 (0.42%) women who received or not LMWH prophylaxis, respectively.
**Conclusions** The risk score model applied in this study proved effective in avoiding pharmacological prophylaxis in almost half of women and safe, since the rate of failure resulted very low (0.2%); furthermore it was easy to apply. A larger study, involving more clinical centers is recommended.

## OC157 **EFFICACIA DELLA CALZA ELASTICA USATA PRECOCEMENTE O DOPO RISOLUZIONE DELLO EDEMA SULLA RICANALIZZAZIONE DOPO TVP**

De Gaudenzi E., Arpaia G., Ciminiello C., Mastrogiacomo O.
*Rep. Medicina Interna - Amb. Angiologia, Ospedale di Domodossola, Domodossola*

**Obiettivo dello studio** Le calze elastiche a compressione graduata sono utili per prevenire la sindrome post trombotica (SPT) dopo una trombosi venosa profonda degli arti inferiori (TVP). Si conosce meno circa la loro efficacia sulla ricanalizzazione dopo la occlusione ed il timing per linizio della compressione questo studio indaga sulla efficacia nel fare indossare le calze subito oppure dopo 2 settimane dalla TVP.
**Metodo** Questo trial randomizzato prospettico ha arruolato 73 pazienti con TVP applicando calze elastiche al momento alla diagnosi oppure 2 settimane dopo. Tutti i pazienti sono stati trattati con EBPM e warfarin secondo i consueti protocolli. Si è misurato dopo 14 e 90 giorni il residuo trombotico mediante ecografia compressiva (CUS) unitamente alla registrazione di reflussi valvolari patologici.
C'è stata una significativamente maggiore ricanalizzazione dei segmenti interessati nel gruppo con elastocompressione precoce.
La ricanalizzazione della TVP poplitea, espressa come riduzione del diametro della vena, come mostrato dal CUS, era migliorata sensibilmente nel gruppo di compressione precoce piuttosto che nel gruppo controllo (giorno 14, 6.5±3 mm vs. 5±2 mm; p=0.035; giorno 90; 3.7±3mm vs. 2.1±1.7 mm; p=0.014).

Il giorno 14 il punteggio medio per la pervietà poplitea era migliorato in modo significativo per i pazienti con compressione precoce (1.0±0.6 vs. 1.5 0.5, P=0.0015)
**Conclusioni** Applicare una calza elastica immediatamente alla diagnosi di DVT era sicuro ed efficace considerando gli end-point dello studio, cioè la ricanalizzazione e le dimensioni dei trombi residui. Un più lungo follow-up su un numero maggiore di pazienti è necessario per verificare il pattern di ricorrenza di DVT e PTS.

## *TROMBOFILIA*

### OC158 **IN KINDREDS WITH INHERITED THROMBOPHILIA THE VENOUS THROMBOTIC RISK OF THE CARRIERS IS DEPENDENT ON THE CLINICAL PHENOTYPE OF THE PROBAND**

Rossi E., Ciminello A., Za T., Betti S., Leone G., De Stefano V.
*Institute of Hematology, Catholic University, Rome*

Laboratory screening for inherited thrombophilia is recommended for patients with venous thromboembolism (VTE) or obstetric complications (OC). Universal screening (US) is discouraged, but a growing number of asymptomatic carriers of thrombophilia is identified by inappropriate laboratory testing. Whether familial screening should be then pursued is debated.
We assessed in kindreds with thrombophilia the thrombotic risk of the carriers according to clinical and laboratory phenotypes of the proband in a retrospective family cohort study on 1,722 relatives (M/F 769/953) of 566 probands diagnosed because of VTE (n=344), OC (n=86), premature arterial thrombosis (PAT, n=31), or US (n=106); 521 probands carried factor V Leiden (FVL) and/or prothrombin G20210A (PTGA), and 45 had antithrombin (AT), protein C (PC) or S (PS) deficiency. The hazard ratio (HR) for deep venous thrombosis (DVT) was estimated comparing the thrombosis-free survival curves of carriers and non-carriers. Thrombophilia was detected in 968 relatives (56.2%); the observation-years were 37,727 for carriers and 29,548 for non-carriers. DVT occurred in 35 carriers and 11 non-carriers (incidence/1000 individual years 0.92 and 0.37, respectively). In carriers the risk for DVT was increased if the proband had VTE (HR 2.40, 95% CI 1.14-4.05), whereas was not if the proband was diagnosed because of OC, PAT, or US. If the probands with VTE had FVL and/or PTGA, the risk for DVT in carriers was marginally increased (HR 1.97, 95% CI 0.89-3.92). If the probands with VTE had AT, PC, or PS deficiency, the HR for DVT was 4.66 (95% CI 0.80-10.21) in the overall carriers, and 12.80 (95% CI 2.46-59.90) in those with AT deficiency.
In conclusion, familial screening for thrombophilia is only justified for probands with history of VTE. In this setting, the risk for DVT is about doubled in carriers of FVL and/or PTGA, and is exceedingly high in those with AT deficiency.

### OC159 **DETECTION OF CIRCULATING ENDOTHELIAL CELLS WITH CD146+ IMMUNO-MAGNETIC ENRICHMENT FOLLOWED BY MULTI-PARAMETER FLOW CYTOMETRY IN ESSENTIAL THROMBOCYTHEMIA (ET) PATIENTS**

Belotti A., Elli E., Speranza T., Lanzi E., Pioltelli P., Pogliani E.M.
*Clinica Ematologica, Ospedale San Gerardo - Università, Monza*

**Introduction** Circulating endothelial cells (CECs) have been studied in cardiovascular disorders and as a marker of angiogenetic activity. Particularly, angiogenesis leads to an increase of endothelial progenitor cells (EPCs). Clinical applications are limited by a lack of consensus on their phenotypic identification. We determined CECs in ET patients (pts), in order to investigate their possible pathogenetic role.
**Methods** CECs are defined in our study as CD146+/CD45– nucleated cells, determined in peripheral blood from 21 healthy controls (median age 47 years) and from 32 ET pts (median age 54 years), performing a combination of preenrichment of CD146+ circulating cells and multiparametric flow cytometry measurement (FCM). CD146+ cells were isolated using CD146-coated magnetic nanoparticles and labelled using CD45-fluorescein isothiocyanate and CD146-PE or isotype control antibody and propidium iodide before FCM. JAK2V617F assessment was performed with ASO-PCR. Mann Whitney test was carried out in order to evaluate differences in CECs levels among ET pts and controls; correlations with clinical variables (age, disease duration, thrombosis, blood count) were evaluated performing linear regression. Data were analysed with GraphPad PRISM 5.
**Results** CECs levels in ET pts were higher respect to controls (median 4,014 CECs/mL vs. 121.3 CECs/mL, $p<0.001$). No differences were found in CECs between JAK2V617 positive (18) and negative (14) pts or in terms of correlations with clinical variables mentioned above. Hydroxyurea treated pts (26) did not display differences in CECs levels respect to pts receiving only disaggregation (6).
**Conclusions** Our data suggest that endothelium in ET is activated; we purpose to confirm this data by assessing other endothelial activation markers, extending our case study in order to better investigate a probable endothelial role in thrombosis in ET and to better identify a possible linkage with JAK2V617F mutation. This observation may therefore reflect a significant role of angiogenesis in chronic myeloproliferative disorders.

### OC160 **YOUNG PATIENTS WITH STROKE, PFO (PATENT FORAMEN OVALE) AND THROMBO-PHILIA**

Lodigiani C.[(1)], Ferrazzi P.[(1)], Librè L.[(1)], Mendolicchio L.[(1)], Mari D.[(2)], Quaglia I.[(1)], Colombo A.[(1)]
[(1)] *Centro Trombosi, IRCCS Ist Clinico Humanitas, Rozzano;* [(2)] *Dipartimento di Scienze Mediche, Univ. di Milano - Unità di Geriatria IRCCS - Ist. Auxologico Italiano, Milano*

In young stroke patients PFO is present in 50%: but the risk for cryptogenic stroke in healthy people with PFO is 0.1%. Unbalanced haemostasis (thrombophilia) could enhance the risk.
**Aim of the Study** To evaluate the prevalence of thrombophilia in patients with PFO (PFO+) and cryptogenic stroke (stroke+)
**Materials and Methods** In 330 consecutive subjects with

cryptogenic stroke 149 (45.15%) were PFO+ and 181 (54.9%) were PFO–.
We performed neurological examination, brain CT and /or MRI scan, extracranial Doppler ultrasonography, ECG, trans thoracic and/or transesophageal echocardiography, standard blood tests and PT, aPTT, fibrinogen, protein C, protein S, antithrombin, APC resistance, homocysteine, LAC, anticardiolipin antibodies, F VIII, test for thrombophilia mutations G1691A Factor V, G20210A Factor II, C677T MTHFR; 433 healthy subjects, matched for age and gender, were control population: 98 were investigated for PFO (43 PFO+, 55 PFO–).
**Results** Stroke is more prevalent in women than in men (F 54.3%, M 42.1%, p= 0.002). Fibrinogen (14.8 vs. 8.9%, p=0.023), homocysteine (fasting: 15.5% vs. 6.0%, p<0.001, post load: 25.7 vs. 5.6%, p<0.001), factor VIII (57.0 vs. 43.0%, p=0.002), LAC (2.7 vs. 0.0%, p=0.002) and anticardiolipin AbIgG (5.6 vs. 0.0%, p<0.001) were higher in patients stroke+ vs. stroke–. The multivariate analysis confirm female gender (OR: 2.38, 95% IC: 1.53-3.68, p<0.001), homocysteine (OR 3.49, 95% IC: 1.92-6.34), FVIII (OR 1.93, 95% IC: 14-3.26) as risk factors. In patients stroke+ and PFO+, APC resistance, homocysteine, MTHFR mutation and LAC have p<0.05. PFO seems to be a risk factor for stroke when right-to-left shunt is >10 microbubbles (34 vs. 45%, p=0.071). In multivariate analysis PFO, age, female gender and APC resistance are significant.
**Conclusions** A hypercoagulable state in association with concomitant risk factors as PFO could contribute to provoke an intracardiac thrombosis with subsequent embolism.

## OC161 HEREDITARY DEFICIENCY OF NATURAL INHIBITORS OF COAGULATION (ANTITROMBIN, PROTEIN C OR PROTEIN S) CONFERS INCREASED RISK OF ARTERIAL THROMBOEMBOLIC EVENTS. RESULTS FROM A PROSPECTIVE FAMILY COHORT STUDY

Tormene D., Ferri V., Facchin M., Carraro S., Noventa F., Simioni P.
*Department of Cardiologic, Thoracic and Vascular Sciences; 2nd Chair of Internal Medicine, University of Padua, Padova*

**Background** Whether hereditary antithrombin (AT), protein C (PC) or protein S (PS) deficiency is associated with arterial thromboembolic events is controversial.
**Methods and Results** The objective of this study was to prospectively assess the incidence of arterial thrombotic events in subjects with a deficiency of natural inhibitors of coagulation. We conducted a prospective cohort study in asymptomatic family members of unselected patients who presented with a venous thromboembolic event and who were found to have a deficiency of antithrombin, protein C, or protein S. All arterial thrombotic events were diagnosed by objective diagnostic tests. A total of 640 consecutive subjects belonging to 86 families with hereditary deficiency of AT, PC or PS with a mean age (at the baseline) of 38 years (range, 15 to 79) in the carrier and in the non carrier group were enrolled in the study. A total of 4,240 and 3,810 patient observation years was obtained respectively in the two groups. Atherosclerosis risk factors were similar in both the two groups. Nineteen arterial thrombotic events occurred in the carrier group (5.6%), compared with seven events in the non carrier group (2.3%) [p=0.07]. The hazard ratio (multivariable analysis) was 4.9 (95% CI 1.5 to 16.3).
**Conclusions** Compared with non-deficient family members, subjects with antithrombin, protein C or protein S deficiency have a higher risk for arterial thrombotic events.

## OC162 INHERITED THROMBOPHILIC ALTERATIONS AND CEREBRAL ISCHAEMIA ASSOCIATED WITH PATENT FORAMEN OVALE

Favaretto E., Sartori M., Legnani C., Cini M., Conti E.
*U.O. Angiologia e Malattie della Coagulazione M. Golinelli, Università di Bologna, Policlinico S. Orsola-Malpighi, Bologna*

**Objectives** To assess the possible association between inherited thrombophilic alterations and patent foramen ovale (PFO)- related cerebral ischaemia.
**Design** A case-control study.
**Methods** We evaluated the presence of G20210A prothrombin (FII) and R506Q FV Leiden mutations in 128 patients with PFO-associated stroke patients (male 47.7%, age 54.2±1.3) and 172 with cryptogenic stroke without PFO (male 48.1%, age 53.2±1.0). As control group, we studied 200 apparently healthy subjects (male 50.0%, age 55.6±0.8).
**Results** The prevalence of FII G20210A FII mutation was significantly higher in patients with stroke versus controls (9.4% vs. 4.1%, p=0.02; OR 2.42 CI 1.08-5.44), but it was similar in patients with PFO-associated stroke and in those without PFO (9.4% vs. 9.3% p=1.0). The prevalence of the FV Leiden mutation was not different in patients with PFO-associated stroke, in those with cryptogenic stroke without PFO, and in controls.
**Conclusion** Our data do not support the assumption that inherited thrombophilic alterations may be a predisposing condition for PFO-related cerebral infarcts.

## *TROMBOSI E TUMORI 2*

## OC163 ENHANCEMENT OF TISSUE FACTOR EXPRESSION IN THE HUMAN BREAST CANCER CELL LINE MCF7 BY LEPTIN: A POSSIBLE ROLE OF TUMOR NECROSIS FACTOR

Napoleone E.[(1)], Cutrone A.[(1)], Latella M.C.[(2)], Cugino D.[(1)], Iacoviello L.[(1)], de Gaetano G.[(1)], Donati M.[(1)], Lorenzet R.[(1)]
*[(1)] Laboratori di Ricerca, Università Cattolica, Campobasso; [(2)] Centro di Medicina Rigenerativa, Università di Modena, Modena*

**Introduction** Obesity and increased adipose tissue mass are associated with enhanced risk of cancer. Leptin, one of the adipokines synthesized by adipose tissue, and whose levels are elevated in obese individuals, is a pleiotropic molecule which inhibits apoptosis, promotes cell proliferation, tumour cell invasion, and angiogenesis. Tissue factor (TF), in addition to its role in blood clotting, is considered a hallmark of cancer progression.
**Aim** Having shown that leptin induces 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 cell line MCF7.

**Materials and Methods** MCF7 cells, grown to confluency, were incubated with the different reagents in various combinations at 37°C in 7.5% $CO_2$. Cells were then disrupted and procoagulant activity was assessed by a one-stage clotting assay. TF and TNF-α antigens were determined by ELISA and TF mRNA levels by real time RT-PCR. Leptin receptor (ObR) was detected by flow-cytometry.

**Results** Leptin increased the constitutive TF expression by MCF7 in a dose-dependent way. The upregulation of TF activity was accompanied by an increase in TF antigen and mRNA levels. The TF increase was prevented by inhibitory anti-leptin or anti-leptin receptor antibodies, suggesting that the effect of leptin was specific and that binding of leptin to its receptor, whose presence was detected on the cell membrane, was responsible for TF enhancement. Experiments with specific inhibitors of MAPK signalling revealed the involvement of ERK1/2 kinase, but not p38 kinase, pathway.

In addition, leptin induced TNF-α mRNA synthesis and TNF-α secretion from MCF7. An anti-TNF-α MoAb completely abolished the leptin-induced TF expression.

**Conclusions** These data support the hypothesis that leptin, by its upregulation of TF, may contribute to processes underlying both cancer and vascular cell disorders.

## OC164 **IMPAIRED FUNCTIONALITY OF THE PROTEIN C ANTICOAGULANT PATHWAY IS ASSOCIATED WITH INCREASED TUMOR NECROSIS FACTOR-ALPHA LEVELS IN PATIENTS WITH METASTATIC COLORECTAL CANCER**

Riondino S.[(1)], Roselli M.[(2)], La Farina F.[(1)], Portarena I.[(2)], Grenga I.[(2)], Laudisi A.[(2)], Martini F.[(1)], Guadagni F.[(1)], Ferroni P.[(1)]

*[(1)] Department of Laboratory Medicine, IRCCS San Raffaele Pisana, Roma; [(2)] Medical Oncology, Department of Internal Medicine, Tor Vergata Clinical Center, Roma*

Tumor cells and/or tumor-associated macrophages may produce inflammatory cytokines, such as TNF-α. Such release is actively involved in the induction of a pro-thrombotic status, possibly due to impaired conversion of protein C to APC. Thus, this study was designed to investigate the possible association between TNF-α and abnormalities in the APC system as a cause of thromboembolic events (VTE) in cancer patients.

To this purpose, TNF-α (by immunoassay) and ThromboPath IL (a novel assay specifically designed to globally evaluate the functionality of the PC anticoagulant pathway) were assessed in 45 metastatic CRC patients (mCRC) undergoing chemotherapy and 45 age and sex-matched controls. VTE events were recorded during follow-up.

TNF-α levels were increased [3.2 pg/mL (1.9-6.6)] and ThromboPath was decreased [74 PICI% (71-80)] in mCRC patients compared to controls (both $p<0.001$). No PC deficiency was found in mCRC. An inverse correlation was observed between TNF-α and ThromboPath values in mCRC (Rho=0.336, p=0.02). Multivariate regression analysis including functionality of the PC anticoagulant pathway as the dependent variable and age, sex, ECOG, platelet counts, hemoglobin, BMI, concurrent treatments and TNF-α levels as the predictor variables showed that an increase of TNF-α [β=–0.332, p=0.029] was the only independent predictor for ThromboPath abnormalities during chemotherapy. Nine mCRC patients experienced VTE during chemotherapy. Survival analysis of patients stratified on the basis of pre-treatment serum TNF-α levels demonstrated a worst cumulative event-free survival (56%) of mCRC patients in the upper (>6.6 pg/mL) compared to those in the lower quartiles (86%, Log-rank 2.3, p=0.02).

The results obtained suggest that the host inflammatory response to cancer cells and/or tumor-derived TNF-α could be responsible for an impairment of the APC system. Low APC levels might explain the VTE episodes experienced by mCRC patients undergoing chemotherapy.

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## OC165 **LONG TERM CLINICAL OUTCOMES OF CANCER ASSOCIATED VENOUS THROMBOEMBOLISM: FINDINGS FROM THE MASTER REGISTRY**

Imberti D.[(1)], Agnelli G.[(2)], Ageno W.[(3)], Moia M.[(4)], Palareti G.[(5)], Pistelli R.[(6)], Verso M.[(2)]

*[(1)] Internal Medicine, University Hospital, Ferrara; [(2)] University Division of Internal and Cardiovascular Medicine, Perugia; [(3)] Department of Internal Medicine, University of Insubria, Varese; [(4)] Ca' Granda Fondazione IRCCS, Milano; [(5)] University Angiology Unit, Bologna; [(6)] Pneumology, Catholic University, Roma*

**Background** The clinical characteristics of venous thromboembolism (VTE) have been reported to be different in cancer and in non cancer patients included in randomised clinical trials. However, sparse information is available on the long-term clinical outcomes of VTE in a large cohort of unselected cancer patients.

**Aim** To prospectively evaluate whether long-term clinical outcomes of VTE are different in cancer and non cancer patients.

**Methods** MASTER is a multicenter registry of consecutively recruited patients with symptomatic, objectively confirmed, acute VTE. Patients were followed-up at 6, 12 and 24 months for many major clinical outcomes.

**Results** 2,119 patients with acute VTE were enrolled in the registry. For the aim of this study, we restricted the analysis to the 1,883 patients followed with at least one visit at 6, 12 or 24 months; 343 patients (19.3%) had cancer. The excess of risk for death or recurrence of VTE were estimated in a Cox model and expressed as Hazard Ratio (HR) between patients affected and not affected by cancer. The risk of death (HR=6.84; 95 CI% 4.32-10.83; $p<0.0001$) and recurrence of VTE (HR=1.62; 95 CI% 0.98-2.68; p=0.056) was higher in cancer patients. We analysed the cumulative incidence of the following major outcomes as Odds Ratio (OR) between patients with cancer and without cancer: post-thrombotic syndrome (25.3% versus 5.9%; OR: 5.39; 95 CI% 3.92-7.41; $p<0.0001$), acute myocardial infarction (1.7% versus 0.5%;OR: 3.17; 95 CI% 1.09-9.21; p=0.02), ischemic stroke (0.6% versus 0.4%; OR: 1.39; 95 CI% 0.28-6.95; p=0.68), any bleeding (5.5% versus 1.8%; OR: 3.1; 95 CI% 1.72-5.58; $p<0.0001$), inferior vena cava (IVC) filter implantation (7.2% versus 4.3%; OR: 1.69; 95 CI% 1.06-2.71; p=0.02).

**Conclusions** During the two year follow-up, the risk of death and recurrence of VTE was higher in patients with cancer than

in patients without cancer. Post-thrombotic syndrome, myocardial infarction, and bleeding were more common in cancer patients.

OC166 **THE RISK OF CANCER AFTER THE FIRST EPISODE OF VENOUS THROMBOEMBOLISM DOES NOT EXCEED THAT EXPECTED IN THE GENERAL POPULATION AFTER THE FIRST SIX MONTHS**

Prandoni P.[(1)], Casiglia E.[(2)], Piccioli A.[(3)], Ghirarduzzi A.[(4)], Pengo V.[(1)], Gu C.[(5)], Douketis J.D.[(6)]
*[(1)] Scienze Cardiotoraciche e Vascolari, Università di Padova, Padova; [(2)] Medicina Clinica e Sperimentale, Università di Padova, Padova; [(3)] Scienze Mediche e Chirurgiche, Università di Padova, Padova; [(4)] Unità di Angiologia Ospedale di Reggio Emilia Reggio Emilia; [(5)] Clinical Epidemiology and Biostatistics, Mc Master University, Hamilton, Canada; [(6)] Department of Medicine, McMaster University, Hamilton, Canada*

Venous thromboembolism (VTE) is a well-known marker of occult malignancy. Indeed, during the initial six months following a thrombotic episode a new cancer is diagnosed in up to 10% of patients, especially in those with idiopathic VTE. To determine whether and to what extent this rate exceeds that expected in the general population beyond this time period, we assessed the risk of subsequent overt malignancy in 1,495 patients who had remained cancer free during the initial six months after the thrombotic episode, and in 1,495 age- and sex-matched control subjects derived from the general population of three regions in Northern Italy.
For both study patients and controls the mean age was 60.0±16.8 years, and 46% were males. In 825 (55%) patients with VTE, the episode was considered idiopathic. All patients and controls had follow-up for 30 months, which began at the 6-month point after VTE. New objectively-confirmed cancer developed in 48 patients with VTE and in 43 control subjects, resulting in a cumulative incidence of 3.2% (95% CI, 2.3 to 4.4) and 2.9% (95% CI, 2.0 to 4.0), respectively. The HR for new cancer in patients with VTE as compared to controls was 1.09 (95% CI, 0.59-1.34). When the analysis was confined to the 825 patients with idiopathic VTE and their controls, the HR for cancer was 1.12 (95% CI, 0.61 - 1.36).
We conclude that in patients with a first VTE, including those with idiopathic thrombosis, the risk for subsequent cancer beyond the initial six months does not appear to exceed that expected in the general population.

OC167 **PROTHROMBOTIC STATE IN GLIOBLASTOMA MULTIFORME: EVALUATION OF CIRCULATING MICROPARTICLES**

Sartori M.T.[(1)], Ballin A.[(1)], Della Puppa A.[(2)], Saggiorato G.[(1)], Radu C.[(1)], Campello E.[(1)], De Bon E.[(1)], Simioni P.[(1)]
*[(1)] Department of Cardiologic, Thoracic and Vascular Science Second Chair of Internal Medicine Padua; [(2)] Neurosurgery, University Hospital of Padua, Padua*

**Background** The relationship between venous thromboembolism (VTE) and cancer is supported by several pathogenetic factors, including circulating microparticles (MPs) of different cell origin and/or tissue factor-bearing. Since VTE often complicates the clinical course of patients with glioblastoma multiforme (GBM; WHO grade IV), in the present prospective study we aimed to evaluate microparticles procoagulant activity (MP-activity) and circulating levels of glial, endothelial and tissue factor-bearing MPs in GBM patients.
**Methods** We enrolled 61 GBM patients aged 56.7±12.4 yrs, who underwent neurosurgery with total (>90%) or partial (≤90%) mass resection followed by combined radio-chemotherapy; 20 healthy volunteers were tested as controls. Blood samples for MP-activity (ELISA) and haemostatic profile were collected before and one week after surgery, then the first, fourth and seventh month of follow-up. Glial (GFAP positive) and endothelial (CD146 and E-selectin positive) derived MPs and tissue factor-bearing MPs were assayed using flow cytometry.
**Results** A significant increase in tissue factor-bearing MPs, in glial derived (GFAP positive) and endothelial derived (both CD146 and E-selectin positive) MPs was observed in GBM patients as compared to controls. A significant increase in MP-activity levels before and seven days after surgery was found in 34/61 (56%) patients. The first and the fourth month controls demonstrated that MP-activity levels had significantly been reduced as compared to presurgical levels (p=0.007 and p=0.018, respectively), but this decrease was confirmed only in the subgroup who received complete mass surgical resection. Among the eleven patients who developed VTE during hospital admission, MP-activity was increased in 7 cases (64%).
**Conclusions** GBM patients may have an increase in MPs-associated procoagulant activity, which might contribute to VTE complications and which seems to regress during the temporary remission of the disease. Flow cytometry analysis showed a different origin of circulating MPs, including glial MPs which were likely released by the neoplasm.

# *POSTER*

## ATEROTROMBOSI: EPIDEMIOLOGIA E FATTORI DI RISCHIO

### P001 PHYSICAL ACTIVITY AND RISK OF COGNITIVE DECLINE: A META-ANALYSIS OF PROSPECTIVE STUDIES

Sofi F.[(1)], Valecchi D.[(2)] Bacci D.[(2)], Gori A.M.[(1)], Abbate R.[(1)], Gensini G.F.[(1)], Macchi C.[(2)]
[(1)] *Area Critica Medico-Chirurgica, Università degli Studi di Firenze, Firenze;* [(2)] *Onlus IRCCS Don Carlo Gnocchi, Firenze*

**Objective** The relationship between physical activity and cognitive function is intriguing but controversial. We performed a systematic review with meta-analysis of all the available prospective studies that investigated the association between physical activity and risk of cognitive decline in nondemented subjects.
**Methods** We conducted an electronic literature search through MedLine, Embase, Google Scholar, Web of Science, The Cochrane Library, and bibliographies of retrieved articles up to January, 2010. Studies were included if they analysed prospectively the association between physical activity and cognitive decline in nondemented subjects.
**Results** After the review process 15 prospective studies (12 cohorts of patients) were included in the final analysis. These studies included 33,816 nondemented subjects followed for a time ranging from 1 to 12 years. A total of 3,210 patients manifested a cognitive decline during the follow-up. The cumulative analysis for all the studies under a random-effects model showed that subjects who performed a high level of physical activity were significantly protected (–38%) versus cognitive decline during the follow-up (HR 0.62, 95% CI 0.54-0.70; p<0.00001). Furthermore, even analysing the low-to-moderate level a significant protection (–35%) versus cognitive impairment was also observed (HR 0.65, 95% CI 0.57-0.75; p<0.00001).
**Conclusion** This is the first meta-analysis that attempted to evaluate the role of physical activity on cognitive decline among nondemented subjects. The present results suggest a significant and consistent protection for every levels of physical activity versus the occurrence of cognitive decline.

### P002 PULMONARY FUNCTION AND METABOLIC SYNDROME: RESULTS FROM THE MOLI-SANI PROJECT

Arcari A.[(1)], Bracone F.[(1)], de Curtis A.[(1)], Costanzo S.[(1)]; Di Castelnuovo A.[(1)], Schünemann H.[(2)], Donati M.B.[(1)], de Gaetano G.[(1)], Iacoviello L.[(1)]
[(1)] *Research Laboratories, John Paul II Centre for High Technology Research and Education in Biomedical Sciences, Campobasso;* [(2)] *Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, Canada*

**Introduction** The present study aimed at investigating the relation between subclinical pulmonary dysfunction and metabolic syndrome in apparently healthy subjects recruited in the Moli-sani project.
**Methods** The Moli-sani Project is an on-going cohort study of subjects aged ≥35 years, randomly recruited in Molise. MS was defined according to NCEP ATP-III. Spirometric tests were performed according to ATS/ERS recommendation. Subjects with CRP levels ≥10 mg/L, history of cardiovascular or pulmonary disease or malignancy were excluded.
Out of 11,379 subjects with HQ Flow/volume manoeuvre [Forced vital capacity (FVC), Forced Expiratory Volume in the first second (FEV1) and FEV1/FVC ratio], 7,071 subjects had HQ Plethysmography [Total lung capacity (TLC), Vital capacity (VC), Functional residual capacity (FRC) and residual volume (RV)] and 3,043 HQ DLCO [lung diffusion of CO (DLCO) adjusted for blood Haemoglobin and Alveolar volume (VA)].
About 25% of HQ populations suffered from MS.
All lung parameters (measured either in L or as normal percent predicted) were associated with MS (p<0.01) in both gender, except FEV1/FVC, RV and DLCO in women. After multivariate analyses (adjusted by age, height, smoking habits, BMI, physical activity and social status) all lung parameters, except RV, remained associated with MS, in men; while FVC, FEV1, FEV1/FVC TLC, VC, FRC remained associated in women. Lung volumes were all lower in MS carriers (–120 mL in men, –58 mL in women, on average). In men all the associations were also independent from CRP and white blood cells, except for DLCO. In women, adjustment for inflammatory factors eliminated the association between FVC, FEV1, VC and MS, but not those for TLC and FRC.
**Conclusions** About a quarter of healthy Moli-sani suffer from MS and present a reduction in lung volumes. A possible effect of inflammation was more evident in women. However, both TLC and FRC remained associated with MS in both gender.

### P003 PULMONARY FUNCTION AND CARDIOVASCULAR RISK PREDICTION: RESULTS FROM THE MOLI-SANI PROJECT

Arcari A.[(1)], Barbato D.[(1)], Magnacca S.[(1)], Di Castelnuovo A.[(1)], Zito F.[(1)], Schünemann H.[(2)] Donati M.B.[(1)], de Gaetano G.[(1)], Iacoviello L.[(1)]
[(1)] *Research Laboratories, John Paul II Centre for High Technology Research and Education in Biomedical Sciences, Campobasso;* [(2)] *Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, Canada*

**Introduction** We studied the association of subclinical pulmonary dysfunction and predicted risk of cardiovascular disease (CVD) in 10 years in subjects from the Moli-sani project.
**Methods** The Moli-sani Project is an on-going cohort study of subjects aged ≥35 years, randomly recruited in Molise. Cardiovascular risk in ten years was estimated by the CUORE score.
Spirometric tests were performed according to ATS/ERS recommendation. Subjects with CRP levels ≥10 mg/L, history of cardiovascular or pulmonary disease or malignancy or with low quality pulmonary tests were excluded. Out of 11,379 subjects with HQ Flow/volume manoeuvre [Forced vital capacity (FVC), Forced Expiratory Volume in the first second (FEV1) and FEV1/FVC], 7,071 subjects had HQ Plethysmography [Total lung capacity (TLC), Vital capacity (VC), Functional residual capacity (FRC) and residual volume (RV)] and 3,043 HQ DLCO [lung diffusion of CO (DLCO) adjusted for blood Haemoglobin and Alveolar volume (VA)].
**Results** The three HQ groups were comparable for all the characteristics, included CUORE risk score: 40% of men and 9% of women had a medium risk.

All lung parameters expressed either in Litres or as normal percent predicted were associated with CUORE score (p<0.0001) in both gender. After multivariate analyses (adjusted by age, height, smoking habits, BMI, physical activity and social status) FVC, FEV1, TLC, VC and VA remained inversely associated with CUORE score both in men and women, while FRC appeared to be associated in women only. Lung volumes were all reduced at increasing CUORE risk score.
Further adjustment for CRP and white blood cells did not change any association, although both parameters were independently associated with pulmonary function and CUORE risk score.
**Conclusion** In apparently healthy subjects, a subclinical reduction of lung function is associated with an increased cardiovascular risk. These results suggest that an early pulmonary monitoring could be important in CVD risk.

P004 **CORONARY ARTERY DISEASE AND-OR CEREBRAL NON-FATAL ISCHEMIC STROKE FOLLOWING RETINAL VEIN OCCLUSION: AN 8-YRS FOLLOW-UP**

Di Capua M. [(1)], Coppola A. [(1)], Cimino E. [(1)], Di Minno M.N.D.[(1)], Russolillo A.[(1)], Loffredo M.[(1)], Cuccaro C.[(2)] Cerbone A.M.[(1)], Di Minno G.[(1)]
[(1)] *Department of Clinical and Experimental Medicine, Federico II University, Naples;* [(2)] *Department of Ophthalmology, Federico II University, Naples*

Forty-five consecutive subjects (26 M, 19 F; mean age 54±14) with a diagnosed retinal vein occlusion (RVO), were followed-up for 8 yrs. As many as 145 sex-age- and blood pressure-matched individuals (78 M, 67 F; mean age 54.4±13.5), that did not experience any vascular event, served as controls. At the time of the RVO, controls and subjects did not differ as to hypercholesterolemia, hypertrigliceridemia, diabetes mellitus, smoking habits, inherited/acquired thrombophilia. At the follow-up completion, they differed as to statin consumption (p=0.016). During the 8-yrs follow-up, in the control population, 11 out of 145 (7.6%) subjects had experienced a major vascular event (8 coronary artery disease; 3 cerebral non-fatal ischemic strokes). In contrast, of the 45 subjects with a history of RVO, as many as 10 (22.2%) had experienced a major vascular event: 4 coronary artery disease; 4 a cerebral non-fatal ischemic stroke; 2 a cardiovascular + cerebrovascular event (p=0.012). A prolonged anti-platelet treatment, prior to the major vascular event, was found in 5/45 cases (11.1%) vs. 23/145 (15.9%) controls (p=0,63). In contrast, a long-lasting administration of antihypertensive drugs, to achieve a control of blood pressure, was found in 83.4% of controls and only in 46.7% of cases (p<0.0001).
In conclusion, in an 8-yr follow up, coronary artery disease and/or non-fatal ischemic stroke were more common in subjects with a history of RVO than in a large setting of subjects comparable for cardiovascular risk factors. These data also argue for RVO as a vascular disease in which aggressive antihypertensive therapy to prevent stroke and/or myocardial infarction is needed.

P005 **T- WAVE AXIS DEVIATION IS ASSOCIATED WITH METABOLIC SYNDROME IN A HEALTHY ITALIAN POPULATION: RESULTS FROM THE MOLI-SANI PROJECT**

Rago L.[(1)], Assanelli D.[(2)], Di Castelnuovo A.[(1)], Gianfagna F.[(1)], Salvetti M.[(2)], Zito F.[(1)], Donati M.B.[(1)], de Gaetano G.[(1)], Iacoviello L.[(1),] on behalf of Moli-sani Investigators
[(1)] *Laboratori di Ricerca, Università Cattolica del Sacro Cuore, Campobasso;* [(2)] *Dipartimento di Medicina Interna, Università di Brescia, Brescia*

**Background** T-axis deviation is a marker of ventricular repolarization, and can help in identifying subjects at risk for arrhythmias and/or major cardiac events. We aimed at investigating the association between ECG T-wave axis deviation and metabolic syndrome (MS), in the Italian population.
**Methods** The Moli-sani Project is a cohort study (N=23,806) in men and women, aged ≥35 years, randomly recruited from subjects living in Molise. After excluding subjects with incomplete ECG data, or history of CVD or cancer, 10,655 women (mean age 55±12) and 9,472 men (mean age 56±12) were analysed. MS was defined using the ATPIII criteria. T-wave axis deviation was measured from the standard 12-lead resting electrocardiogram. ECG was measured using a Cardiettear2100-view electrocardiograph storing ECG in SCP format.
**Results** T-wave was categorized in normal (15 to 75 degree; prevalence: 75.1% in women and 67.7% in men), borderline (–15 to 15; 23.4% and 30.5%) and abnormal (–180 to –15 or 75 to 180; 1.5% and 1.8%). Age, hypertension, smoking, left ventricular hypertrophy and MS increased from normal to abnormal categories, in multivariate analysis, both in men and women. In women, MS was prevalent in 21%, 35% and 39% from normal to abnormal categories, P<0.0001. In men, MS was prevalent in 25%, 33% and 38% from normal to abnormal categories, P<0.0001. Among components of MS, elevated waist, blood pressure and glucose levels were strongly associated with T-wave axis deviation, whereas HDL and triglycerides only marginally increased.
**Conclusion** Our results show that abnormal T-wave axis deviation is strongly associated with MS, in a large adult Italian population. Our findings suggest that ECG monitoring for people with MS can help to early identify T-wave axis deviation to reduce the risk of major cardiac events.

P006 **ASSOCIATION OF SOCIAL STATUS INDICATORS WITH METABOLIC SYNDROME AND INDIVIDUAL GLOBAL CARDIOVASCULAR RISK: FINDINGS FROM THE MOLI-SANI PROJECT**

De Lucia F., Di Castelnuovo A., Costanzo S., Bonaccio M.L., Barisciano P., Sciarretta A., Bonanni A., Donati M.B., de Gaetano G., Iacoviello L.
*Laboratori di Ricerca, Università Cattolica del Sacro Cuore, Campobasso*

**Background** Indicators of social status (SES) are reportedly linked to cardiovascular disease (CVD). We evaluated the association of indicators of social and marital status with metabolic syndrome (MS) and cardiovascular risk, in the apparently healthy population of the MOLI-SANI project.
**Methods** The Moli-sani Project is a cohort study in men and

women (N=23,806), aged ≥35 years, randomly recruited in Molise. After the exclusion of subjects with incomplete data or history of CVD or cancer, 9,855 women (mean age 55±12) and 8,822 men (50±12) were analysed. MS was defined using the ATPIII criteria. Global cardiovascular risk was evaluated using the Progetto-CUORE equation. A score of SES was obtained from people room density, dwelling ownership, education level and in-home hot-water availability during childhood. In addition, profession and marital status (married/live in partner or separated/divorced or single) were considered.
**Results** Prevalence of MS increased from high to low quartiles of SES score (17.4%, 23.4%, 28.8%, 31.0%, P<0.0001 in multivariate analysis adjusted for age and sex). MS prevalence was 18.0%, 23.1% and 27.4% (P<0.0001) in non-manual work, manual work and housewife categories, and 24.9%, 18.8% and 17.4% (P<0.0001) in married, separated and singles, respectively. Global individual CVD risk increased from low to high quartiles of SES score (5.6±6.3 and 3.3±4.7, P<0.0001 in multivariate analysis, first vs. fourth quartile), in manual vs. non manual work (4.1±4.4 and 3.5±4.5, P<0.0001), and in married vs. separated vs. single (4.7±5.8, 3.6±4.7, 3.0±4.2, P<0.0001). C-reactive Protein (CRP) increased from low to high quartiles of SES score (2.2±2.0 and 1.7±1.7, P<0.0001), and in married vs. separated vs. single (2.0±1.8, 1.8±1.8, 1.7±1.7, P<0.0001).
**Conclusion** Indicators of poor social status are associated with higher MS prevalence, and higher global individual CVD risk and CRP. The association between being married and both MS and global CVD strongly suggests family-based public health interventions.

## P007 POOR MUSCLE STRENGTH AND THE RISK OF SYMPTOMATIC ARTERIAL ISCHEMIC EVENTS IN THE ELDERLY: THE INCHIANTI STUDY

Di Nisio M., Di Iorio A., Porreca E., Abate M., Ferrante N.
*Department of Medicine and Aging, "University G. D'Annunzio", Chieti*

**Background** Studies have suggested that low muscle strength, which often occurs with ageing, is associated with an unfavourable cardiovascular risk profile. The aim of the present study was to establish the relationship between measures of muscle strength including hand grip, strength of knee extensors, and muscle power, and the 6-year incidence of first symptomatic arterial ischemic events and cardiovascular mortality in a prospective cohort of individuals 65 years or older.
**Methods** The study population included 748 participants enrolled in the InCHIANTI (Invecchiare in Chianti, aging in the Chianti area) study.
**Results** Overall, there were 178 (24%) fatal and non-fatal ischemic events. Hand grip and muscle power, but not strength of knee extensor, were linearly associated with the risk to develop the study outcomes. After adjustment for age and cardiovascular risk factors, muscle power remained the only significant predictor of ischemic events. For every 10-watt reduction in muscle power the risk of cardiovascular mortality, non-fatal ischemic events, or both increased by 14% (RR 1.14; 95% CI: 1.07-1.21), 4% (RR 1.04; 95% CI: 1.01-1.08), and by 6% (RR 1.06; 95% CI: 1.03-1.09) respectively. Participants with muscle strength remaining in or worsening towards the lowest tertile during follow-up experienced a significant increase in risk compared to those who remained or improved to the second-third upper tertiles of strength (RR 6.28; 95% CI 1.80-39.76). The association between muscle power and ischemic events was partially explained by increased plasmatic levels of interleukin-6.
**Conclusions** Poor muscle strength is a strong risk factor for cardiovascular mortality and arterial ischemic events in elderly individuals. Changes in muscle power are accompanied by significant inverse variations in the risk of events.

## P008 ENDOPAT 2000: NON-INVASIVE ASSESSMENT OF ENDOTHELIAL FUNCTION IN RENAL TRANSPLANT RECIPIENTS

Cioni G.[(1)], Fatini C.[(1)], Sticchi E.[(1)], Cesari F. [(1)], Gori A.M.[(1)], Bertone E.[(2)], Zanazzi M.[(2)], Salvadori M.[(1)], Abbate R.[(1)], Gensini G.F.[(1)]
[(1)] *Medical and Surgical Critical Thrombosis Centre, Florence;* [(2)] *Dipartimento delle Biomedicine, Unità di Nefrologia e Dialisi, Florence*

**Background** Endothelial dysfunction, which is associated with an increased risk of cardiovascular events, may be assessed through a non-invasive technology [peripheral arterial tonometry (PAT) device]. We test this technology in stratifying cardiovascular risk in renal transplant recipients (RTRs) who are at high risk of cardiovascular complications.
**Methods** We used EndoPAT 2000 (Itamar Medical LTD Caesarea, Israel), based on measurement of pulse wave amplitude (PWA) in the fingertip at rest and following induction of reactive hyperemia, in order to evaluate the Reactive Hyperemia Index (RHI). Higher RHI values are representative of a better hyperemic response. We investigated 109 RTRs (75 men, median age 51 yrs and 34 women, median age 54 yrs), at least 12 months post-transplant with stable and normal renal function, under pharmacological control of traditional risk factors and under immunosuppressive treatment.
**Results** RHI mean value was higher in females (2.32±0.73) than in males (2.05±0.53), whereas no difference was observed according to age. In hypertensives a lower RHI mean value in comparison to that observed in non-hypertensive subjects was found (2.10±0.60 vs. 2.32±0.59, p=0.07); RHI values were lower, even not significantly, in diabetics (1.98±0.52), smokers (2.06±0.62) and subjects with a BMI>25 kg/m$^2$ (2.06±0.60), whereas no difference in RHI values according to the presence or absence of dyslipidemia was observed. RHI mean value was progressively lower according to the absence or presence of one or more traditional risk factors (none: 2.53±0.44; one or two risk factors: 2.180.56; more than two risk factors: 2.07±0.66, p=0.07).
**Conclusions** The use of peripheral arterial tonometry may represent a non-invasive test contributing to better characterize the risk profile in RTRs, and may permit an accurate clinical assessment.

## P009 RHEOLOGICAL PARAMETERS IN ELDERLY: RESULTS FROM THE INCHIANTI STUDY

Gori A.M.[(1)], Sofi F.[(1)], Alessandrello Liotta A.[(2)], Ricci I.[(1)], Molino Lova R.[(3)], Bandinelli S. [(4)], Ferrucci L.[(5)], Macchi C.[(3)], Gensini G.F.[(1)], Mannini L. [(2)]
[(1)] *Medical and Surgical Critical Care, University of Florence,*

*Florence; [2] Heart and Vessels, Atherothrombotic Disease Unit, Azienda Ospedaliero Universitaria Careggi, Florence; [3] Centro S. Maria agli Ulivi, Don Gnocchi Foundation, Pozzolatico; [4] Geriatric Unit, Azienda Sanitaria di Firenze, Florence; [5] Longitudinal Studies Section, Clinical Research Branch National Institute on Aging, Bethesda, MD, USA*

Advancing age is an important risk factor for cardiovascular disease among men and women. Hyperviscosity is caused by alterations of blood cells, mainly red blood cells, and plasma components (mainly fibrinogen) and it is associated with increased risk of cardiovascular diseases. Several factors are able to influence whole blood and plasma viscosity. Scarce data are available about the relation between plasma, whole blood viscosity and advanced age. We used data from the 6-year follow-up of the InChianti Study (n=948) to determine the relation between advanced age and blood rheology parameters. In the InChianti Study whole blood viscosity (WBV) was measured at 37°C by using a Rotational Viscosimeter at share rate of 0.512 second-1, plasma viscosity (PLV) at shear rate of 20.40 second-1. Whole blood viscosity values adjusted for a haematocrit of 44% in men and of 40% in women are termed HCT-corrected WBV.
WBV and HCT-corrected WBV were significantly related with age, body mass index, lipid parameters, systolic and diastolic blood pressure, physical activity levels and cigarette pack-years. PLV values significantly correlated with fibrinogen levels, but no correlation with age was observed. WBV and hematocrit values were lower in the highest age quartile with respect to lowest quartile, whereas HCT-corrected WBV were higher in the highest age quartile with respect to lower quartiles. General linear model adjusted for sex, cardiovascular risk factors and lipid parameters demonstrated that age is a predictor of WBV and hematocrit.
In conclusion, our study provide further insight into the complex interaction between rheologic parameters, cardiovascular risk factors and age, suggesting that advanced age, in addition to several cardiovascular risk factor, is a significant determinant of WBV.

P010 **TELEVISION WATCHING, BUT NOT INTERNET SURFING, IS ASSOCIATED WITH METABOLIC SYNDROME IN A HEALTHY ADULT ITALIAN POPULATION: RESULTS FROM THE MOLI-SANI STUDY**

Bonaccio M. [1], Di Castelnuovo A.[1], Costanzo S.[1], De Lucia F.[1], Olivieri M.[1], Donati M.B.[2], de Gaetano G.[2], Iacoviello L.[1], Bonanni A.[1]
*[1] Laboratory of Genetic and Environmental Epidemiology, John Paul II Centre for High Technology Research and Education in Biomedical Sciences Research Laboratories, Campobasso; [2] Research Laboratories, John Paul II Centre for High Technology Research and Education in Biomedical Sciences Research Laboratories, Campobasso*

**Aim of the study** We sought to investigate the association between television watching or Internet surfing and metabolic syndrome (MetS) in a healthy adult Italian population.
**Methods** A specific questionnaire on media exposure was elaborated, validated and administered to a sub-sample of the Moli-sani Project cohort (men and women, aged ≥35 years, randomly recruited from subjects included in the city-hall registries of Molise). Subjects with history of cancer or cardiovascular disease were excluded. Altogether 704 subjects completed the self-administered questionnaire (mean age 54±10, 50% men). MetS was defined according to ATP III criteria. A specific TV-exposure score was created considering time spent watching TV daily (two categories, less or more than 3 hrs/day). Surfing the Internet was scored as users and non-users.
**Results** Overall prevalence of MetS was 35%. Subjects exposed more than 3 hrs/day to TV (n=236) showed a higher prevalence of MetS (43%) in respect to those spending less time watching TV (30%) (P=0.0007). The association persisted in multivariate analysis adjusted for age, sex, physical activity, total caloric intake and social status (P=0.007), but was reduced when body mass index (that was higher in TV viewers) was included in the multivariate analysis (P=0.049). No association (P=0.52) was found in contrast between MetS and surfing the Internet (n=387).
**Conclusion** Prolonged TV watching was associated with MetS in a sample of healthy adult Italian population. The association was explained at least in part by higher BMI (linked to lower physical activity) in TV viewers. However, absence of association of MetS with a similar sedentary activity as surfing the Internet suggests that, in addition to overweight, TV watching is associated with other unfavourable variables such as consumption of junk foods and snacks.

## *NUTRIZIONE E TROMBOSI: STUDI EPIDEMIOLOGICI E SPERIMENTALI*

P011 **EFFECTS OF DIET ON INR VARIABILITY**

Sottilotta G.[1], Consonni D.[2], Romeo E.[1], Siboni S.M.[3], Latella C.[1], Oriana V.[1], Ramirez F.[1], Trapani Lombardo V.[1]
*[1] Centro Emofilia - Servizio Emostasi e Trombosi, Azienda Ospedaliera Bianchi-Melacrino-Morelli, Reggio Calabria; [2] Dipartimento di Medicina Preventiva, Unità Operativa di Epidemiologia, Milano; [3] Department of Medicine and Medical Specialties "A. Bianchi Bonomi", Haemophilia and Thrombosis Centre, Milano*

**Introduction** The potential association between dietary vitamin K intake and International Normalized Ratio (INR) variability in patients on oral anticoagulants treatment (OAT) has been evaluated in several studies. Changes in diet composition are known to lead to INR variability. In this study we evaluated INR over time in married couples on OAT (presumably sharing the same diet) and non-cohabitant couples on OAT (presumably having different diets), to assess clinical relevance of the diet.
**Materials and Methods** Among outpatients receiving OAT at our Centre we selected 31 married couples aged 49-89 years. Husbands and wives were then matched by demographic and clinical characteristics (age, sex, diagnosis, study period, and numbers of visits) to 31 men and 31 women on OAT not married nor living together. We used multiple mixed regression models with random intercept and slope to analyze INR trends within and between couples over time, while adjusting for possible seasonal effects.
**Results** We analyzed 6,357 INR measurements recorded from February 1998 to November 2009. We found similar average INR values within married couples (men: 2.5; females: 2.5) and also within non-cohabitant couples (men: 2.6; women:

2.6). We found slightly lower INR values in December-February (2.5) and March-May (2.5) than in June-August (2.6) and September-November (2.6). Using mixed models we confirmed INR differences between seasons ($p<0.0001$) and the slightly lower INR in non-cohabitant couples compared to married couples ($p=0.02$); although statistically significant, they were of marginal clinical significance. Within both married and non-cohabitant couples, we did not find statistically or clinically significant differences between men and women over time.
**Conclusion** The lack of INR differences over time within non-cohabitant couples indicates that diet is not an important determinant of INR over time. Also seasonal INR variations and differences between married and non-cohabitant couples were of little practical importance.

P012 **TYPICAL BREAKFAST FOOD CONSUMPTION AND RISK FACTORS FOR CARDIOVASCULAR DISEASE IN A LARGE SAMPLE OF ITALIAN ADULTS: RESULTS FROM THE MOLI-SANI PROJECT**

di Giuseppe R.[(1)], Di Castelnuovo A.[(1)] Melegari C.[(2)], De Curtis A.[(1)], Zito F.[(1)], Krogh V.[(3)], Donati M.B.[(4)], de Gaetano G.[(4)], Investigators on behalf of the Moli-sani Project
*[(1)] Laboratory of Genetic and Environmental Epidemiology, Catholic University, Campobasso; [(2)] Nutrition Science Unit, Barilla Spa, Parma; [(3)] Nutritional Epidemiology Unit, Cancer National Institute, Milano; [(4)] RE ARTU Research Laboratories, Catholic University, Campobasso*

**Background** Breakfast consumption has been associated with a better global nutritional quality, with a reduced risk to develop obesity during lifetime and a higher probability to achieve a healthy body weight in children, adolescents or adults. We hypothesized that the consumption on a regular basis of foods typical of the Italian breakfast even if consumed throughout the day, could be associated with a better cardiovascular risk profile, in an adult Italian population.
**Methods** 10,048 healthy subjects (50.7% women), aged ≥35 yrs (mean age 50±10 yrs), randomly selected from the population of the Moli-sani Project were studied.
The EPIC Food Frequency questionnaire was used for dietary assessment. To derive breakfast pattern, an *a priori* approach was used: first, foods typical of the Italian breakfast were identified (milk, coffee, tea, yoghurt, crisp bread/rusks, breakfast cereals, brioches, biscuits, honey, sugar and jam). Then the amount in grams/day for each food was standardized to mean zero and standard deviation 1. A standardized breakfast score was obtained adding all the standardized amounts of the selected foods.
**Results** Subjects showing a higher breakfast score were younger, women, with higher social status and physical activity ($p<0.01$ for all). In a fully adjusted model, subjects with a higher breakfast food consumption had a lower risk to have high BMI, WH ratio, systolic and diastolic blood pressure, blood glucose, triglycerides, total cholesterol and CRP ($P<0.001$ for all). These associations were unrelated to age, sex, smoking, obesity, physical activity and social status. Subjects with a high food breakfast score also showed a better physical healthy status score, and a lower risk of metabolic syndrome (OR=0.68; 0.54-0.84 95% CI) and men had also a lower risk of future CVD ($p<0.001$).
**Conclusions** Consumption of typical Italian breakfast foods positively affects CVD risk factors profile in an adult Italian population.

P013 **EFFECTS OF DIETARY ADVICES AND POLYGLUCOSAMINES ON METABOLIC AND INFLAMMATORY PARAMETERS IN SUBJECTS ON AN ATHEROGENIC DIET**

De Curtis A.[(1)], Rago L.[(1)], Persichillo M.R.[(1)], Verna A.[(1)], Spinelli M.[(1)], Savarè R.[(2)], Donati M.B.[(1)], De Gaetano G.[(1)], Iacoviello L.[(1)], Zito F.[(1)]
*[(1)] Laboratory of Genetic and Environmental Epidemiology, John Paul II Centre for High Technology Research and Education in Biomedical Sciences Research Laboratories, Campobasso; [(2)] Clinical Study Manager, Direzione Medica, Farma Bracco, S. Donato Milanese*

**Background** Chitosans are polysaccharides derived by deacetylation from chitin, a structural element in crustaceans exoskeleton. ARD Lipiban (Bracco, Spa) contains polyglucosamines, a low molecular weight chitosans.
Our aim was to test the effects of polyglucosamines on metabolic and inflammatory parameters in subjects on atherogenic diet.
**Methods** 102 volunteers (25-69 yrs) were randomised to receive Mediterranean diet-based advice without (n=45) or with ARD-Lipiban administration (n=53). ARD Lipiban was taken 6 day/week for 4 weeks, 2 tablets/day (polyglucosamine 89.21 g/100 g, vitamin C 5.88 g/100 g and tartaric acid as acidifier for a total of 510 mg/tablet). All measures were performed both at the admission and at the end of the trial.
The atherogenicity of each individual's diet was calculated by the cholesterol/saturated-fat index (CSI) that provides an estimate of the serum cholesterol-raising tendency of the diet.
**Results** At the baseline, there was no significant difference between characteristics of the two groups. After one month trial, the following parameters showed a statistically significant reduction in both groups: body weight, BMI, waist and hip circumferences, SBP, total cholesterol and LDL-cholesterol (that ranged from –0.6 to –12 after one month of dietary advice only; and from –0.8 to –13 after one month of ARD-Lipiban; P=NS, difference between groups). In contrast, a significant decrease in CRP levels (from 3.1±2.5 to 2.1±1.4 ng/dL, $p<0.0010$) was observed after dietary advice plus ARD-Lipiban only, but not after dietary advice alone (from 2.7±2.4 to 2.2±2.2 ng/dL, P=0.25). The CUORE global cardiovascular risk score was reduced more in Lipiban group (–15%) than in diet group (–6%), but the difference between the two groups was not statistically significant.
**Conclusions** Mediterranean diet-based advice were able to improve metabolic risk factors for cardiovascular disease in a period as short as one month. The addition of ARD-Lipiban showed a favourable effect on inflammation markers.
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P014 **LIFESTYLE MODIFICATIONS IN PATIENTS AFTER ACUTE CORONARY SYNDROMES ENROLLED IN A SUBSET OF THE AMI-FLORENCE 2 STUDY: WHICH CHANGES AFTER THE ACUTE EVENT?**

Sofi F.[(1)], Fabbri A.[(1)], Marcucci R.[(1)], Balzi D.[(2)], Barchielli A.[(2)],

Santoro G.M.[3], Marchionni N.[1], Abbate R.[1], Gensini G.F.[1]
*[1] Area Critica Medico-Chirurgica, Università degli Studi di Firenze, Firenze; [2] Unità di Epidemiologia, Azienda Sanitaria Firenze; [3] Divisione di Cardiologia, Ospedale S. Giovanni di Dio, Firenze*

**Background and aims** Recent findings showed a poor adherence in terms of modifications of lifestyle habits and drugs prescription among patients with a prior vascular event. Aim of this study was to evaluate the adherence to recommendations for secondary prevention of cardiovascular diseases in patients with acute coronary syndromes (ACS) after an acute event.
**Methods and Results** Physical examination, a careful medical interview with assessment for lifestyle habits, adherence to pharmacological therapy and blood analyses were performed in 130 patients (33 F; 97 M, with a median age of 72.5 years) at the time of the acute event and after 6 months of follow-up. At follow-up examination, 7 patients persisted to smoke (5.4%), 41 (31.5%) continued to have high blood pressure, 34 (26.1%) had high levels of total cholesterol, 38 (29.2%) high levels of triglycerides, 64 (49.2%) high levels of LDL-cholesterol and 46 (35.4%) low levels of HDL-cholesterol, despite all the treatments did not significantly change over the follow-up period and laboratory parameters showed a significant decrease after 6 months. A high percentage of patients (47%) reported a lower daily consumption of fruit and vegetables with respect to the recommended daily portions, nearly the whole population (92.3%) did not reach the recommended portions of legumes per week recommended, and a consistent percentage of patients (81.5%) did not consume fish twice a week, as recommended.
**Conclusion** These findings demonstrate the difficulty of modifying the lifestyle habits in patients with ACS even after an acute vascular event.

## P015 TOTAL DIETARY ANTIOXIDANT CAPACITY AND LUNG FUNCTION IN A FEMALE ITALIAN POPULATION: RESULTS FROM THE MOLI-SANI PROJECT

di Giuseppe R.[1], Arcari A.[1], Serafini M.[2], Sieri S.[3], Pellegrini N.[4], Schünemann H.J.[5], Donati M.B.[6], de Gaetano G.[6], Iacoviello L.[7], Investigators on behalf of the Moli-sani Project[7]
*[1] Laboratory of Genetic and Environmental Epidemiology, Catholic University, Campobasso; [2] Antioxidant Research Laboratories, Istituto Nazionale di Ricerca, Roma; [3] Nutritional Epidemiology Unit, National Cancer Institute, Milano; [4] Department of Public Health, University of Parma, Parma; [5] Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, Canada; [6] RE ARTU Research Laboratories, Catholic University Campobasso; [7] Laboratory of Genetic and Environmental Epidemiology, Catholic University, Campobasso*

**Background** The measure of total antioxidant capacity (TAC) in the diet summarizes the capacity of the different food antioxidants in scavenging preformed free radicals. It may be an appropriate approach to assess the cumulative antioxidant properties of plant foods and to investigate the association between diet and oxidative stress-induced disease in a more comprehensive way. Our study was aimed at examining possible associations between dietary TAC and pulmonary function in an Italian population.
**Methods** The association between TAC of the diet and pulmonary function was investigated in apparently healthy women (5,786) and men (5,807), randomly recruited from the general population in the framework of the Moli-sani project. The European Investigation into Cancer and Nutrition (EPIC) Food Frequency questionnaire was used for dietary assessment.
**Results** In women pulmonary function was associated with dietary ferric reducing-antioxidant power (FRAP), the better predictor of pulmonary function. Compared to the first quintile of FRAP, those in the fifth quintile had a +37 mL higher Forced Expiratory Volume in the first second (FEV1) and +52 mL higher Forced Vital Capacity (FVC), after adjustment for confounders ($P<0.03$ for all). A significant interaction was found with smoking habits and menopausal status, being the association present only in non-smoker/premenopausal women ($P<0.001$ for all). In the latter group the increment in pulmonary function was equivalent to an improvement in pulmonary age of ~3 years. In men no association was found between pulmonary function and TAC.
**Conclusions** Dietary TAC may play a role in respiratory health, with a more obvious effect in premenopausal, non-smoker women. Since reduced pulmonary function is a risk factor for chronic disease and mortality, these findings highlight the importance of diets rich in antioxidant to help reducing such a risk.

## P016 WINE AND BEER CONSUMPTION IN RELATION TO VASCULAR RISK: AN UPDATED META-ANALYSIS

Costanzo S., Di Castelnuovo A., Donati M.B., Iacoviello L.
*Laboratori di Ricerca, Università Cattolica del Sacro Cuore, Campobasso*

**Background** Several epidemiological studies have evaluated whether moderate consumption of different alcoholic beverages protects against cardiovascular disease. We performed an updated meta-analysis of 15 studies on the relationship between wine or beer consumption and fatal and non fatal vascular events (acute myocardial infarction, stroke, coronary heart disease).
**Methods** Articles were retrieved through April 2010 by search in PubMed and Embase. A weighed, least-squares regression analysis of second-order fractional polynomial models was applied to pooled data derived from 15 studies that gave quantitative estimation of the vascular risk associated with either wine or beer consumption. Studies were excluded if only one category of alcoholic beverage intake was reported, or if it was not possible to extract quantitative data.
**Results** From 14 pooled studies (9 prospective studies involving 247,141 persons and 5 case-control studies involving 2,621 cases and 5,086 controls) there was strong evidence supporting a J-shaped relationship between different amounts of wine intake and vascular risk. A significant maximal protection -average 32% (95% CI: 18-44%)- at 24 grams of alcohol per day (2 glasses of wine) was defined.
From 12 pooled studies (7 prospective studies involving 209,063 persons and 5 case-control studies comprising 2,525 cases and 4,401 controls) an inverse J-shaped relationship between different amounts of beer intake and vascular risk was found. A significant

maximal protection -average 48% (95% CI: 20-68%) at 40 grams of alcohol per day (3 cans of beer) was assessed.
**Conclusion** This updated meta-analysis gives evidence for a J-shaped significant inverse association between moderate wine or beer consumption and vascular risk. Excess of drinking is clearly associated with an unhealthy profile. Dose-response curves appear substantially similar for wine and beer.

## *OMOCISTEINA E VITAMINE*

### P017 **GENOTYPE-INDEPENDENT *IN VIVO* OXIDATIVE STRESS FOLLOWING A METHIONINE LOADING TEST. MAXIMAL PLATELET ACTIVATION IN SUBJECTS WITH A HISTORY OF EARLY-ONSET THROMBOSIS**

Di Minno M.N.D. [(1)], Tremoli E.[(2)], D'Angelo A.[(3)], Coppola A.[(1)], Tufano A.[(1)], De Gregorio A.M.[(1)], Di Palo M.G.[(1)], Cerbone A.M.[(1)], Di Minno G.[(1)]
*[(1)] Department of Clinical and Experimental Medicine Federico II University Naples; [(2)] Department of Pharmacological Sciences, Milan University, Milan; [(3)] Coagulation Service and Thrombosis Research Unit IRCCS H S. Raffaele, Milan*

**Background** The methionine loading test identifies individuals in whom fasting plasma homocysteine (tHcy) may be normal but the post-methionine load tHcy is abnormally high.
**Methods** In 96 subjects [54 males, 42 females, mean age 40.4±12.3 yrs; 28 with the 68bp844 ins polymorphism of the Cystathionine-β-synthase (CBS ins+) gene; 20 homozygotes for the C677T methylene-tetrahydrofolate reductase gene mutation (MTHFR++); 13 with the combination of the two, and 35 without any of these variations], we evaluated in vivo oxidative stress (reflected by urinary excretion of 8-iso-PGF2α) and platelet activation (reflected by the urinary excretion of 11-dehydro-TXB2), before and after the ingestion of 100 mg/kg methionine (PML).
**Results** Baseline: tHcy was higher in MTHFR ++ carriers ($p<0,05$); 8-iso-PGF2α was independent of sex, genotype, and a history of thrombotic events; 11-dehydro-TXB2 was not different according to the presence/absence of MTHFR++ and/or CBS ins+ (p always >0.05). PML: A~3-fold increase in tHcy occurred, reaching a plateau within 6-8 hrs (T6,T8) (p always <0.01). Mean PML-tHcy (T4,T6,T8) was maximal in MTHFR++ carriers ($p=0.000$). 8-iso-PGF2α increase ($p<0.01$) reached a maximum at T4. The increase in 11-dehydro-TXB2 was maximal at T4 and highest ($p=0.023$) in subjects with a history of thrombosis (52/94, 18 arterial, 32 venous, 2 both). No difference in T4 PML 11-dehydro-TXB2 was found with respect to a history of arterial or venous thrombosis ($p>0.05$). The PML increase in either 8-iso-PGF2a or in 11-dehydro-TXB2 excretion was comparable in the different genotypes analyzed ($p>0.05$). Baseline 11-dehydro-TXB2 levels and thrombotic events independently predicted PML 11-dehydro-TXB2 levels ($\beta=0.287$, $p=0.000$ and $\beta=0.308$, $p=0.026$, respectively).
**Conclusion** *In vivo* oxidative stress and platelet activation, independent of genotypes associated with moderate hyperhomocysteinemia, occur following a methionine loading test. *In vivo* platelet activation is maximal in subjects with a history of early-onset thrombosis and comparable in those with a history of arterial or venous events.

### P018 **HOMOCYSTEINE LOWERING AND GLUTATHIONE INCREASING EFFECTS OF N-ACETYLCYSTEINE IN ACUTE CORONARY SYNDROME**

Squellerio I.[(1)], Marenzi G.[(1)], Veglia F.[(1)], Falcucci G.[(1)], Crisci M.[(1)], Songia P.[(1)], Boccotti L.[(2)] , Eligini S.[(1)], Tremoli E.[(3)]
*[(1)] IRCCS Centro Cardiologico Monzino, Milano; [(2)] Dipartimento di Scienze Cardiovascolari, Università degli Studi di Milano, Milano; [(3)] Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Milano*

Several studies have suggested that increased homocysteine (Hcy) is a risk factor for cardiovascular (CVD) and atherothrombotic diseases because it affects both the vascular wall structure and the blood coagulation system. However, large randomised clinical trials have shown that, even though B-vitamin and folic acid supplementation reduced Hcy levels, there was no significant effect on CVD risk. Oxidative stress is another contributory factor to the etiology of many CVD and we have previously reported low concentrations of reduced glutathione (GSH) in CVD. GSH is an endogenous tripeptide with antioxidant properties and the ratio between reduced and oxidized (GSSG) forms is a major mechanism by which cells maintain redox balance.
N-acetylcysteine (NAC), a drug used to prevent contrast-nephropathy after angiography, is a GSH synthesis precursor. Recent studies have also evidenced a Hcy-lowering activity of NAC in healthy subjects and in renal insufficiency bearing patients. In the present study we investigated the effect of NAC on Hcy and GSH levels in acute coronary syndrome (ACS) patients.
Plasmatic Hcy and whole blood GSH and GSSG were measured in patients with ACS (n=20) before and 2, 6 and 24 hrs after an intravenous bolus (600 mg) of NAC.
Mean Hcy concentrations significantly decreased up to 6 hours after bolus (–40.1%, $p<.0001$), returning to basal levels at 24 hrs, while the profile of NAC concentrations showed an opposite behaviour. Whole blood GSH significantly increased after 24 hrs (+18.4%, $p=0.015$) with a concomitant GSSG decrease (–23.8%, $p=0.03$).
These preliminary results suggest a beneficial effect of NAC administration in ACS patients. In conclusion, NAC may represent a therapeutic tool able to counteract both the oxidative stress status, by increasing GSH levels, and hyperhomo-cysteinemia condition, by reducing Hcy plasmatic levels.

## *SINDROME CORONARICA ACUTA*

### P019 **DIFFERENTIAL GENE EXPRESSION PROFILES OF WHOLE PERIPHERAL BLOOD IDENTIFY THREE GROUPS OF ACUTE CORONARY SYNDROME PATIENTS UNDERGOING PCI**

Giusti B.[(1)], Rossi L.[(1)], Lapini I.[(1)], Magi A.[(1)], Marcucci R.[(1)], Cordisco A.[(1)], Antoniucci D.[(2)], Giglioli C.[(2)], Gensini G.F.[(1)], Abbate R.[(1)]
*[(1)] Dpt. Medical and Surgical Critical Care - Atherothrombotic Diseases Unit, AOU Careggi University of Florence, Florence; [(2)] Department of Heart and Vessels, AOU Careggi, Florence*

The pathophysiological mechanisms underlying the acute coronary syndrome (ACS) in its multiple manifestations

remain to be finely understood. Aim of our study was to evaluate the peripheral gene expression profile of ACS patients undergoing percutaneous coronary intervention (PCI) on dual antiplatelet therapy. We evaluated the gene expression profiles by Affymetrix technology (47,000 transcripts) on RNA extracted from whole peripheral blood of patients.
By an unsupervised clustering analysis of 29 patients, we identified three different groups of patients (A, n=9; B, n=11; and C, n=9). The three groups were comparable for sex, and significantly differed for age [A=76 (71-89); B=74 (63-89); C=68 (55-74)]. Group A, B and C showed respectively 66.7%, 27.3% and 33.3% of patients with ≥3 vessels with a stenosis >50%, and 55.6%, 9.1% and 44.4% of patients with ≥3 traditional cardiovascular risk factors. Interestingly, in group A we observed 88.9% of patients with hypertension compared with 45.4% in B and 44.4% in C; in group C 66.7% of patients were dyslipidemic with respect to 33.3% in A and 18.2% in C; in group B 90.9% of patients had residual platelet reactivity on antiplatelet treatment by both ADP- and arachidonic acid-induced platelet aggregability with respect to A (66.7%) and C (33.3%).
The Significance Analysis of Microarrays identified 8,464 differentially expressed genes between group A and B, 8,945 between A and C, and 3,223 between B and C.
Interestingly, among genes with a different profile in the 3 groups there were genes coding components of the methionine metabolism (MTHFR, MTHFD2, MTR, MTRR, FOLR1, SLC19A1 DHFRL1, AHCYL1), of the TGF pathway (TGFB, TGFBR1, TGFBR2, SMAD1, SMAD2, SMAD4), and of the superfamily of the phospholipase A2 (PLA2G7/Lp-PLA2, PLA2G6/iPLA, PLA2G4A/cPLA2α, PLA2G12A/sPLA2, PLAA). Our data identify differential profiles underling different pathophysiological mechanisms in ACS patients and biological markers to consider in the diagnosis and prognosis.

## *ARTERIOPATIE PERIFERICHE*

### P020 **THROMBOPHILIC RISK FACTORS AND OUTCOME IN PATIENTS UNDERGOING ENDOVASCULAR INTERVENTION FOR PERIPHERAL ARTERIAL DISEASE**

Sartori M., Conti E., Favaretto E., Legnani C., Cini M., Pili C., Palareti G.
*U.O. Angiologia e Malattie della Coagulazione "Marino Golinelli", Policlinico S. Orsola-Malpighi, Bologna*

**Objectives** Few data are available on thrombophilic risk factors and clinical outcome in patients undergoing percutaneous transluminal angioplasty (PTA) for peripheral arterial disease (PAD). We investigated the role of homocysteine, fibrinogen, Factor VIII (FVIII), lupus anticoagulant (LAC), FII G20210A, and FV R506Q (FV Leiden) mutations, known to be associated with thrombotic risk, as prognostic factors in 197 patients who underwent PTA for PAD (Fontaines stages: II through IV; aged 69±10 years, male/female 119/78).
**Design and Methods** A longitudinal study. End-points of the study were total mortality, cardiovascular events and restenosis after PTA. Patients were followed up for an average time of 32+2 months.
**Results** During the follow-up, total mortality was 16%, 45.5% of patients had a cardiovascular event. According to Cox regression analysis, age and the presence of critical limb ischaemia were predictors of mortality and cardiovascular events, whereas diabetes, hyperlipidemia, homocysteine, and LAC were predictor of cardiovascular events. Considering as dichotomous the following variables: fibrinogen, homocysteine, FVIII, presence of LAC, FII G20210A, and FV Leiden mutations, the frequency of patients with at least two thrombophilic alterations was 31%. During the follow-up, cardiovascular events were more frequent in the patients with at least two thrombophilic alterations versus those with one or without thrombophilic alterations (37 vs. 17% log-rank p=0.001). Rates of restenosis during the follow-up were not different in the two groups (26 vs. 20%, p=ns).
**Conclusion** The presence of two or more thrombophilic risk factors in patients who underwent PTA for PAD is associated with increased risk of arterial thrombotic events. Intervention trials are required to show the benefit of different therapeutic approaches in such patients at high risk of clinical deterioration.

## *FIBRILLAZIONE ATRIALE E CARDIOEMBOLISMO*

### P021 **ANTITHROMBOTIC PROPHYLAXIS IN ELDERLY PATIENTS ATRIAL FIBRILLATION ADMITTED TO INTERNAL MEDICINE WARDS IN ITALY: ADHERENCE TO GUIDELINES**

Iorio A.[(1)], Marcucci M.[(1)], Pasina L.[(2)] Marengoni A.[(3)], Salerno F.[(4)], Corrao S.[(5)], Nobili A.[(2)], Mannucci P.M.[(6)]
*[(1)] Medicine, Internal Vascular Medicine, Perugia; [(2)] Istituto di Ricerche Farmacologiche Mario Negri, Milano; [(3)] Medical and Surgery Sciences, University of Brescia, Brescia; [(4)] Medicine, Policlinico San Donato, Milano; [(5)] Medicine, University of Palermo, Palermo; [(6)] Medicine, IRCCS Fond. Ospedale Maggiore, Milano*

**Background** Elderly patients with atrial fibrillation (AF) often fulfil the indication for vitamin K antagonists (VKA), but age, compliance, frailty, logistics and co-morbidities represent barriers to the adoption of VKA.
**Aims** To verify adherence to guidelines (ACCP 2008) when indication to VKAs is present as assessed by the CHADS2 score at admission and discharge in a cohort of elderly patients.
**Materials and Methods** The project was a collaboration of the Italian Society of Internal Medicine and the Pharmacological Research Institute Mario Negri. During each of four selected weeks in 2008, 10 patients were recruited in any participating Centre and demographical and clinical information collected. Patients with AF (ICD9 427.31/32) and those treated with VKAs (ATC B01AA) or antiplatelet drugs (AP, ATC B01AC) were included in this analysis. The role for haemorrhage and cancer was also evaluated.
**Results** 35 centres enrolled 1,333 patients, of which 1,156 were analysed. AF was diagnosed in 18.7%, of which 79.7% was >75 years, 57.0% had hypertension, 25.7% diabetes, 26.2% heart failure, 0.8% previous stroke. In patients with CHADS2 ≥2, VKA has been withdrawn in 19.6% and started in 13.7%; among those discharged not on VKAs, 5/108 were admitted for bleeding and 1/44 bled during hospitalization. 31% of untreated and 16% of treated patients had cancer (OR=0.43, p 0.03).

**Conclusions** In elderly patients, clinical practice was found far away from guidelines. VKA treatment was largely underused and hospitalization did not have a large effect on adherence to guidelines.

| | All | VKA | AP | VKA+AP | No treatment |
|---|---|---|---|---|---|
| **All** | | 33.2 | 37.0 | 2.1 | 27.7 |
| | | (34.7) | (30.6) | (1.8) | (32.9) |
| **$CHADS_2$ ≥2** | 68.9 | 30.2 | 40.1 | 2.5 | 27.2 |
| | (69.4) | (33.1) | (35.7) | (2.6) | (28.6) |
| **$CHADS_2$ = 1** | 23.4 | 42.4 | 32.2 | - | 25.4 |
| | (25.2) | (39.3) | (17.9) | | (42.9) |
| **$CHADS_2$ = 0** | 5.9 | 28.6 | 21.4 | 7.1 | 42.9 |
| | (5.4) | (33.3) | (25.0) | (0) | (41.7) |

P022 **BLEEDING AND STROKE RISK IN ATRIAL FIBRILLATION PATIENTS IN A PRIMARY PREVENTION SETTING**

Poli D.[(1)], Testa S.[(2)], Antonucci E.[(3)], Grifoni E.[(3)], Paoletti O.[(2)], Lip G.Y.H.[(4)]
*[(1)] Dept of Heart and Vessels, Thrombosis Centre, Azienda Ospedaliero-Universitaria Careggi, Florence; [(2)] Haemostasis and Thrombosis Centre, AO Istituti Ospitalieri di Cremona, Cremona; [(3)] Department of Medical and Surgical Critical Care, University of Florence, Florence; [(4)] Centre for Cardiovascular Sciences, University of Birmingham, Birmingham, United Kingdom*

The absolute benefit of antithrombotic therapy in atrial fibrillation (AF) patients depends on both stroke and bleeding risk. Several clinical characteristics have been associated with an increase of both risks. A history of previous stroke identifies high risk secondary prevention patients who undoubtedly benefit from warfarin, but prior stroke also increases bleeding risk. Given that the balance between benefit and risk is less clear in a primary prevention setting, our aim was to evaluate if a relationship between stroke and bleeding risk also exists for such patients.
We prospectively followed-up 3,302 AF patients treated with warfarin for primary prevention (follow-up 10,019 patients/years; mean 3.0±2.8 years), median age at the beginning of follow-up 74 (37-100) years. We classified our patients for stroke risk using the CHADS2 score and for bleeding risk using the Out-patient Bleeding Risk Index (OBRI).

| | | | CHADS2 score | | | | |
|---|---|---|---|---|---|---|---|
| | | | **0** | **1** | **2** | **3** | **4** |
| **OBRI** | **0** | **n** | 109 | 221 | 23 | 0 | 0 |
| | | **%** | 30.9 | 62.6 | 6.5 | .0 | .0 |
| | **1** | **n** | 179 | 843 | 987 | 221 | 0 |
| | | **%** | 8.0 | 37.8 | 44.3 | 9.9 | .0 |
| | **2** | **n** | 13 | 89 | 249 | 234 | 65 |
| | | **%** | 2.0 | 13.7 | 38.3 | 36.0 | 10.0 |
| | **3** | **n** | 0 | 1 | 23 | 33 | 8 |
| | | **%** | .0 | 1.5 | 35.4 | 50.8 | 12.3 |
| **Total** | | **n** | 301 | 1,154 | 1,282 | 488 | 73 |
| | | **%** | 9.1 | 35.0 | 38.9 | 14.8 | 2.2 |

CHADS2 score and OBRI was closely correlated (r=0.51, p=0.000). Among patients with OBRI=0 almost all patients have CHADS2 score ≤1, whilst with OBRI=3, almost all patients had a CHADS2 score ≥2.
Bleeding and stroke risk are closely related and patients at high risk for stroke are at the same at a similarly high risk for bleeding. A new approach in determining the optimal net clinical benefit for thromboprophylaxis for AF patients is needed.

P023 **A GENETIC AND CLINICAL APPROACH TO PROFILE THE ELDERLY IN ORAL ANTICOAGULANT THERAPY**

Ogliari G.[(1)], Giordani I.[(2)], Mihalich A.[(3)], Castaldi D.[(3)], Di Blasio A.[(3)], Dubini A.[(3)], Messina E.[(2)], Archetti F.[(2)], Mari D.[(1)]
*[(1)] Department of Medical Sciences, Università degli Studi di Milano, Milano; [(2)] DISCO - Dipartimento di Informatica, Università degli Studi di Milano, Milano; [(3)] I.R.C.C.S. Istituto Auxologico, Milano*

**Objectives** The response to anticoagulants is marked by high inter-individual and inter-temporal variability, which can lead to serious adverse events especially in elderly patients. Polymorphisms of genes CYP2C9 and VKORC1 account for a part of this variability. Objectives of our study are: 1) introducing a new index called drug sensitivity (Dsens) to capture the dose-INR relationship which better characterizes the patient behaviour, 2) Profiling patients on the basis of their genetic, personal, clinical and therapeutic.
**Materials and Methods** We have collected in a structured database data of 1,013 elderly patients (from 65 to 99 years). Each patient is characterized by the following features: age, sex, anticoagulant drug used, medical evidence to anticoagulation therapy, concomitant therapy and a time series of INR and anticoagulant doses measurements. So far we have genotyped 325 patients according to polymorphism of CYP2C9 and VKRC01 genes. The Dsens of each patient is computed by the ratio between dose and INRs variation as follows: Dsens = σδINRi/σδdi. In our work we have classified patients in three Dsens classes (Wrong Responders, Correct Responders, Excess Responders); lower value of Dsens means that patient is responding in a wrong way to therapy. On the other side higher values of Dsens indicate that patient is responding to therapy in an excessive way. Finally, patients falling in correct responders' class have a more predictable drug response behaviour. We also evaluated CYP2C9 and VKORC1 allelic variant frequencies distribution in the population of the three Dsens classes. Finally we have applied different machine learning classification algorithms taking into account genetic feature comparing obtained results with those obtained by classical INR based classification algorithms.
**Conclusion** We show that classification methods can identify groups of patients homogeneous with respect to the dynamics of INR taking into account genetic, personal, clinical and therapeutic patient's features.

## *MALATTIE CEREBROVASCOLARI*

P024 **HUMAN CAROTID PLAQUE COMPOSITION: CORRELATION BETWEEN HISTOLOGY AND MULTIDETECTOR COMPUTED TOMOGRAPHY (MDCT)**

Facchinetti L.[(1)], Spirito R.[(2)], Ballerini G.[(2)], Bona V.[(2)],

Formenti A.[2], Fusari M.[2], Tremoli E.[3], Camera M.[3]
[1] *Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Milano;* [2] *Centro Cardiologico Monzino IRCCS, Università degli Studi di Milano, Milano;* [3] *Dipartimento di Scienze Farmacologiche, Centro Cardiologico Monzino IRCCS, Milano*

**Background** Plaque morphology is an important predictor of stroke risk. Although plaque morphology is not used, so far, in the decision making of whether to perform carotid endoarterectomy (CEA) or not, having a non-invasive technique capable of diagnosing vulnerable plaque would be very useful in the recognition of patients at high risk of cerebrovascular disease.
**Aim** To compare human carotid plaques composition obtained by means of histological analysis and MDCT.
**Methods** 37 patients (22 male, 15 females; mean age: 70±7 years) undergoing CEA due to high grade carotid artery stenosis (82±9%) were evaluated with MDCT for non-invasive plaque morphology assessment prior to CEA. Plaques removed during surgery were divided into 5 mm pieces. The part corresponding to the point of maximum stenosis was formalin fixed and paraffin embedded for histology. Sections were stained with Haematoxilin-Eosin and Masson-Trichrome to assess plaque morphology and composition (collagen, smooth muscle cell, lipids and calcium). Kappa statistics was used for the degree of agreement between the histological and MDTC images.
**Results** Images of histological sections were computer-reconstructed with Zeiss-Panorama software after being photographed under a microscope (90 frames for each section on average). The area occupied by collagen, smooth muscle cells, lipids, calcium and thrombus was then quantified (absolute amount, $mm^2$, and percentage over the total area) using the Zeiss-Axiovision-measurement software. According to the composition thus obtained, plaques were classified into four classes: lipidic (10%), fibrotic (45%), mixed (35%), and calcific (10%). DSCT plaque analysis was performed on a image of the vessel area at the highest degree of stenosis. A good correlation between histology and MDCT was found (k=0.76).
**Conclusion** MDCT angiography of the carotid arteries is feasible and the evaluation of carotid plaque composition allows non-invasive assessment of different plaque components. This may have an impact on the non-invasive differentiation of vulnerable plaques.

## P025 PREDICTIVE VALUE OF CARDIOVASCULAR RISK FACTORS, PATENT FORAMEN OVALE, AND PROTHROMBOTIC GENOTYPES ON MIGRAINE SUBTYPES IN YOUNG ADULTS WITH ISCHEMIC STROKE

Pezzini A.[1], Lodigiani C.[2], Patella R.[3], Gandolfo C.[4], Casoni F.[5], Musolino R.[6], Calabrò R.S.[7], Lodovici M.L.[8], Del Sette M.[9], Iacoviello L.[10]
[1] *Dip. Scienze Mediche e Chirurgiche - Clin. Neurologica, Spedali Civili, Brescia;* [2] *Centro Trombosi, IRCCS Ist. Clinico Humanitas, Rozzano;* [3] *Stroke Unit, Az. Osped. S. Andrea, Roma;* [4] *Dip Neuroscienze, Oftalmologia e Genetica, Università di Genova, Genova;* [5] *Stroke Unit, Nuovo Ospedale Civile S. Agostino Estense, Modena;* [1] *Dip. Neuroscienze, Clinica Neurologia, Università Messina, Messina;* [7] *Centro Neurolesi Bonino-Pulejo, Policlinico Universitario, Messina;* [8] *Unità di Neurologia, Università Insubria, Varese;* [9] *Unità Neurologia, Ospedale S. Andrea, La Spezia;* [10] *Lab. Ricerca, Centro Ricerca e Formazione ad Alta Tecnologia nelle Scienze Biomediche, Univ. Cattolica del Sacro Cuore, Campobasso*

**Background** The mechanisms underlying the relation between migraine and ischemic stroke remain uncertain. The aim of the present study was to investigate the predictive value of major cardiovascular risk factors, cardiac interatrial abnormalities, and additional biologic markers on migraine subtypes in a large series of young adults with ischemic stroke.
**Methods** Ischemic stroke patients aged ≤45 years were consecutively enrolled as part of the Italian Project on Stroke in Young Adults (IPSYS). A comprehensive evaluation was performed including assessment of self-reported migraine and cardiovascular risk factors, patent foramen ovale (PFO), and genotyping to detect factor V Leiden and the G20210A mutation in the prothrombin gene.
**Results** 981 patients (mean age, 36.0±7.6 years; 50.7% women) were included. The risk of migraine with aura (MA)-related infarcts increased with decreasing number of cardiovascular risk factors (OR, 0.50; 95% CI, 0.24 to 0.99 for two factors or more), increasing number of thrombophilic variants (OR, 2.21; 95% CI, 1.05 to 4.68 for carriers of at least one of the two), and the presence of PFO (OR, 2.41; 95% CI, 1.37 to 3.45), as compared to stroke patients without migraine. None of these factors had influence on the risk of migraine without aura (MO)-related infarcts.
**Conclusions** In young adults with ischemic stroke, low cardiovascular risk profile, PFO and an underlying procoagulant state are strong predictors of MA. The biologic effects of these factors should be considered in future studies aimed at investigating the mechanisms linking migraine to brain ischemia.

## P026 BIOLOGICAL AND INSTRUMENTAL MARKERS OF ENDOTHELIAL DYSFUNCTION IN CADASIL (CEREBRAL AUTOSOMAL DOMINANT ARTERIOPATHY WITH SUBCORTICAL INFARCTS AND LEUKOENCEPHALOPATHY)

Giusti B.[1], Pescini F.[2], Cesari F.[1], Valenti R.[1], Zicari E.[3], Bianchi S.[3], Federico A.[3], Gori A.M.[1], Pantoni L.[2], Parodi O.[4], Inzitari D.[2], Abbate R.[1]
[1] *Dpt. Medical and Surgical Critical Care, Atherothrombotic Diseases Unit, AOU Careggi University of Florence, Florence;* [2] *Dpt. of Neurological and Psychiatric Sciences, University of Florence, Florence;* [3] *Neurometabolic Unit, Institute of Neurological Sciences, University of Siena, Siena;* [4] *Cardiovascular Department, Niguarda Ca' Granda Hospital, Milan*

CADASIL is an inherited microangiopathy with a highly variable phenotype. Endothelial dysfunction could play a role in modulating the expressivity. Recently, we found an association between low bone marrow-derived circulating cells levels and the disease, particularly in patients with the most severe picture.
To evaluate the relationship among CADASIL and two markers, endothelial and circulating progenitor cells (EPCs and CPCs) and flow-mediated vasodilatation (FMD),

associated with endothelial dysfunction.
We evaluated 27 genetically-diagnosed CADASIL patients (13 M; mean age 52.1±2.6 yrs). Cells were measured in peripheral blood using flow cytometry. EPCs were defined as positive for CD34/KDR, CD133/KDR and CD34/CD133/KDR; CPCs as positive for CD34, CD133 and CD34/CD133. FMD was assessed by non invasive pletismographic method of pulse arterial volume at the fingertips, before and after reactive hyperemia induced by occlusion of the brachial artery at the non dominant forearm level (EndoPAT2000, Itamar, Israel). The ratio between the average post-occlusion and baseline signal amplitude (PAT score) was assumed as FMD index.
Median PAT score was 1.78 [1.12-3.60]. Patients in the first and second PAT score tertiles (range 1.12-2.20) presented significantly reduced CPCs levels compared with those in the third tertile (range 2.26-3.60) [CD34: 1.60 vs. 3.45 cells/mL, p=0.05; CD133: 1.45 vs. 3.15 cells/mL, p=0.03; CD34/CD133: 1.50 vs. 3.12 cells/mL, p=0.03]. At the multivariate logistic regression analysis, the difference remained significant after adjusting for age and gender [OR (95% CI) and p values: 3.0 (1.0-9.1), p=0.049 for CD34, 3.7 (1.1-12.2), p=0.035 for CD133, 3.7 (1.1-12.8), p=0.035 for CD34/CD133]. EPCs were not significantly lower in the same patients groups. Even if not significantly, patients in these groups presented worse cognitive performances.
We documented an association between CPCs and FMD in CADASIL. Even if preliminary, these results support the potential role of endothelial dysfunction in the disease.

P027 **DIFFERENTIAL GENE EXPRESSION PROFILES OF CAROTID ARTERY STENOSIS BIOPSIES OF HCV+ AND HCV– PATIENTS SUGGEST PECULIAR MOLECULAR MECHANISMS IN THE PATHO-GENESIS OF THE DISEASE**

Rossi L.[(1)], Lapini I.[(1)], Magi A.[(1)], Boddi M.[(1)], Zignego A.L.[(2)], Pratesi G.[(3)], Pulli R.[(4)], Pratesi C.[(4)], Abbate R.[(1)], Giusti B.[(1)]
*[(1)] Dpt. Medical and Surgical Critical Care - Atherothrombotic Diseases Unit, AOU Careggi University of Florence, Florence; [(2)] Department of Internal Medicine, University of Florence, Florence; [(3)] Vascular Surgery Unit, Department of Surgery, University of Rome Tor Vergata, Rome; [(4)] Department of Vascular Surgery, University of Florence, Florence*

Clinical and experimental evidence suggests that hepatitis C virus (HCV) infection shows peculiar characteristics that strongly support a role in the development of atherosclerosis. Recently, we demonstrated the presence of HCVRNA sequences within carotid plaques of HCV seropositive (HCV+) patients and showed that HCV infection facilitates the occurrence of carotid atherosclerotic lesions.
In order to evaluate whether peculiar molecular mechanisms might support a different pathogenesis of carotid artery disease in HCV+ patients with respect HCV– patients, we performed the gene expression profile by GeneChip technology (47,000 transcripts) of carotid biopsies of 6 HCV+ and 6 age and gender comparable HCV– patients undergoing carotid revascularization.
The intima-media thickness in carotid bifurcation and prevalence and severity of plaques in internal carotid artery as well as HCVRNA sequences in plaque tissues and serum, atherosclerotic risk profile, inflammation markers and main liver function tests were investigated. 278 genes resulted differentially expressed in HCV+ with respect to HCV– patients. After the application of an unsupervised hierarchical clustering, HCV– and HCV+ cases resulted co-clustered, and we were able to separate by the gene expression profile HCV+ and HCV– patients.
Interestingly, among genes with increased expression in HCV+ patients we found: ITIH4, an anti-inflammatory protein associated with diagnosis and prognosis of acute ischemic stroke; thioredoxin, a gene involved in oxidative stress and associated with intraplaque haemorrhage of coronary culprit lesions or atherosclerotic plaques; IRAK3, HLAB and C2, involved in the establishment of an immune response; ADAM23, a member of the a disintegrin and metalloprotease domain family; WNK1, showed to play a role in angiogenesis and heart development in mice.
In conclusion, our data identified differential gene expression profiles in HCV+ and HCV– patients affected by carotid artery disease suggesting the presence of peculiar mechanisms of disease in the two groups of patients.

P028 **TISSUE FACTOR PATHWAY INHIBITOR: A POSSIBLE MARKER OF ENDOTELIAL DAMAGE ASSOCIATED TO HYPERHOMOCYSTEINEMIA IN ALZHEIMER'S DISEASE PATIENTS**

Piazza F.[(1)], Speranza T.[(2)], Ferrarese C.[(3)], Pogliani E.[(1)]
*[(1)] Department of Neuroscience and Biomedical Technologies, University of Milano-Bicocca, Monza; [(2)] Department of Clinical Medicine, Prevention and Sanitary Biotechnology, University of Milano-Bicocca, Monza; [(3)] Department of Neurology, University of Milano-Bicocca, Monza*

Although Alzheimer's Disease (AD) is mainly considered a neuronal disease, much evidence points to a vascular pathogenetic involvement in its etiology. Many vascular risk factors have been associated to AD; i.e. Hyperhomocysteinemia (HHcy) is one of the strongest independent risk factors for vascular and cerebrovascular disorders and has recently been associated to the risk of develop AD in elderly people. Moreover, β-amyloid peptide (Aβ), which plays a central role in AD, not only exerts harmful effects on the vessel walls, increasing the risk of silent hemorrhagic and ischemic strokes, but also facilitates the ultrastructural degeneration of the vessels. Conversely, vascular damage can influence APP processing, modulating the expression of enzymes responsible for Aβ production.
Based on these evidence, we investigated the possible involvement of vascular damage in the pathogenesis of AD, by assessment of plasma levels of tissue factor pathway inhibitor (TFPI), a serine protease inhibitor induced by endothelial injury; homocysteine (Hcy); and folate levels, the most important co-factors involved in methionine metabolism. Plasma levels of tissue factor (TF), and thromboxane B2 (TXB2) were also evaluated.
110 probable AD, 38 mild cognitive impairment, 31 patients affected by idiopathic Parkinson's disease (without dementia) and 100 healthy controls, who displayed no vascular disorders were enrolled. TFPI and Hcy were significantly higher in AD

patients with respect to other groups. The levels of TFPI and Hcy were positively correlated in hyperhomocysteinemic AD and mild cognitive impairment subjects, and were negatively correlated with folate levels.
Our findings suggest that an impairment of endothelial function associated with high Hcy levels may occur in AD patients, despite the absence of manifest cerebrovascular lesions. Therefore, TFPI may represent a candidate marker of endothelial damage in AD and might be used for the identification and monitoring of patients that would benefit from folate supplementation treatment.

## *TROMBOEMBOLISMO VENOSO: DIAGNOSI*

### P029 **VENOMETER® IN THE DIAGNOSIS OF LOWER LIMB DEEP VEIN THROMBOSIS**

Lombardo M.S.[(1)], Ciuti G.[(1)], Grifoni E.[(1)], Zanasi L.[(1)], Pavellini A.[(1)], Abbate R.[(2)], Moggi Pignone A.[(1)]
*[(1)] SOD Medicina Interna ad Orientamento Immunologico, AOU Careggi, Firenze; [(2)] SOD Malattie Aterotrombotiche, AOU Careggi, Firenze*

**Introduction** Venometer® is a non-invasive technician operated machine that uses automated strain gauge plethysmography for the diagnosis of lower limb deep vein thrombosis (DVT). Initial studies on this technology were promising, but more recent works show lower sensitivity than originally reported, even for proximal DVT.
Aim of the study. To evaluate Venometer® accuracy in the diagnosis of lower limb DVT using an integrated approach including also DVT pretest clinical probability (PCP) assessment and D-dimer (DD) measurement.
**Methods** Fourteen patients with documented lower limb DVT on echocolordoppler (ECD) and 14 controls with negative ECD underwent Venometer®, PCP assessment by Wells Score, and DD measurement.
**Results** Venometer® showed a sensitivity of 79% and a specificity of 86% with a negative predictive value (NPV) of 80% and a positive predictive value of 85%. Sensitivity and NPV were higher when Venometer® was used in combination with PCP and DD measurement (90 and 88%, respectively). In the subgroup of patients with proximal DVT Venometer® sensitivity and NPV reached 100%.
**Conclusions** Venometer, especially in combination with PCP assessment and DD measurement, is a useful non-invasive method to rule out the diagnosis of lower limb DVT. Since it is quick and easy to use, it may be useful both in inpatient and outpatient settings to reduce DVT diagnosis related costs.

## *TROMBOEMBOLISMO VENOSO: EPIDEMIOLOGIA E FATTORI DI RISCHIO*

### P031 **PREGNANCY-RELATED VENOUS THROMBO-EMBOLISM AND HAPLOTYPE M2 IN THE ANNEXIN A5 GENE**

Tiscia G.L.[(1)], Colaizzo D.[(1)], Favuzzi G.[(1)], Chinni E.[(1)], Sarno M.[(1)], Margaglione M.[(1,2)], Grandone E.[(1)]
*[(1)] Laboratorio di Emostasi e Trombosi, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo; [(2)] Scienze Biomediche-Genetica Medica??, Università degli Studi di Foggia, Foggia*

Pregnancy itself is a risk for venous thromboembolism (VTE). ANXA5 plays an important antithrombotic role. A haplotype (M2) in ANXA5 gene significantly reduces the expression of ANXA5, as shown by *in vitro* and ex vivo experiments. In addition, it has been shown to be a risk factor for recurrent foetal losses and hypertensive disorders of pregnancy. The aim of our study was to investigate if the M2 haplotype is significantly associated with the occurrence of pregnancy-related VTE.
Eighty-three not anticoagulated patients (median age: 35; range: 18-75) with a documented deep venous thrombosis in a leg during pregnancy or in the puerperium (6 weeks after pregnancy) and 195 controls (median age: 32; range 17-58) were investigated. Medical history was collected. Venous thrombosis was diagnosed by ultrasonography; pulmonary embolism by angiography or ventilation-perfusion lung scan. The median age at the time of first thrombotic event was 30 yrs (range: 17-42).
As expected, patients had a higher prevalence of FV Leiden (17.1% vs. 5.2 in controls) and FII A20210 mutations (18.3% vs. 5.7 in controls). Twenty-seven patients (32.5%) carried the M2 haplotype. Among them, 17 (63.0%) had a history of VTE in puerperium and 10 (37%) during pregnancy. The prevalence of the M2 haplotype was different as compared to that recorded among controls (OR: 2.7, 95% CI: 1.5-4.9, $p<0.001$). A logistic regression analysis, correcting for potential confounders showed a significant increase (OR: 3.4, 95% CI: 1.7-6.7) of the occurrence of VTE in carriers of the M2 haplotype as compared to non-carriers.
If further studies will confirm these data, the investigation of this haplotype could be considered in the thrombophilic work-up in patients with a previous pregnancy-related VTE, as for thrombophilic factors FV Leiden and FII A20210 gene variants.

## *TROMBOEMBOLISMO VENOSO: EPIDEMIOLOGIA E FATTORI DI RISCHIO*

### P032 **FACTOR VIII, IX AND XI IN PATIENTS WITH RETINAL VEIN OCCLUSION**

Marcucci R.[(1)], Sofi F.[(2)], Grifoni E.[(3)], Degl'Innocenti G.[(2)], Lami D.[(4)], Sodi A.[(4)], Giambene B.[(4)], Menchini U.[(4)]; Abbate R.[(3)], Prisco D.[(3)]
*[(1)] Dipart. di Area Critica, Università di Firenze, Firenze; [(2)] Dipartimento Area Critica Medico-Chirurgica, Università di Firenze, Firenze; [(3)] Dipartimento Area Critica Medica, Università, Firenze; [(4)] Clinica oculistica, Università, Firenze*

Retinal vein occlusion (RVO) is a multicausal disease. Known acquired risk factors include advanced age, hypertension and hyperviscosity, i.e. risk factors commonly associated with arterial disease. Among thrombophilic risk factors, an established role has been found for elevated levels of Homocysteine (Hcy). Elevated levels of FVIII, factor IX and FXI have been reported to be risk factors for DVT. Factor IX plays a key role in hemostasis; it is a vitamin K-dependent

glycoprotein, which is activated through the intrinsic pathway as well as the extrinsic pathway. On the other hand, factor XI is a component of the intrinsic pathway of coagulation which contributes to the generation of thrombin and it is involved both in the formation of fibrin and protection against the fibrinolysis.
In the framework of a project aimed to evaluate the possible parameters associated with retinal vein occlusion, we report data on the levels of factor VIII, factor IX and FXI in 684 patients with retinal vein occlusion (413 M/ 271 F; age: 61.5±13.8 yrs). 50 healthy subjects comparable for age and sex (with no history of thrombotic disease and not on oral contraceptives) were used as controls (30 M/ 20 F; age: 62±16).
Mean FVIII, FIX and FXI levels in patients and controls were respectively: 127.4±47.5% vs. 114.4±31.9% ($p<0.005$); 122.7±27.5% vs. 107.6±15.3% ($p<0.001$); 119±28.3% vs. 107±20.6% ($p<0.001$).
According to Leiden Thrombophilia Study, elevated levels of FVIII, FIX and FXI were considered respectively values above 150%, 129% and 120% ($90^{th}$ percentiles of distribution).
At multivariate analysis adjusted for age and sex, elevated levels of FVIII, FIX and FXI were all associated with an increased risk of RVO: OR 2.6 (95% CI 1.1-6.3), $p<0.02$; OR 9.8 (95% CI 3.0-31.9), $p<0.001$; OR 3.2 (95% CI 1.6-6.5), $p<0.001$.
These data on a large number of patients stress the role of hypercoagulability in RVO.

## P033 CEREBRAL VENOUS THROMBOSIS: RISK FACTORS

Tufano A., Di Palo M.G., Cimino E., Cerbone A.M., Di Minno G.
*Department of Clinical and Experimental Medicine, University Federico II, Naples*

**Background** The role of thrombophilia in determining venous thromboembolism is well established, even in cerebral vein thrombosis (CVT). CVT is more common in women (3:1 ratio), oral contraceptive and pregnancy/puerperium being important risk factors.
**Patients** We screened for G20210A prothrombin gene and G1691A Factor V (FV-Leiden) polymorphisms; antiphospholipids, hyperhomocysteinemia, protein S, C, and/or antithrombin deficiency, 49 consecutive patients (13 men and 36 women; mean age 34.19±10.33 years) with early-onset-CVT, compared to 50 age-and-sex-matched controls (mean age 35.72±1.71 years), from the same ethnic background.
**Results** 12/49 (24.5%) CVT patients showed G20210A FII polymorphism, compared to 4% of controls (OR 7.78, 95% CI 1.64-36.94; p=0.004). The FV Leiden frequency was 4.10% in patients and 10% in controls (p=0.437). Only one patient and one control carried both polymorphism. None of patients was affected by anticoagulants deficiency. The 25.6% of cases and 22.2% of controls is affected by hyperhomocysteinemia, (p=NS). As to antiphospholipids antibodies not significant difference among cases and controls were observed. Cigarette smoking (OR 1.03; 95% CI 0.46-2.13 P=1.000) and arterial hypertension (p=1.000) were not significant risk factors. No risk of recurrence is related to genetic polymorphism. 19/35 female developed CVT while assuming oral contraceptives (54.3%), and 4/35 during pregnancy/puerperium (11.4%). None of these patients presented thrombophilic mutations. In 91% of our sample we evaluated estrogens assumption: 9.1% of patients with prothrombin gene mutation did not assume estrogens, while 12.1% of them assumed such therapy (p=1.000).
**Conclusion** Despite the limitations of the sample size, these data confirm the role of the FII G20210A mutation and underline the importance of acquired factors like oral contraceptives. Screening for selected inherited and acquired thrombophilic risk factors might be justified to identify highest risk for early onset CVT.

## P034 RISK OF VENOUS THROMBOEMBOLISM IN PATIENTS NURSED AT HOME OR IN LONG-TERM CARE RESIDENCES

Ambaglio C., Arpaia G., Serras A., Mastrogiacomo O., Cimminiello C.
*Medicina Generale II, Azienda Ospedaliera di Desio e Vimercate, Vimercate*

**Background** Little is known even today about the risk of venous thromboembolism (VTE) in patients with chronic diseases, bedridden or with greatly limited mobility, cared for at home or in long-term residential facilities. The aim of this study was to evaluate the prevalence of deep venous thrombosis (DVT) in these patients, and the impact of risk factors for DVT in these settings.
**Methods** We enrolled 221 chronically ill patients, all over 18 years old, with markedly limited mobility or total immobility, nursed at home or in two long-term residential nursing facilities in the Vimercate area. Two experienced angiologists screened all the patients at the bedside by simplified compression ultrasound (CUS) examination using a portable US machine. Nursing staff blind to the CUS findings recorded socio-demographic details and VTE risk factors.
**Results** The prevalence of asymptomatic proximal DVT was 18% (95% confidence interval - CI, 13%-24%); there were no cases of symptomatic DVT or pulmonary embolism. The best model with a maximum of four risk factors included: previous VTE, time of onset of reduced mobility, home or nursing home care for the lack of mobility and causes of reduced mobility. In particular the risk of DVT for patients with causes of reduced mobility other than cognitive impairment was about half that of patients with cognitive impairment/dementia.
**Conclusions** This study offers a first estimate of the prevalence of DVT among bedridden or low-mobility chronic patients in a selected population. Some of the risk factors that came to light, such as home care as opposed to long-term residential care and cognitive deficit as causes of reduced mobility are not among those usually observed in acutely ill patients.

## P035 ASYMPTOMATIC DEEP VEIN THROMBOSIS IN ELDERLY BEDRIDDEN

De Carlo P., Rechichi A., De Rui M., Marigo L., Granziera S., Nante G., Manzato E.
*Clinica Geriatrica, Scienze Mediche e Chirurgiche, Università di Padova, Padova*

**Background and Objective** Ultrasound imaging, D-Dimer test (DD) and assessments of clinical disease have proved safe in patient with suspected Deep Vein Thrombosis (DVT). Our study examines the development of DVT in a geriatric unit in asymptomatic pts immobilized for more than a week.
**Methods** 199 pts were recruited in the Geriatrics unit of Padova Hospital. All pts were immobilized since at least 7 days. Admittance diagnosis, RF, origin, concomitant pathologies were recorded. Excluding criteria: previous DVT, concomitant anticoagulant therapy, life expectation; presence of DVT's symptoms or signs. Within 24 hrs from admittance, DD was tested and a lower extremities venous with ultrasonographic compression (CUS) performed. After one week, DD was tested again in those pts with negative CUS (CUS–) in phase 1. Statistical analysis involved the use of Students t test and the determination of Odds Ratio (OR), between CUS+ and CUS– pts. The interval of confidence was set at 95%.
**Results** The age=86.4±6.7 yrs. In phase 1, CUS+ was found in 12.6% of pts, with a mean DD of 2164. There wasn't a significant association between the value of the DD and the results of the CUS in phase 1. There was an association between a CUS+ and pathologies like cancer (OR 1.72) and BPCO (2.30). In phase 2, 2.9% developed a DVT, with mean DD of 3,161.
**Conclusion** Data show that the duration of the setting, the age and not correlated with the occurrence of venous thrombosis. In patients with cancer or COPD, there was a significant OR for the diagnosis of DVT, then bedridden patients asymptomatic confirms the usefulness of prophylaxis only during concomitant acute disease or Doppler study if known malignancy. DD in patients with less than 500 (<225 ug/L) were not found DVT.

## P036 INCREASING LEVELS OF FREE THYROXINE AS A RISK FACTOR FOR A FIRST VENOUS THROMBOSIS: A CASE-CONTROL STUDY

Squizzato A.[(1)], Van Zaane B.[(2)] Huijgen R.[(2)], Van Zanten A.P.[(3)], Fliers E.[(4)], Cannegieter S.C.[(5)], Buller H.[(6)], Gerdes V.E.A.[(7)], Brandjes D.P.M.
*[(1)] Medicina Clinica, Università dell'Insubria, Varese; [(2)] Vascular Medicine Academic Medical Centre, Amsterdam, The Netherlands; [(3)] Clinical Biochemistry, Slotervaart Hospital, Amsterdam, The Netherlands; [(4)] Endocrinology, Academic Medical Centre, Amsterdam, The Netherlands; [(5)] Clinical Epidemiology, Leiden University Medical Centre, Leiden, The Netherlands; [(6)] Vascular Medicine, Academic Medical Centre, Amsterdam, The Netherlands; [(7)] Internal Medicine, Slotervaart Hospital, Amsterdam, The Netherlands*

**Introduction** There is a hypercoagulable state in hyperthyroidism, but the association with venous thrombosis (VT) is not fully explored. We aimed to investigate VT risk for different plasma levels of thyroid hormones and thyroid antibodies.
**Methods** We used a case-control study on leg vein thrombosis conducted between September 1999 and August 2006 at the Academic Medical Centre, Amsterdam, The Netherlands. Parameters of thyroid function were assessed in 190 cases (mean age 57 years, range 19-90) and 379 gender-matched controls (mean age 56 years, range 18-93). Odds ratios (ORs) and 95% confidence intervals (CIs) for VT risk were estimated according to several cut-off levels derived from plasma levels observed in controls.
**Results** We found the risk of venous thrombosis to gradually rise with increasing levels of free thyroxine (FT4). In the absence of traditional acquired risk factors, FT4 levels above 17 pmol/L yielded a gender- and age-adjusted OR of 2.2 (95% CI 1.2-4.2) for deep venous thrombosis, which further increased up to an OR of 13.0 (95% CI 1.1-154.1) for FT4 levels above reference range.
**Conclusions** Our data suggest increasing levels of free thyroxine to be a risk factor for venous thrombosis and may have implications for both the prevention and management of this disease.

## P037 RISK FACTORS AND MANAGEMENT OF VTE IN A COHORT OF 163 CONSECUTIVE WOMEN TREATED IN A SINGLE OBSTETRIC INSTITUTION

Donvito V.[(1)], Maina A.[(2)], Arrotta M.[(2)], Cicogna L. [(2)], Di Prima S.[(2)]
*[(1)] Dipartimento Ginecologia, Servizio di Medicina Interna, ASO OIRM Sant'Anna, Torino; [(2)] Servizio di Medicina Interna, ASO OIRM Sant'Anna, Torino*

From 1994 until 2009, 163 consecutive women were referred to the Internal Medicine Unit of our obstetric institution, a third level referral centre with around 8,000 deliveries per year.
Out of 163 thromboembolic events, 39 were pulmonary embolism (PE), 122 were venous thromboembolism (VT), 2 of them cerebral VT.
106 events occurred during pregnancy and 57 in the post-partum period.
If we consider the 57 patients who experienced VTE in puerperium, 43 (75%) delivered by caesarean section (CS) versus 14 (25%) by spontaneous vaginal delivery (SVD).
CS seems to be the major risk factor for VTE in our patients (OR=6).
We compared the figures from the first 8 years (1994-2001) of our observational period with the second part of this period (2002-2009): we found that the ratio of puerperal VTE/total VTE decreased from 46% of earlier period to 27% of the latter period of observation.
Considering only puerperal VTE, the ratio post CS VTE/puerperal VTE decreased from 87% to 60%, respectively in the two periods of observation (p=0,033).
Two considerations arise from these figures:

- in the last 10 years the standard care has changed with regard of thromboprophylaxis after CS and the widespread diffusion of neuraxial anesthesia and peridural analgesia. Our data are in agreement with other European Maternal Institutions and show a definite reduction in the VTE in puerperium and after CS;
- number of VTE during pregnancy remains consistent and constant over the period observed. Sound measures to identify women at risk are needed and should be applied in advance or in early gestation, because occurrence of VTE is increased since the first weeks of pregnancy or even earlier in case of *in vitro* fertilization.

Management of women with VTE in our patients required the application of caval filter in 9 cases, with two main indications:

concomitant haemorrhagic risk or impending labour and delivery. Complications were rare and successful pregnancies with healthy babies were seen in the majority of the cases.

P038 **TRATTAMENTO DELLE TROMBOSI VENOSE RARE: REVISIONE DELLA LETTERATURA ED ESPERIENZA DI UN CENTRO PERIFERICO (DOMODOSSOLA)**

De Gaudenzi E.
*Rep. Medicina Interna, Amb. Angiologia, Ospedale di Domodossola, Domodossola*

**Introduzione** La TVP interessa prevalentemente gli arti inferiori con o senza EP. Più raramente può colpire le vene degli arti superiori, i vasi splacnici e i seni cerebrali. La sintomatologia è a volte subdola e trascurata, ma spesso clinicamente severa e caratterizzata da importante morbilità e mortalità.
**Scopo del lavoro** Nella letteratura non vi sono studi multicentrici estesi che riguardano la terapia delle trombosi venose rare: recenti linee guida suggeriscono raccomandazioni limitate al grado C. I reparti che gestiscono tali patologie sono vari con interventi terapeutici non univoci. Per tale motivo in questo lavoro tentiamo di focalizzare aspetti patogenetici e di trattamento che riguardano la TV dei seni cerebrali, delle v. splacniche e degli arti superiori, omettendo le trombosi retiniche che costituiscono la forma più comune della patologia vascolare dell'occhio e quindi non possono essere considerate rare.
**La nostra esperienza** Nell'ambulatorio di gestione dei pz anticoagulanti e di diagnostica vascolare del reparto di medicina interna del nostro ospedale abbiamo seguito negli ultimi 10 anni di attività anche pz con trombosi venose rare e soprattutto dell'arto superiore. I pz trattati per tale patologia sono stati 25; di questi sono state valutate le percentuali di complicanze (embolie polmonari, recidive e sindromi post trombotiche), di patogenesi trombofilica e la durata della terapia anticoagulante effettuata.

P039 **PREVALENCE OF INHERITED AND ACQUIRED HYPERCOAGULABLE STATES IN YOUNG PATIENTS WITH THROMBOSIS**

Sottilotta G., Latella C., Luise F., Oriana V., Piromalli A., Ramirez F., Romeo E., Trapani Lombardo V.
*Azienda Ospedaliera Bianchi-Melacrino-Morelli, Centro Emofilia, Servizio Emostasi e Trombosi, Reggio Calabria*

**Introduction** The prevalence of thromboembolic diseases increases with age. Aim of this study was to evaluate the prevalence of several congenital and acquired risk factors for arterial and venous thrombosis in a group of young patients.
**Materials and Methods** We analysed data from 153 patients (42 males, 111 females) with less than 40 years (age 14-39, median age 30) referred to our centre in the last ten years because of thrombotic events. All subjects were screened for G20210A prothrombin mutation (FII G2010A), factor V Leiden (FVL) and other causes of inherited and acquired thrombophilia.
**Results** Deep venous thrombosis (DVT) was found in 53 patients (34.6%), pulmonary embolism (PE) in 42 (27.4%); thirty-one patients had DVT/EP (20.3%). Acquired hypercoagulable states were found in 112/153 subjects (73.2%): in 72 of them (21 in post-partum, surgery: 12, oral contraceptive: 10, pregnancy: 7, trauma: 6, cancer: 4, arterial dissection: 3, congenital cardiopathy: 2, other causes: 7) no other prothrombotic condition was evidenced. In 22/153 cases (14.4%) we didn't found any thrombosis risk factor. Of all patients, 13 were heterozygous and 4 homozygous for the FVL. The FII G20210A was found in 21 patients (all heterozygous). 5 patients were heterozygous for both mutations. The FII G20210A had the higher prevalence in patients with DVT, PE, and DVT/PE: 20.7%, 16.7% and 25.8% respectively. FVL had the highest prevalence in patients with thrombosis in atypical sites: in 83.3% of these subjects FVL was associated with other acquired hypercoagulable states.
**Conclusion** Venous thromboembolism was confirmed as the most frequent form of thrombosis in subjects up to age 40. Our results also evidenced a strong association between venous thrombosis and thrombophilia in young people and the importance of screening for thrombophilia especially in presence of familial thrombosis history.

P041 **LONG-TERM EVALUATION OF THE RISK OF RECURRENCE AFTER CEREBRAL SINUS-VENOUS THROMBOSIS**

Martinelli I., Bucciarelli P., Passamonti S.M., Battaglioli T., Mannucci P.M.
*Dipartimento di Medicina Interna, Centro Emofilia e Trombosi "A. Bianchi Bonomi", Milano*

**Background** The clinical course of cerebral sinus-venous thrombosis (CSVT) is largely unknown because prospective studies are lacking with a long follow-up and with the goal to assess thrombosis recurrence rate and predisposing factors for recurrence.
**Methods and Results** 145 patients with a first CSVT were followed-up for a median time of 6 years after discontinuation of anticoagulant treatment. End points were recurrent CSVT or other clinical manifestations of venous thromboembolism. CSVT recurred in 5 patients (3%) and other manifestations of venous thromboembolism (deep vein thrombosis of the lower limbs or pulmonary embolism) in 10 additional patients (7%), for a recurrence rate of 2.03 per 100 person-years (95% CI 1.16-3.14) for all manifestations of venous thromboembolism and 0.53 per 100 person-years (95%CI 0.16-1.10) for CSVT. Nearly half of the recurrences occurred within the first year after discontinuation of anticoagulant therapy. Risk factors for recurrent venous thrombosis were male sex (adjusted hazard ratio 9.66, 95% CI 2.86-32.7) and, for thromboses other than CSVT, severe thrombophilia due to antithrombin, protein C, protein S deficiency, antiphospholipid antibodies or combined abnormalities (adjusted hazard ratio 4.71, 95% CI 1.34-16.5).
**Conclusions** The risk of recurrent CSVT is low, being higher in the first year after discontinuation of anticoagulant treatment and among men. Mild thrombophilia abnormalities are not associated with recurrent CSVT, but severe thrombophilia entails an increased risk of deep vein thrombosis of the lower limbs or pulmonary embolism.

P042 **CEREBRAL VEIN THROMBOSIS: DDIMER LEVELS AFTER ORAL ANTICOAGULANT**

**WITHDRAWAL AND RISK OF RECURRENCE**

Poli D.[1], Antonucci E.[2], Grifoni E.[2], Lami D.[2], Abbate R.[2], Prisco D. [2]
[1] *Dept of Heart and Vessels, Azienda Ospedaliero-Universitaria Careggi Thrombosis Centre, Firenze;* [2] *Dept of Medical and Surgical Critical Care, University of Florence, Firenze*

Cerebral vein thrombosis (CVT) is a rare disease and scanty information is available on the risk of recurrence after treatment with oral anticoagulants (OA). Available data are in agreement in reporting that recurrences of CVT and venous thromboembolic events (VTE) after a first episode of CVT are uncommon. It is well known that among patients with a first episode of VTE of the lower limbs elevated Ddimer (DD) levels after OA withdrawal are related with an increased risk of recurrent VTE. Aim of our study was to measure, in patients with a first episode of CVT treated with a standard course of OA (at least 3 months, INR 2.0-3.0), DD levels 1 month after OA discontinuation and search if they are related with recurrent CVT or VTE.
We prospectively followed-up 17 patients (12 F, 71%) with a first CVT episode; median age 44 (19-69) years. In 8/17 (47%) patients a haemorrhagic complication was detected at presentation; all patient had full neurological recover (Rankink scale=0). Patients were treated with OA for a median time of 12 months (3-36); follow-up after OA withdrawal was 42.5 months (3-194); 16/17 patients had idiopathic CVT, 10/17 (10/12 F) were on treatment with oral contraceptives (OC) at CVT occurrence. Neuroradiological examination after treatment showed persistent occlusion of cerebral veins in 3/17 patients (18%) and incomplete re-canalization in 5/17 (29%). All patients were studied for thrombophilia: one was heterozygous for prothrombin gene polymorphism and 2 for factor V Leiden mutation. During follow-up no recurrent events were recorded. All patients had normal DD levels after OA withdrawal.
In conclusion, in our patients no recurrence was recorded after a first CVT episode. In agreement with the low risk of recurrence in these patients, DD levels after OA withdrawal were found normal in all cases.

P043 **DOES THE CLINICAL PRESENTATION AND EXTENT OF VENOUS THROMBOSIS PREDICT LIKELIHOOD AND TYPE OF RECURRENCE? A PATIENT LEVEL META-ANALYSIS OF 2,554 UNSELECTED PATIENTS AFTER A FIRST THROMBOSIS**

Marcucci M.[1], Tosetto A.[2], Baglin T.[3], Douketis J.[4], Cushman M.[5], Eichinger S.[6], Palareti G.[7], Poli D.[8], Tait C.[9], Iorio A.[1]
[1] *Medicine, Internal Vascular Medicine, Perugia;* [2] *Haematology, St. Bortolo Hospital, Vicenza;* [3] *Haematology, Addenbrookes Hospital, Cambridge;* [4] *Medicine McMaster University, Hamilton, Canada;* [5] *Medicine, University of Vermont, Colchester, USA;* [6] *Medicine, University of Vienna, Vienna, Austria;* [7] *Medicine, University of Bologna, Bologna;* [8] *Thrombosis Centre, Azienda Ospedaliera Universitaria Careggi, Firenze;* [9] *Haematology, Royal Infirmary, Glasgow, United Kingdom*

**Aim** To determine if the mode of presentation of a first episode of venous thromboembolism (VTE) predicts likelihood and type of recurrence.
**Materials and Methods** Patient-level meta-analysis of seven prospective cohort studies. Time-to-event analysis was performed by Kaplan-Meier estimates with cumulative recurrence rates reported at different years of follow up and annualized rates presented as events per 100 patient-years. Hazard ratios (HR) were calculated by study-stratified Cox regression models by including two- and three-level variables for clinical presentation and extent of disease and adjusting for other putative confounders (age, sex, provoked or unprovoked VTE, hormone therapy).
**Results** In 869 patients presenting with symptomatic PE the cumulative rate of recurrence at 5 years was 22.0% and recurrence as PE was 10.6%. In 1,365 patients presenting with symptomatic proximal DVT without symptomatic PE the recurrence rate at 5 years was 26.4% and recurrence with PE was 3.6%. The risk of recurrence as PE was about 3-fold greater in patients presenting with symptomatic PE compared to proximal DVT (hazard ratio 3.1, 95% confidence interval [CI] 1.9 to 5.1) and tended to be greater in patients presenting with proximal DVT compared to distal DVT, even if without a statistical significance (hazard ratio 4.5, 95% CI 0.6 to 33.9). Patients with clot confined to the calf had an almost 5-fold lower cumulative recurrence rate than patients with proximal DVT without PE (hazard ratio 4.8, 95% CI 2.1-11.0).
**Conclusion** Whilst DVT and PE are manifestations of the same pathology the phenotype of the disease is predetermined. Patients presenting with symptomatic PE are 4-times more likely to suffer recurrence as PE compared to patients presenting with DVT alone. Patients presenting with DVT confined to the calf veins are at low risk of recurrence and of recurrence as PE.

P045 **ABSENCE OF RESIDUAL VEIN THROMBOSIS AFTER AN EPISODE OF FIRST IDIOPATHIC VTE**

Malato A.[1], Saccullo G.[1], Lo Coco L.[1], Abbadessa V.[2], Siragusa S.[1]
[1] *U.O.S. Interdipartimentale di Emostasi, Policlinico Universitario di Palermo, Palermo;* [2] *Ematologia, Policlinico Universitario di Palermo, Palermo*

**Background** The optimal duration of Oral Anticoagulant Therapy (OAT) for Deep Vein Thrombosis (DVT) can be tailored by Residual Vein Thrombosis (RVT). However, in patients with idiopathic DVT the safety of early interruption of OAT, because of absence of RVT, is still debated. 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. 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. 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 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 recurrent events [n/100 person-year (%)] between patients with and without RVT were 63/191.3 (32.9%) and 2/184.7 (1.08%), respectively. This difference was statistically significant ($p<0.0005$). Major bleeding occurred in 3/312 (0.9%) of patients who continued OAT for 1 year; no events were recorded in those who stopped anticoagulation after 3 months.
**Conclusions** This investigation shows that in patients without VT, three months of OAT are safe even after an episode of idiopathic DVT. This approach carries also a negligible risk for bleeding.

## P046 D-DIMER TO PREDICT RECURRENCE AFTER A FIRST EPISODE OF UNPROVOKED VENOUS THROMBOEMBOLISM: COMPARISON OF AGGREGATE AND INDIVIDUAL PATIENT DATA META-ANALYSIS

Marcucci M.[(1)], Douketis J.[(2)] Tosetto A.[(3)], Baglin T.[(4)], Cushman M.[(5)], Eichinger S.[(6)], Palareti G.[(7)], Poli D.[(8)], Tait C.[(9)], Iorio A.[(1)], on behalf of the REPOSI investigators
*[(1)] Medicine, Internal Vascular Medicine, Perugia; [(2)] Medicine McMaster University, Hamilton, Canada; [(3)] Haematology, St. Bortolo Hospital, Vicenza; [(4)] Haematology, Addenbrookes Hospital, Cambridge, United Kingdom; [(5)] Medicine, University of Vermont, Colchester, USA; [(6)] Medicine, University of Vienna, Vienna, Austria; [(7)] Medicine, University of Bologna, Bologna; [(8)] Thrombosis Centre, Azienda Ospedaliera Universitaria Careggi, Firenze; [(9)] Haematology, Royal Infirmary, Glasgow, United Kingdom*

**Background** Individual patient data (IPD) meta-analysis, even if more resource demanding, as compared to aggregate data (AD) meta-analysis, can more rigorously elaborate time-to-event data and investigate sources of heterogeneity.
**Methods** We compared the performance of 2 meta-analyses pooling the same set of studies on the efficacy of D-dimer to stratify the risk of thrombosis recurrence after anticoagulation stopping in patient with a first unprovoked venous thromboembolism (VTE). AD meta-analysis provided annualized recurrent rates and a pooled risk ratio for positive versus negative D-dimer patients by a mixed-effects Poisson model. In addition to annualized rates, in the IPD meta-analysis a Kaplan-Mayer survival analysis was performed to obtain cumulative hazard for recurrence 1, 3 and 5 years after stopping anticoagulation according to D-dimer status, either as defined in each source study or basing on pre-specified cut-off points (250 and 500 ng/mL), also for age and D-dimer test timing subgroups. IPD-based study-stratified multivariable Cox regression was compared to meta-regression based on aggregate data.
**Results** Overlapping annualized VTE recurrence rates were found by the two approaches (8.8-8.9 for positive, 3.5-3.7 for negative D-dimer patients). IPD-based cumulative hazard after 3 years was 25.4 (95% confidence interval [CI] 21.3-30.4) for positive and 9.3 (95% CI 7.1-12.1) for negative D-dimer patients. AD-based pooled risk ratio and IPD-based hazard ratio suggested a 2.2-2.5-fold higher recurrence risk for positive versus negative D-dimer patients. Subgroup analyses and Cox regression showed that none of hypothetical confounders (age, BMI, sex, hormonal therapy, genetic thrombophilia, timing of D-dimer testing, qualitative/ quantitative definition of D-dimer status) affected the D-dimer prognostic efficacy. Meta-regression was not able to demonstrate it.
**Conclusions** The AD and IPD meta-analyses on D-dimer yielded comparable findings but only IPD was able to explore the trend over time of recurrence risk and the effect of patient-level confounders on the prognostic value of D-dimer.

## P047 RATIONALE AND STUDY DESIGN OF THE SURVET TRIAL (SULODEXIDE IN SECONDARY PREVENTION OF RECURRENT DEEP VEIN THROMBOSIS)

Andreozzi G.M.[(1)] Palareti G.[(2)], on behalf of the SURVET investigator group
*[(1)] UOC Angiology, University Hospital, Padua; [(1)] Angiology and Blood Coagulation, University Hospital S. Orsola-Malpighi, Bologna*

Prevention and acute-phase therapy of venous thromboembolism (VTE) are well codified and standardized. Post-acute therapy, also known as secondary prevention, which is designed to avoid recurrent events (that affect up to 17-20% of patients two years after stopping anticoagulant therapy, and 30% within 5 years) is not well established. The guidelines indicate an optimal duration of anticoagulant therapy from 3 months to 6 months on the base of patients' clinical profile. In some cases it is suggested to prolong the treatment until 12 months (ACCP 2008). After this period the risk of haemorrhagic events increases and the risk-benefit balance is towards the risk. For this reason, in most of cases, the therapy is interrupted. Starting from this time, the still present risk of VTE recurrence could be decreased by sulodexide, a glycosaminoglycan with antithrombotic properties and devoid of bleeding side effects.
**Methods** A randomized, double blind, placebo controlled trial designed to study long-term treatment with sulodexide for the prevention of recurrent VTE after the standard anticoagulation therapy with oral vitamin K antagonists administered for 3 to 12 months. SURVET trial will examine the efficacy of sulodexide given for two years to almost 800 patients in 50 centres in Czech Republic, Italy, Poland, Portugal, Romania, Slovak Republic and Russia. The primary outcome is the occurrence, within 24-month treatment period, of any of the following events: proximal deep vein thrombosis (DVT) of lower limb; pulmonary embolism; death due to documented VTE episode. The secondary efficacy endpoints include: time to the qualifying event; occurrence of isolated DVT of the legs; occurrence of superficial vein thrombosis of the legs; occurrence of post-thrombotic syndrome; occurrence of major cardiovascular events (AMI, stroke). The SURVET trial will document whether therapy with sulodexide is able to reduce recurrences of VTE episodes.
**Keywords** Sulodexide, VTE, DVT, glycosaminoglycans.

## TROMBOEMBOLISMO VENOSO: TERAPIA

### P048 SAFETY OF THROMBOLYSIS IN CEREBRAL VENOUS THROMBOSIS

Dentali F.[1], Squizzato A.[1], Gianni M.[2], De Lodovici M.L.[3], Venco A.[1], Paciaroni M.[4], Crowther M.[5], Ageno W.[1]
[1] *Medicina Clinica, Università dell'Insubria, Varese;* [2] *Cardiologia, Ospedale di Tradate, Tradate;* [3] *Neurologia, Ospedale di Circolo, Varese;* [4] *Medicina Cardiovascolare, Università di Perugia, Perugia;* [5] *Haematology, McMaster University, Hamilton, Canada*

**Background** Several small series have demonstrated the efficacy of thrombolysis in patients with cerebral vein thrombosis (CVT). However, since no randomized controlled trials have compared the use of thrombolysis with anticoagulant treatment in these patients, the risk to benefit ratio of this approach remains uncertain. The aim of this study is therefore to assess the safety of thrombolysis in CVT estimating mortality and major bleeding complications.
**Data Sources** MedLine and Embase databases were searched up to August 2009.
**Study Selection** Two reviewers performed study selection independently. Studies providing data on mortality and/or on the incidence of major bleeding complications were potentially eligible for the study.
**Data Extraction** Two reviewers independently extracted data on study and population characteristics, type, dose and administration route of thrombolytic treatment; use and dose of concomitant heparin. Weighted mean proportion of the mortality rate and of the rate of major and non-major bleeding complications were calculated.
**Results** Fourteen studies for a total of 148 patients were included. Twelve patients died after thrombolysis (weighted mean 7.3%; 95% CI 2.6, 14.3%) and 15 patients had a major bleeding complication (weighted mean 10.4%; 95% CI 5.6, 16.5%). Twelve haemorrhages were intracranial (weighted mean 8.0%; 95% CI 3.6, 14.0%) and 7 of these patients died (58.3%; 95% CI 32.0, 80.7%).
**Conclusions** Our results suggest that thrombolysis is associated with a non-negligible incidence of major bleeding complications, including intracranial bleeding potentially affecting patients' outcome.
Future studies are necessary to evaluate the efficacy and safety of thrombolysis in comparison to more conservative strategies.

## EMBOLIA POLMONARE E IPERTENSIONE POLMONARE

### P049 FIBRINOLYTIC INHIBITORS AND FIBRIN CHARACTERISTICS DETERMINE A HYPOFIBRINOLYTIC STATE IN PATIENTS WITH PULMONARY EMBOLISM

Grifoni E.[1], Cellai A.P. [2], Lami D.[1], Fiorillo C.[3], Becatti M.[3], Miniati M.[1], Poli D.[2], Abbate R.[1], Prisco D.[1]
[1] *Department of Medical and Surgical Care, University of Florence, Florence;* [2] *Department of Heart and Vessels, Thrombosis Centre, Azienda Ospedaliero Universitaria Careggi, Florence;* [3] *Department of Biochemical Sciences, University of Florence, Florence*

**Introduction** Mechanisms determining pulmonary embolism (PE) and its resolution are not well known, and many studies on fibrinolytic system have been so far inconclusive. We recently demonstrated in studies on purified fibrinogen exposed to thrombin to obtain fibrin clots, subsequently digested with plasmin up to 6 hours, that fibrin degradation was significantly slower in PE patients than in controls. Aim of the study. In 33 patients with PE without pulmonary hypertension, previously evaluated for fibrin resistance to plasmin digestion, and 23 healthy controls we evaluated fibrinolytic system and correlated these data with those previously obtained.
**Methods** Fibrinolytic system was studied by both global functional tests and by measuring a number of its components in plasma.
**Results** PE patients showed a more prolonged clot lysis time (at least $p<0.001$) and significantly higher plasma levels of TAFI activity, PAI-1 antigen, t-PA antigen and factor XIII ($p<0.05$) than controls. Clot lysis time in PE patients was influenced by both the levels of PAI-1 and TAFI and the impairment of plasmin-mediated lysis of fibrin β-chain ($p<0.001$). Moreover, in patients with incomplete restoration of pulmonary perfusion PAI-1 and ECLT values were significantly higher than in those without residual perfusion defect ($p=0.007$ and $p=0.001$, respectively).
**Conclusions** A hypofibrinolytic state, determined by both fibrin resistance to plasmin digestion and excess of fibrinolysis inhibitors, is present in most patients with previous PE, and can influence the restoration of pulmonary perfusion after PE.

### P050 FIBRIN RESISTANCE TO LYSIS IN PULMONARY HYPERTENSION OTHER THAN THROMBOEMBOLIC

Miniati M.[1], Fiorillo C.[2], Becatti M.[2], Monti. S.[3], Marini C.[4], Grifoni E.[1], Poli D.[5], Abbate R.[1], Prisco D.[1]
[1] *Area Critica Medico Chirurgica, Università di Firenze, Firenze;* [2] *Scienze Biochimiche, Università di Firenze, Firenze;* [3] *Fisiologia Clinica, CNR, Pisa;* [4] *Cardiotoracico, Università di Pisa, Pisa;* [5] *Malattie Aterotrombotiche, Azienda Ospedaliero-Universitaria, Firenze*

**Rationale** Reportedly, fibrin isolated from patients with chronic thromboembolic pulmonary hypertension (CTEPH) is resistant to lysis. Persistence of regions within the fibrin β chain, which mediate cell signalling and migration, could trigger the organization of pulmonary thromboemboli into chronic intravascular scars.
**Objectives** Ascertain whether fibrin resistance to lysis occurs in patients with pulmonary arterial hypertension (PAH) other than CTEPH, and in those with prior pulmonary embolism (PE) and no pulmonary hypertension.
**Methods** Fibrinogen was purified from 96 subjects (17 with CTEPH, 14 with PAH, 39 with prior PE, and 26 healthy controls) and exposed to thrombin to obtain fibrin clots. Plasmin-mediated cleavage of fibrin β chain was assessed hourly over a 6-hour period by polyacrilamide gel electrophoresis. Fibrin band intensity was measured by densitometry of stained gels. Data were normalized to the

band intensity of the undigested protein (t=0).
**Measurements and main results** By one hour of digestion, the fibrin band intensity had decreased by a median of 25% (interquartile range [IQR], 20 to 27%) in controls, and by 15% (IQR, 11 to 18%) in patients with prior PE (p<0.0001). The one-hour median reduction in band intensity was 2% (IQR, 1 to 3%) in CTEPH, and 4% (IQR, 2 to 7%) in PAH (p<0.0001 vs. controls and PE). The decline in fibrin band intensity remained significantly different among the 4 groups up to 6 h of incubation with plasmin (p<0.0001).
**Conclusions** Fibrin resistance to lysis occurs in pulmonary hypertension other than CTEPH and, to a smaller extent, in patients with prior PE and no pulmonary hypertension.

## *EMOFILIA E ALTRI DISORDINI (EMORRAGICI)*

### P051 **RECOMBINANT ACTIVATED FACTOR VII (RFVIIA) FOR TREATMENT OF PATIENTS WITH FACTOR V DEFICIENCY: A CASE REPORT AND REVIEW OF THE LITERATURE**

Coppola A.[(1)], Feola G.[(2)], Di Capua M.[(1)], Conca P.[(1)], Di Palo M.[(1)], Marrone E. [(1)], Ruocco A.L.[(1)], Di Minno M.N.D.[(1)], Tomaiuolo M.[(3)], Margaglione M.[(3)]
*[(1)] DACM, Centro di Riferimento Regionale per la Cura dell'Emofilia, Azienda Ospedaliera Universitaria, Napoli; [(2)] Immunoematologia e Centro Emofilia, Ospedale S. Luca, Vallo della Lucania; [(3)] Genetica Medica, Università di Foggia*

**Introduction** Patients with Factor V deficiency show heterogeneous, mainly muco-cutaneous, bleeding tendency, for which fresh frozen plasma (FFP) still represents the only available replacement treatment. Aside from the residual risk of blood-borne infections and of volume overload, the need for alternative haemostatic treatment is particularly important in patients developing FFP-related immunologic complications, including inhibitors, allergy/anaphylaxis and transfusion related acute lung injury.
**Report** We report the challenging gynecological history (severe menorrhagia and two episodes of haemoperitoneum due to haemorrhagic follicle cysts) and a successful pregnancy of a woman with severe FV deficiency (residual activity 1%, compound heterozygous defects p.Arg712Stop and c.3510-3511 delAA), whose treatment was complicated by transfusion anaphylactic reactions. Leukodepleted FFP from a single related donor (her brother) enabled prophylaxis and treatment of bleeding in several occasions. When the patient became pregnant and prolonged haemostatic coverage was needed at Caesarean section, a single infusion of her brothers apheresed plasma was followed by rFVIIa bolus injections (90 µg/kg every 4-6 hrs for 4.5 days). No excessive bleeding or adverse events occurred.
**Review of literature** Other 6 patients with FV deficiency, all but two with FFP-related immunological complications, were successfully treated with rFVIIa, even at home. Eleven joint bleeds, four muscle and one breast haematomas, one intestinal bleeding and four surgical procedures were reported. In all cases standard rFVIIa doses (80-120 µg/kg) as bolus injections (1-31) and interval of administration similar to haemophilia with inhibitors were used. FFP was associated only in one post-operative treatment.
**Conclusions** Management of FV-deficient patients, especially women at risk for menstrual bleeding and pregnancy, may be highly challenging, because of the limitations of the only available therapeutic option. Although clinical experience is still limited, treatment with rFVIIa may provide an alternative approach, in particular for patients with FFP-related immunologic complications.

### P052 **CLOTTING FACTOR CONCENTRATES GIVEN TO PREVENT BLEEDING AND BLEEDING-RELATED COMPLICATIONS IN PEOPLE WITH HAEMOPHILIA A OR B**

Marchesini E.[(1)], Marcucci M.[(2)], Stobart K.[(3)], Chan A.[(3)], Iorio A.[(2)]
*[(1)] Internal and vascular medicine, University of Perugia, Perugia; [(2)] Haemophilia Centre, University of Perugia, Perugia; [(3)] Hemophilia Centre, University of Edmonton, Canada*

**Introduction** The hallmark of severe hemophilia (factor VIII/IX <1%) is recurrent bleeding into joints and soft tissues with a progressive joint damage, which was shown to be prevented by prophylaxis by Swedish pioneering observational studies and may other later on.
**Objectives** To determine the effectiveness of clotting factor concentrate prophylaxis in the management of people with hemophilia A (HA) or B (HB).
**Methods** We searched the Cystic Fibrosis and Genetic Disorders Group's Trials Register comprising references from comprehensive electronic database searches and handsearches of journals and abstract books. Reference lists of relevant articles were reviewed. Last update was updated on June 2009. Randomised or quasi-randomised clinical trials reporting rates of bleeding in severe HA or HB, receiving prophylactic treatment regimens were included.
**Results** Six studies (including 142 participants) were eligible for inclusion. Five studies evaluated secondary and one primary prophylaxis. Five enrolled HA, one HB patients. The results of the four older studied were not pooled and reported in a Cochrane review. Studies included in the current update compared prophylaxis versus on demand regimens and showed a statistically significant reduction of total hemorrhages (mean difference 10.89) and joint bleeds (mean difference 4.22).
**Conclusion** New evidence from randomised controlled trials recently arose to confirm effectiveness of prophylaxis to prevent bleeding episodes in severe HA patient. The advantage was highest when treating patients very early.
**Contribution to the practice/evidence base of haemophilia and bleeding disorders** Prophylaxis has to be considered the treatment of choice to prevent bleed joints in hemophilia patients.

### P053 **MOLECULAR CHARACTERIZATION OF FOUR ITALIAN FAMILIES WITH INHERITED SEVERE FXIII DEFICIENCY**

Giacomelli S.H.[(1)], Sanna S.[(1)], Molinari A.C.[(2)], Valdrè L.[(3)], Rodeghiero F.[(1),] Castaman G.[(1)]
*[(1)] Department of Cell Therapy and Hematology, Hemophilia*

*and Thrombosis Centre, San Bortolo Hospital, Vicenza; [2] Hemostasis and Thrombosis Unit, Department of Hematology and Oncology, Giannina Gaslini Children's Hospital, Genova; [3] Angiology and Coagulation Department, S. Orsola-Malpighi Hospital, Bologna*

Hereditary factor XIII (FXIII) deficiency is a very rare autosomal recessive bleeding disorder mostly due to mutations in FXIII A subunits. We have studied the molecular basis of severe FXIII deficient patients from four unrelated Italian families. The coding region, intron-exon boundaries and 5-3F untranslated regions of the FXIII A subunit gene were amplified and sequenced. Patient 1 and 2 are respectively heterozygous and homozygous for the Gly562Arg variation, which has been already reported in Italian patients with FXIII deficiency. However, the mutation seems to segregate with different haplotypes. The second mutation was not found in patient 1. Probably it could be due to heterozygous gene/exons deletion recognizable through other methods (MLPA and/or mRNA analysis). Patient 3 (FXIII:C <5%) is compound heterozygote for two novel deletions, in intron 11 (c.1460 -2 Del A) and in exon 12 (c.1809-1811 Del AAG).
In silico analysis by web software (www.fruitfly.org/seq_tools/splice.html and www.cbs.dtu.dk/services/NetGene2/) of the first deletion in predicts the abolition of acceptor splice site of exon 12 probably leading to exon skipping. The c.1809-1811 Del AAG mutation is an in-frame deletion causing the loss of the amino acid lysine at position 569 in the Barrel 1 domain of the protein. The remaining patients (FXIII:C 4%) are two brothers resulting compound heterozygotes for two missense mutations, one previously reported in exon 7 (Tyr283Cys) and one novel in exon 15 (c.2212 CGG>CAG Arg703Gln). To date, this is the second sequence change to be found at codon 703. With this novel mutation the basic amino acid arginine in the Barrel 2 domain is substituted by the hydrophilic glutamine. Moreover the Arg 703 is an amino acid residues conserved among different species. In conclusion, all the variations found in these patients can be considered disease-causative. Molecular modelling will help in elucidating the structural role of these mutations.

## P054 CHOOSING IMMUNE TOLERANCE INDUCTION IN PATIENTS WITH HAEMOPHILIA AND HIGH-RESPONDING INHIBITORS: WHY OR WHY NOT?

Coppola A.[1], Rocino A.[2], Mancuso M.E.[3] Scaraggi F.A.[4], Castaman G.[5], Tagliaferri A.[6], Latella C.[7], Schinco P.[8], Messina M.[9], Santoro R.[10], Gandini G.[11], Santagostino E.[3]
*[1] Regional Reference Centre for Coagulation Disorders, Federico II University, Napoli; [2] Haemophilia and Thrombosis Centre, S. Giovanni Bosco Hospital, Napoli; [3] Dep. of Medicine and Medical Specialties, IRCCS Ca' Granda Foundation, Maggiore Hospital, "A. Bianchi Bonomi" Haemophilia and Thrombosis Centre, Milano; [4] University Hospital Haemophilia Centre, Dep. of internal Medicine Bari; [5] Centre for Thrombotic and Haemorrhagic Disorders, Dep. of Haematology, S. Bortolo Hospital, Vicenza; [6] Regional Reference Centre for Congenital Bleeding Disorders, University Hospital, Parma; [7] Malacrino Bianchi Morelli Hospital Haemophilia Centre Reggio Calabria; [8] Regional Reference Centre for Thrombotic and Haemorragic Disorders, University Dep. of Haematology, Torino; [9] Regional Reference Centre for Haemorragic Disorders, Regina Margherita Children Hospital, Torino; [10] Pugliese-Ciaccio Hospital Haemophilia Centre Catanzaro; [11] Transfusion and Haemophilia Centre, University Hospital, Verona*

**Introduction** Immune tolerance induction (ITI) is presently the only therapeutic approach able to eradicate inhibitors in haemophilia A patients and represents the first choice in children with recently onset inhibitors. However, ITI is a highly demanding treatment and compliance and cost-utility evaluations may often influence clinical choices, particularly in adults.
**Methods** In the frame of the Italian ITI Registry, participating Centres were asked to register all high-responding inhibitor patients followed between 1996 and 2009. For patients who did not undergo ITI, reasons for clinical choices were reported.
**Results** Eighteen Centres provided data on 149 patients (140 severe), of which 88 (59%) underwent ITI over the study period. ITI was attempted in almost all children (<14 yrs; 65/74, 88%). The lack of parents consent and/or concerns for poor adherence were reported as reasons hampering ITI in four children (5%). ITI was deferred to achieve inhibitor titres <10 BU/mL in the remaining 5 children. Twenty-three/88 ITI patients (23%) were aged >14 yrs. In this age group, ITI was not carried out in 52/75 patients (69%). The perception of poor prognosis (long-standing inhibitors, high historical inhibitor peak titre) was the main reason in 21 (40%) patients, whereas inadequate adherence/refusing treatment and mild bleeding tendency were reported in 16 (31%) and 14 (27%) cases, respectively. Venous access was a major problem only in one patient. Among patients not undergoing ITI, 6 adults (12%) died because of bleeding complications.
**Conclusions** Data from the Italian ITI registry confirm that ITI is attempted virtually in all compliant inhibitor children. This choice currently applies to approximately 30% of patients with long-standing inhibitors. Individual cost-utility and long-term prognostic evaluations, including the risk of severe and fatal bleeding, should be carefully considered in these patients.

## P055 CANCER PREVALENCE IN A COHORT OF HAEMOPHILIC PATIENTS

Santoro R., Iannaccaro P., Prejanò S.
*Centro Emofilia, Emostasi e Trombosi, Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro*

Haemophilia is no longer a disorder of children and young adolescents. Thanks to the introduction of clotting factor concentrates and prophylactic treatment, life expectancy of patients with haemophilia in industrial countries has increased from 7.8 years to over 70 years. Because of this increased life expectancy (age-related) co-morbidity is becoming a common occurrence in haemophilia patients.
Elderly haemophilia patients have different problems compared with the younger generation. They not only have to live with premature arthropathy due to lack of treatment, and with HCV and/or HIV infection, they are also confronted with age-related ailments. So a growing number of haemophilia patients will, besides co-morbility related to haemophilia, suffer from co-morbidity like cardiovascular disease, urological problems and cancer.
We report the experience in haemophilic patients followed in

our institution. Height patients had a diagnosis of cancer. The median age at diagnosis was 64 years (range 39-75). In three patients (all with HCV-related hepatitis) Hepatocellular Carcinoma was diagnosed. Of remaining 5 patients, 2 of them had a diagnosis of a low grade malignancy non Hodgkin Lymphoma (one patient was suffering of HCV-related hepatitis). In 2 patients the diagnosis was Prostatic Cancer. Finally one patient (with HIV and HCV infections) had a diagnosis of Testis Cancer (Seminoma) when he was 39 years old and then, 5 years after, a diagnosis of Colorectal Spinocellular Carcinoma.
The prevalent diagnosis of Hepatocellular Carcinoma in our patients reflects high prevalence of HCV infection in older haemophiliacs treated with plasma-derived Factor VIII concentrates until the mid Eighties. All patients survive (except 2 with Hepatocellular Carcinoma).
Haemophilia does not influence surgery, chemotherapy or radiotherapy when necessary. Better life-expectancy advises a careful surveillance for neoplasms in haemophilic patients.

## P056 MANAGING THE DOUBLE HAEMORRAGIC AND THROMBOEMBOLIC RISK IN PATIENTS WITH ACQUIRED HAEMOPHILIA

Tufano A., Coppola A., Cimino E., Quintavalle G., Ruocco A.L., Marrone E., Ieranò P., Arturo C., Conca P., Cerbone A.M.
*Department of Clinical and Experimental Medicine, Federico II University, Naples*

**Introduction** Most patients with acquired haemophilia (AH) are in the elderly age and carry thromboembolic risk factors or history of vascular disease, requiring antithrombotic therapy.
**Case reports** We report three cases of AH patients in whom the coexistence of bleeding and cardiovascular risk influenced clinical choices. A 80 yr-old man, with a history of coronary artery disease on oral anticoagulation because of atrial fibrillation and ventricular tachiarrhythmias, was diagnosed with AH because of severe post-traumatic haematomas, in spite of cessation of warfarin. Because of cardiovascular risk, a severe iliopsoas haematoma and massive haemothorax were treated with rFVIIa at lower doses (60 ug/kg every 4 hrs for 5 days). Inhibitor disappeared within 6 weeks of steroid treatment. This patient suddenly died probably because of arrhythmic complications.
A 59-yr old man with a history of multiple myeloma and a recently-onset bleeding tendency leading to diagnose AH, showed a severe haematoma of the arm, treated with desmopressin and rFVIII concentrate. During eradication treatment, unstable angina occurred, treated only with anti-ischemic drugs, as the coagulation abnormality led to deferral of coronary angiography and antithrombotic treatment. Inhibitor was negative after 8 weeks. The patient undergone coronary angioplasty and stenting, and started low-dose aspirin with frequent clinical and laboratory follow-up.
AH was diagnosed in a 70 yr-old man with previous myocardial infarction on antiplatelet treatment, evidence of a kidney mass and a history of recurrent bleeding (melena, hematuria; hematomas). Clopidogrel was stopped and a single 90 ug/kg rFVIIa infusion controlled a post-traumatic gluteal haematoma. During eradication treatment, when FVIII levels became >30%, prophylaxis with low-molecular weight heparin was introduced.
**Conclusions** Management of AH patients requires an accurate evaluation of coexisting cardiovascular risk factors/disease. In particular, inhibitor eradication should be obtained as early as possible in order to minimize bleeding risk and to adopt antithrombotic strategies when needed.

## P057 BONE MINERAL DENSITY IN HEMOPHILIA PATIENTS: A META-ANALYSIS

Marcucci M.[(1)], Marchesini E.[(1)], Fabbriciani G.L.[(2)], Brozzetti B.[(1)], Luciani M.[(3)], Iorio A.[(1)]
*[(1)] Internal and Vascular Medicine, University of Perugia, Perugia; [(2)] Emergency Medicine, ASL1 C. Castello Hospital, Città di Castello; [(3)] ASL2, Norcia Hospital, Norcia; Hemophilia Centre, Bambin Gesù Hospital, Roma*

**Introduction** Bone mineral density (BMD) accounts for 60-80% of skeletal mechanical resistance. Hemophilia patients are at risk of osteoporosis because of decreased physical activity, HCV and HIV infections. Aim of the study was to evaluate BMD reduction in severe hemophilia A patients through a systematic review of the literature.
**Methods** We performed a meta-analysis of 7 case-control studies on relationship between hemophilia and osteoporosis providing lumbar BMD values [g/cm$^2$] (all studies), anthropometric data (BMI [kg/m$^2$]: 5/7 studies), and HCV seropositivity (6/7 studies) of severe hemophilia patients and age-matched controls. The random effects method for standardized mean differences (SMD) was used to compare BMD in cases vs. controls, grouping studies by patient age (children or adults). The effect of BMI (BMI SMD cases vs. controls) and HCV infection (% of positive patients) on BMD SMD was investigated by meta-regression.
Results One-hundred-one adult cases (age 33±8.9) with 101 controls and 111 paediatric cases (age 8±3.6) with 307 controls were available for analysis. Lumbar BMD was significantly lower in severe hemophilia patients than in controls, both in adult (pooled SMD –1.379, 95% CI –2.355 to –0.403, p=0.006) and children (pooled SMD –0.438, 95% CI –0.686 to –0.189, p=0.001). The reduction in BMD in patients versus controls was not significantly correlated with the reduction in BMI or with the percentage of HCV infected patients.
**Discussion** This meta-analysis confirms the association between severe hemophilia and low BMD. Future studies should investigate fracture rates and interventions to prevention bone loss in persons with hemophilia.

## P058 INHERITED FIBRINOGEN DEFICIENCY CAUSED BY TWO NOVEL HETEROZYGOUS FIBRINOGEN Bβ AND γ CHAIN MUTATIONS

Sanna S., Giacomelli S.H., Rodeghiero F., Castaman G.
*Department of Cell Therapy and Hematology, Hemophilia and Thrombosis Centre, San Bortolo Hospital, Vicenza*

Fibrinogen is a complex dimeric protein of 340 KD, synthesized in hepatocytes as a hexamer composed of 3 pairs of non identical polypeptides designated as Aα-, Bβ- and γ-chains, involved in the final step of the coagulation cascade. Inherited disorders of fibrinogen are classified according to a complete (afibrinogenemia) or partial (hypofibrinogenemia) fibrinogen deficiency and finally to an abnormal circulating

molecule (dysfibrinogenemia) in plasma.
We report here the identification of two novel mutations occurring in two families. The first patient is asymptomatic while the second had an episode of cryptogenic stroke. Thrombin time (ratio) was 1.58, PT-derived fibrinogen 97 mg/dL and Clauss-derived fibrinogen 90 mg/dL). A novel heterozygous missense mutation in exon 8 of FGG was detected, a transition c.1108 CTC>TTC resulting in a Leu344Phe substitution. Leu344 is highly conserved amino-acid in several species. With this variation the linear and hydrophobic leucine is replaced by the aromatic phenylalanine, probably leading to a steric hindrance at protein level. The second patient (TT=1.49, Reptilase time=1.23, PT-derived fibrinogen 55 mg/dL and Clauss-derived fibrinogen 87 mg/dL) shows a novel heterozygous splice site mutation in intron 5 of FGB (c.832 +2 T>A).
The analysis by web software predicts that this change leads to the abolition (www.fruitfly.org/seq_tools/splice.html and www.cbs.dtu.dk/services/NetGene2/) or reduction (www.umd.be/HSF/) of the original donor splice site probably leading to the some exon-skipping. mRNA study is ongoing to clarify this issue.
In conclusion, we confirm that patients with hypofibrinogenemia (fibrinogen level >50 mg/dL) caused by missense heterozygous mutations do not show a bleeding tendency. The thrombotic risk associated with this splicing mutation leading to a true quantitative fibrinogen deficiency remains to be clarified. The genetic analysis of patients with inherited fibrinogen disorders will help to elucidate the structure-function relationship in the fibrinogen molecule.

## P059 DESCRIBING OCCURRENCE OF CARDIO-VASCULAR EVENTS AND TREATMENT IN HAEMOPHILIA: THE ITALIAN DOCET_H REGISTRY

Coppola A.[1] Tagliaferri A.[2] Zanon E.[3] Giuffrida A.[4], Rocino A.[5], Valeri F.[6], Radossi P.[7], Lapecorella M.[8], Santoro C.[9], Banov L.[10], Di Perna C.[11], Santoro R.[12]
*[1] Regional Reference Centre for Coagulation Disorders, Federico II University, Napoli; [2] Regional Reference Centre for Congenital Bleeding Disorders, University Hospital, Parma; [3] Haemophilia Centre, University Dep. of Internal Medicine, Padova; [4] University Hospital Transfusion and Haemophilia Centre Verona; [5] Haemophilia and Thrombosis Centre, S. Giovanni Bosco Hospital, Napoli; [6] Regional Reference Centre for Thrombotic and Haemorragic Disorders, University Dep. of Haematology, Torino; [7] Transfusion and Haemophilia Centre, Ulss 8 Hospital, Castelfranco Veneto; [8] Internal Medicine and Haematology, Haemophila and Thrombosis Centre, L'Aquila; [9] La Sapienza University Dep. of Haematology Roma; [10] Thrombosis and Haemostasis Unit, Giannina Gaslini Institute, Genova; [11] Regional Reference Centre for Congenital Bleeding Disorders, University Hospital, Parma; [12] Haemophilia Centre, Pugliese-Ciaccio Hospital, Catanzaro*

**Introduction** Although haemophiliacs are likely to be protected from cardiovascular disease, the increasing patient's life expectancy and several case-reports raise questions on the management of ischemic heart disease (IHD) in these patients, particularly concerning percutaneous coronary interventions (PCI) and antithrombotic treatment.
**Methods** A retrospective registry collecting data on management of IHD in haemophilic patients was established in 2009 by the Italian Association of Haemophilia Centres. Eleven Centres provided data on 18 cases (7 severe, 1 moderate and 10 mild haemophilia).
**Results** Acute myocardial infarction (AMI) occurred in 8 cases, unstable angina in 4 and stable angina (SA) in 6, at a mean age of 63 years (range, 42-81). Five patients died, in two cases within 30 days from the acute episode. SA and one AMI were treated only with nitrates in severe patients. In the remaining cases, haemophiliacs received antithrombotic drugs (heparin and/or single or combined antiplatelet treatment). Fourteen patients (6 severe) underwent PCI with concomitant factor replacement (bolus administration, in one continuous infusion), in two cases with bleeding complications. Diffuse multi-vessel coronary disease was shown in 3 severe and 2 mild patients, leading to coronary by-pass surgery in 4 (2 severe) of them. Five patients (one severe) underwent coronary angioplasty and stenting. Data on long-term antiplatelet treatment are available for one severe and three mild patients. All the latter experienced bleeding complications: severe haematoma in one patient on aspirin, recurrent bleeding in two patients on aspirin plus clopidogrel. One of the latter and the severe patient received long-term low-dose FVIII prophylaxis, enabling antiplatelet treatment.
**Conclusions** Haemophilic patients may experience severe IHD. Balancing thrombotic and haemorrhagic risk in this setting remains an open issue, as treatment was often not aggressive because of the fear of bleeding complications. Administering adequate factor replacement may enable PCI and long-term antiplatelet treatment.

## P060 SUCCESSFUL HAEMORRHAGE MANAGEMENT WITH HIGHLY PURIFIED VWF/FVIII COMPLEX CONCENTRATE AS FIRST LINE THERAPY IN TWO PATIENTS AFFECTED BY ACQUIRED HAEMOPHILIA A

Brandolin B., Barbar S., Spiezia L., Saggiorato G., Zanon E.
*Department of Cardiologic, Thoracic and Vascular Sciences Second Chair of Internal Medicine, Padua*

Bleeding control is the first priority in acquired hemophilia A (AHA). International guidelines recommend bypassing agents (BA) as first line therapy and, only if these are not available, FVIII concentrates. Here we describe two cases of bleeding in AHA patients successfully treated with highly purified (HP) VWF/FVIII concentrate (Fanhdi, Grifols).
A 69 years old man with history of coronary disease presented with severe anaemia (Hb 79 g/L) due to rectus abdominis haematoma, aPTT 102 sec, FVIII 0.3%, inhibitor 6 BU. Patient was transfused with packed red blood cells (PRBC) and treated with a bolus of HP VWF/FVIII (20,000 IU; 263 IU/kg) followed by continuous infusion (1.3 IU/kg/h, adjusted to attain FVIII levels of 60-80%) for 11 days.
A 65 years old man, discharged after a pancreatic jejunal anastomosis for chronic pancreatitis, was admitted, one month later, with severe anemia (Hb 46 g/L) due to large bilateral hematoma of upper limbs, aPTT 60s, FVIII 10.4%, inhibitor 1 BU. Patient was transfused with PRBC and treated with 100 IU/kg of HP VWF/FVIII for 14 days. In both cases hemorrhage immediately stopped, followed by progressive hematomas reabsorption; inhibitor disappearance was obtained in two weeks by starting therapy with prednisone (1mg/kg)

and cyclophosphamide simultaneously.
Our clinical experience shows that the highly purified VWF/FVIII concentrates are effective in controlling hemorrhage in AHA patients presenting with low inhibitor titre (<5 BU) and could be a less costly alternative to BA especially when contraindications exist as thrombotic risk factors.

## P061 RECOMBINANT FACTOR VIIA CONCENTRATE VERSUS PLASMA DERIVED CONCENTRATES FOR THE TREATMENT OF ACUTE BLEEDING EPISODES IN PEOPLE WITH HAEMOPHILIA AND INHIBITORS.

Matino D.[(1)], Makris M.[(2)], D'Amico R.[(3)], Iorio A.[(1)]
*[(1)] Internal and Vascular Medicine, University of Perugia, Perugia; [(2)] Academic Unit of Haematology, University of Sheffield, Sheffield, United Kingdom; [(3)] Department of Oncology, hematology and respiratory diseases, University of Modena and Reggio Emilia, Modena*

**Introduction** Inhibitors remains the challenge in the treatment of hemophilia patients. During ITI or after its failure, "by-passing" agents are available for the treatment of bleeding. This Cochrane review investigates which is the most effective treatment of acute bleeding in haemophiliacs with inhibitors.
**Objective** To determine the clinical effectiveness of recombinant FVIIa concentrate compared to plasma-derived concentrates for the treatment of acute bleeding episodes in people with haemophilia and inhibitors.
**Methods** Relevant trials were searched for on the Group's Coagulopathies Trials Register. We considered for inclusion randomised (RCTs) and quasi-randomised controlled clinical trials. Two authors independently selected the trials to be included in the review and extracted data.
**Results** A total of ten trials were identified; two trials, with a total of 69 participants, were eligible for the analysis. The two treatments showed a similar efficacy, ranging from 40% and 80% of successful treatments. No pooled analysis was possible due to heterogeneity of outcomes assessed.
**Conclusion** We conclude that both rfVIIa and aPCC can be used to treat patients with haemophilia and inhibitors. The trials did not show a difference in efficacy of the two products and were equally safe in terms of tolerability and absence of thrombotic complications.
**Contribution to the practice/evidence base of haemophilia and bleeding disorders** The analysis of published trials confirms the efficacy and safety of the two treatments and points to the need for further trials. There is a need for standardization of outcome measures in order to allow pooling of results.

## P062 ACQUIRED HEMOPHILIA A AND NON-HODGKIN LYMPHOMA: EFFECT BEFORE CAUSE?

Sottilotta G., Luise F., Latella C., Oriana V., Romeo E., Cuzzola F., Trapani Lombardo V.
*Centro Emofilia - Servizio Emostasi e Trombosi, Azienda Ospedaliera Bianchi-Melacrino-Morelli, Reggio Calabria*

Acquired hemophilia is a rare coagulation disorder caused by the development of autoantibody to factor VIII (FVIII), causing factor deficiency, abnormalities of coagulation tests, and bleeding tendency of variable severity. About 15% of acquired haemophilia is associated with malignant diseases (lymphoproliferative disease or solid neoplasia).
We report the case of a 63-year-old man referred to our Centre because of an isolated prolonged activated partial thromboplastin time (aPTT) (75 seconds; ratio=2.5), mild thrombocytopenia and absence of bleeding symptoms. The presence of lupus anticoagulant (LAC) was excluded. Acquired haemophilia A was diagnosed because of a reduced FVIII activity level (15%) and presence of anti FVIII inhibitor: 4.4 Bethesda Unit (BU).
Autoimmune diseases, malignancies, drug reactions were excluded. His medical history included hypertension and diabetes. After diagnosis the patient was periodical screened: in the first year we observed stability of Factor VIII level and Inhibitor titre (FVIII activity range: 8-33%; FVIII Inhibitor range: 2.6-5.7 BU). No bleeding episodes occurred. In consideration of the age and his clinical history we didn't start any immunosuppressive therapy. The inhibitor disappeared spontaneously one year after diagnosis, the FVIII activity normalized, but the patient was found to have LAC. One month after, an abdominal echography evidenced diffused abdominal nodules. A biopsy under general anesthesia was performed: the histological sample showed a nodal marginal zone B-cell lymphoma, then the patient started chemotherapy.
In this patient we observed an acquired coagulation disorder before the clinical onset of a lymphoproliferative disease. Our case report confirms the importance of periodical screening for malignant disease in patients with acquired coagulation disorders without apparent causes. The disappearance of the FVIII inhibitor before any immunosuppressive or chemotherapic treatment is an uncommon event, but the development of LAC after disappearance of the inhibitor is a very rare condition.

## P063 A CASE OF HAEMOPHILIA SUCCESSFULLY TREATED WITH ORTHOTOPIC LIVER TRANSPLANTATION

Santoro R., Iannaccaro P., Prejanò S.
*Azienda Ospedaliera Pugliese-Ciaccio Centro Emofilia, Emostasi e Trombosi Catanzaro*

**Introduction** Patients with severe haemophilia suffer from recurrent bleeding episodes. Factor replacement therapy in haemophilic patients made the bleeding management easier. However, in the past, the use of contaminated factors from the multi-donor pooled factors made the patients vulnerable to the risk of viral infections, liver cirrhosis and hepatocellular carcinoma (HCC). We had a successful experience of liver transplantation for a young haemophilic B patient (FIX<1%) with liver cirrhosis.
**Patient and methods** The patient, 25 years old, had no factor IX inhibitor; he was prepared with Factor IX replacement using a continuous infusion regimen after initial bolus dosing, plus platelet transfusion. No intra-operative or post-operative surgical complications were observed. Standard post transplantation immunosuppressive therapy included NEORAL and steroids.
Factor IX ranged from about 75% to 98% after discontinuing concentrate replacement.
After two years of recurrent HCV positivity, the patient was treated with ribavirin for chronic hepatitis.
Median Factor IX concentration is about 75% after ten years from liver transplantation.

**Conclusion** We believe that liver transplantation offers an effective therapeutic option to eradicate haemophilia, although there is no known trial of liver transplantation for a haemophilic patient without liver cirrhosis and/or hepatocellular carcinoma in the word.

P064 **DYNAMIC WHOLE BLOOD COAGULATION PROFILE IN EIGHT SEVERE (HOMOZYGOUS) FACTOR V DEFICIENT SUBJECTS**

Spiezia L., Bertini D., Radu C., Campello E., Bon M., Gavasso S., Fadin M., Simioni P.
*Department of Cardiologic, Thoracic, and Vascular Sciences, Università di Padova, Padova*

**Background** Severe factor V (FV) deficiency (parahaemophilia) is a rare hemorrhagic disorder characterized by a discrepancy between plasma FV levels (less than 1%) and bleeding phenotype (ranging from mild to severe). Rotation thromboelastometry, performed by ROTEM (Pentapharm, Germany), is a useful tool for studying the simultaneous and integrated effects of plasmatic factors, platelets, leukocytes, and red blood cells involved in the dynamic process of clot formation and lysis. Aim of our study was to perform WB coagulation profile in 8 severe (homozygous) factor FV deficient subjects and to compare thromboelastographic profile with bleeding symptoms.
**Patients and Methods** Four ROTEM assays (INTEM, EXTEM, FIBTEM, and NATEM) in eight subjects with severe (homozygous) FV defect (FV mean activity 1.8%) were performed. The following ROTEM parameters were analyzed: clotting time (CT), clotting formation time (CFT), maximum clot firmness (MCF), α-angle, and the Area Under Curve (AUC).
**Results** In all subjects considered in the study ROTEM depicted a hypocoagulable profile as for CT and CFT. On the contrary no statistically significant difference were found as for MCF and α-angle in INTEM, EXTEM, and FIBTEM between study population and a control group of 50 healthy individuals. No correlation between ROTEM profile and the history of hemorrhagic manifestations referred by patients at the time of sampling were found.
**Conclusions** WB ROTEM profiles revealed a retardation of initiation and propagation phases of clot formation in coagulative pattern of subjects with homozygous FV defects. The superimposable values of clot firmness between cases and controls find a possible explanation because of MCF, α-angle, and AUC are mainly influenced by platelet count and fibrinogen plasma levels than by FV plasma levels. Further studies, evaluating the follow-up of bleeding manifestations in homozygous FV deficient subjects, are needed to clarify the potential role of ROTEM in the management of parahemophilic patients.

P065 **TRANSITION TO A WEB-BASED ARCHITECTURE OF THE ITALIAN HEMOPHILIA REGISTRY NETWORK**

Oliovecchio E.[(1)], Marchesini E.[(1)], Rodorigo G.[(2)], Marietta M.[(3)], Di Minno G.[(4)], Perricone C.[(5)], Macchi S.[(6)], Castaman G.[(7)], Morfini M.[(8)], Iorio A.[(1)]
*[(1)] Internal and Vascular Medicine, University of Perugia, Perugia; [(2)] Hemophilia Centre, Bologna Hospital, Bologna; [(3)] Hemophilia Centre, Modena Hospital, Modena; [(4)] Hemophilia Centre, Federico II Hospital, Napoli; [(5)] Hemophilia Centre, Pausillipon Hospital, Napoli; [(6)] Hemophilia Centre, Ravenna Hospital, Ravenna; [(7)] Hemophilia Centre, Vicenza Hospital, Vicenza; [(8)] Hemophilia Centre, Azienda Ospedaliera Universitaria Careggi, Firenze*

**Introduction** In 2003 the Italian Association of Hemophilia Centres (AICE) started a national hemophilia registry program based on the stand-alone software EmoCard to support medical and administrative activities of the Centre, which also originated three peer-reviewed publications.
**Objectives** To describe a new web-based registry program (EmoWeb) and a related software (EmoPro) intending to improve Centre management and data collection and to optimize support to research.
**Description** The new software was designed based on a new relational database refining data storage and management and to comply with ISTH/SSC recommendations. EmoWeb was realized on a web-based architecture, to allow consultation and data entry from any web access site. At variance with EmoCard, EmoWeb offers a streamlined interface and allows customizing program screens and functionalities, organizing the most used tasks and tools accordingly to the particular needs of different users. With the aim of making easy to collect research-related data about the patient population of the Italian Registry Network, a companion software called EmoPro was designed aside Emoweb. The administrator module of EmoPro allows to easily building up electronic Case Record Forms, including both EmoWeb managed fields and newly created ad hoc fields, to be released on a dedicated website. The user module of EmoPro allows compiling the CRF, making it easy collection of data for the AICE sponsored trials. EmoPro based-CRFs automatically select the patients potentially eligible for any specific study.
**Contribution to the practice** EmoWeb and EmoPro are expected to optimize Hemophilia Centre management and to make easier research activities.

P066 **STAGING OF LIVER FIBROSIS WITH NON INVASIVE METHODS IN HAEMOPHILIC PATIENTS INFECTED BY HCV**

Demartis F.
*Agenzia Regionale Coagulopatie, Azienda Ospedaliera Universitaria Careggi, Firenze*

In Haemophilic patients infected by HCV staging of liver fibrosis is essential for predicting prognosis and planning antiviral treatment. Liver biopsy is the golden standard but it is rarely used in these patients because of safety concerns.
Non-invasive markers of liver fibrosis (NIM) have been proposed as an alternative to liver biopsy for hepatitis staging. Scores using routine biochemical parameters, such as APRI, FORNs, and FibroIndex, are attractive for routine clinical use. Liver stiffness (LS) measured by transhepatic elastometry (TE) has also been proposed for the staging of liver fibrosis in HCV patients.
We analyzed the concordance between TE and NIM in the assessment of liver staging in 48 anti-HCV male patients affected by haemophilia A (n=32), B (n=13) and von Willebrand disease (n=3), 28 were found serum HCV-RNA+ve. Among them, LS was measurable in 25. APRI,

Forns, and Fibro-index cut-off values for either recognizing or excluding significant fibrosis (Metavir score > F2 and < F2 respectively) were used as suggested by the authors. The APRI score (AST/Platelet ratio index) is calculated according to the formula:
APRI = [(AST/ULN) x 100]/platelet count $10^9$/L
where ULN = the upper limit of normal. The Forns score, calculated according to the formula:
Forns score = 7.811 – 3.131 x ln [platelet count ($10^9$/L)] + 0.781 x ln [(GGTP (IU/L)] + 3.467 x ln [age (years)] – 0.014 [cholesterol (mg/dL)] the Fibro-index applies platelets AST and gammaglobulin ratio. Overall concordance of APRI, Forns and Fibroindex scores with LS were 56%, 36%, and 48%.
In this group of Haemophilic patients infected by HCV the overall concordance of APRI, Forns and Fibroindex with LS values was low suggesting that other serological test are need for a better staging of liver fibrosis.

## *MALATTIA DI VON WILLEBRAND*

### P067 **HOMOZYGOUS TYPE 2N R854W VON WILLEBRAND FACTOR IS POORLY SECRETED AND CAUSES A SEVERE VON WILLEBRAND DISEASE PHENOTYPE**

Castaman G.[(1)], Giacomelli S.H.[(1)], Obser T.[(2)], Budde U.[(3)], Rodeghiero F.[(1)], Schneppenheim R.[(2)]
*[(1)] Department of Cell Therapy and Hematology, Hemophilia and Thrombosis Centre, San Bortolo Hospital, Vicenza; [(2)] University Medical Centre, Hamburg-Eppendorf Department of Pediatric Hematology and Oncology, Hamburg, Germany; [(3)] AescuLabor Hamburg Coagulation Laboratory, Hamburg, Germany*

**Background** Von Willebrand Disease type Normandy (VWD 2N) is caused by mutations at the Factor VIII (FVIII) binding site of von Willebrand Factor (VWF), located in the D and D3 domain on the amino-terminus of mature VWF. The R854Q mutation is the most frequent cause of this phenotype.
**Objectives** We report the characterization of a homozygous VWD 2N mutation, R854W, detected in a patient with a severe VWD phenotype.
**Methods** Plasma VWF phenotype was studied and transient expression of recombinant mutant full-length VWF in 293 EBNA cells was performed and the results compared with those obtained with wild type (wt) VWF.
**Results** The multimeric analysis of plasma VWF shows the lack of the typical triplet structure, with the presence of the central band only, and a relative decrease of the high molecular weight multimers. The R854W mutation leads to the change of a basic and linear amino acid (arginine) into a hydrophobic and aromatic one (tryptophan), leading to a severe reduction of the binding of FVIII to VWF (<2 IU/dL). Homozygous expression of recombinant R854W VWF results in normal amount of cellular VWF, but with a severe reduction of secretion in the medium. The glycoprotein Ib binding activity and collagen binding of secreted W854 were severely reduced and reproduced the phenotypic pattern observed in plasma.
**Conclusions** Our results demonstrate that homozygous R854W mutation in the D' domain of VWF induces impaired secretion and activity of the protein, justifying the severe phenotype of the patient.

### P068 **ACQUIRED VON WILLEBRAND SYNDROME TYPE 2A ASSOCIATED WITH JAK2 POSITIVE MYELOPROLIFERATIVE DISORDER IN ONE AFFECTED MEMBER OF A LARGE FAMILY WITH AUTOSOMAL DOMINANT A1716P MUTATION OF THE VWF GENE**

Giannini S.[(1)], Solimando M.[(2)], Fierro T.[(1)], Baronciani L.[(2)], Federici A.B.[(2)], Gresele P.[(1)]
*[(1)] Department of Internal Medicine Division of Internal and Cardiovascular Medicine Perugia; [(2)] Department of Internal Medicine, "Angelo Bianchi Bonomi" Hemophilia and Thrombosis Centre, Department of Medicine and Medical Specialties, Milano*

Acquired von Willebrand syndrome (AVWS) is a rare bleeding disorder occurring in subjects with no personal or family history of bleeding and is similar to inherited VWD in terms of laboratory findings and clinical symptoms. A 2A-like AVWS has been found to be associated with myeloproliferative disorders (MPD), especially in patients with very high platelet count, and the VWF defect can be corrected with reappearance in plasma of HMW VWF multimers when platelet count is normalized upon cytostatic treatment.
We have followed-up a 41 year-old male with increased platelet count ($6.5x10^8$/mL) and a JAK2 positive MPD who met WHO criteria for Essential Thrombocythemia (ET). At diagnosis, PFA-100 C/Epi closure time was prolonged and the ristocetin-induced platelet aggregation (RIPA) reduced. VWF activities were also reduced with low VWF:RCo/VWF:Ag (0.34), VWF:CB/VWF:Ag (0.48) and loss of the HMW multimers, suggesting VWD2A. Considering the absence of other cardiovascular risk factors and the VWF defect, the patient was left untreated until an episode of acute myocardial infarction occurred; he was then immediately started on anti-platelet and cytostatic therapy (aspirin 100 mg/die, hydroxyurea 500 mg/die).
During his follow-up, sixteen family members were studied and 6/16 showed reduced levels of VWF activity with all the HMW multimers in plasma suggesting VWD1. Sequencing analysis of the VWF gene revealed a novel missense mutation in exon 29 (c.5146 GCT > CCT) leading to an alanine to proline substitution in the collagen binding domain (A3) of VWF (A1716P) in affected members, including the JAK2-positive patient. Following treatment the patient had the platelet count normalized ($3.5x10^8$/mL) and the acquired 2A-like defect turned into VWD1.
We conclude that bleeding defects can be present in the families of patients with MPD and should be always evaluated with appropriate tests to exclude inherited defects underlying acquired platelet disorders.

## *MALATTIE EMORRAGICHE: ASPETTI DIAGNOSTICI E CLINICI*

### P069 **REDUCED DOSE OF RVIIA IN LIFE THREATENING HAEMORRHAGE AFTER CARDIAC SURGERY**

Russo U.[(1)], Dellefave A.[(2)], Crivelli S.[(1)], Vanelli C.[(1)], Cassin R.[(1)], Ferrari S.[(1)], Antona C.[(3)], Condemi A.M.[(2)], Federici A.B.[(1)]
*[(1)] Division of Haematology and Transfusion Medicine, L. Sacco University Hospital, Milan; [(2)] Division of Cardio*

*Anesthesia, L. Sacco University Hospital, Milan; (3) Division of Cardio Surgery, L. Sacco University Hospital, Milan*

**Background** From 1999 rVIIa was employed for off-label treatment of patients with life threatening haemorrhage, at doses from 20 to 200 µg/kg. In 2006 an increased risk of thrombotic complication was reported in these patients[1] and off label use of rVIIa in haemorrhagic patients was discouraged.
**Aim** To evaluate the efficacy of a reduced dose of rVIIa in managing untreatable haemorrhage after cardiac surgery.
**Patients** From 2004 to 2009, 21 patients (16 males, age 28 85) who underwent cardiac surgery (coronary-artery by-pass and/or valvular repair or replacement) developed an untreatable haemorrhage after intervention and were treated with rVIIa.
**Methods** rVIIa was administered at a dose of 45 µg/kg, eventually repeated after 3 hours. Patients were enrolled to treatment if fulfilled the following criteria[2]: 1) blood loss >100 mL/hour in the 3 hours after surgery; 2) exclusion of surgical cause of haemorrhage; 3) attainment of the following parameters: PT and aPTT <1.5, fibrinogen >100 mg/dL, hematocrit >24%, platelet count >50x$10^9$/L, pH >7.2.
**Results** 16/21 patients showed a marked decrease of blood loss after treatment and transfusion requirement drop from 11.8±3.6 to 2.8±2.7 RBC units ($p<0.001$) in the 48 hours after treatment; 5/21 patients did not respond to rVIIa administration and died in the 24 hours after treatment (1 haemorrhagic shock, 1 acute heart failure, 1 aortic dissection, 1 hepatic haemorrhage, 1 ARDS). Among patients, 16 underwent an extra corporeal circulation >120 min; 17 were treated with rVIIa once and 4 twice. Of the 16 patients who responded to rVIIa administration, 4 died in the 4 weeks after surgery because of septic shock. None patient showed thrombotic events in the 4 weeks after treatment with rVIIa.
**Conclusion** rVIIa at a dose of 45 µg/kg is an effective and safe measure for management of untreatable haemorrhage in cardiac surgery.
**References**
1) A 2006;295:293-298.
2) Hematology 2006:426-431.

## P070 RAPID CYTOFLUORIMETRIC EVALUATION OF REFRACTORINESS TO PLATELETS CONCENTRATES IN PATIENTS WITH GLANZMANN'S THROMBOASTHENIA

Gallo L., Lattuada A., Sacchi E., Libera L., Ferrari S., Cassin R., Vanelli C., Rossi V., Russo U., Federici A.B.
*Division of Hematology and Transfusion Medicine, Luigi Sacco University Hospital, Milan*

**Background** Glanzmann's Thromboastenia (GT) is a rare autosomal recessive bleeding disorder characterized by quantitative or qualitative defects of the glycoprotein IIb-IIIa (GPIIb-IIIa) receptor on platelet surface, prolonged bleeding time with normal platelet count, no response to agonists in platelet aggregation. Flow cytometry is a sensitive and specific technique to distinguish platelets with normal or reduced levels of GPIIb-IIIa receptors. Standard therapy for GT is platelet concentrates (PC) transfusion and/or administration of recombinant activated factor VII (rFVIIa) in refractory patients.
**Aim of the study and Methods** The rapid evaluation of PC refractoriness in GT patients exposed to PC as tested by cytofluorimetric assays with mAbCD41 (clone p2).
**Patients** Two patients with type I GT (GPIIb-IIIa <5%) were followed-up at the Division of Hematology and Transfusion Medicine, L. Sacco University Hospital.
**Case 1** 44-years-old woman with severe gastrointestinal bleeding was treated with 42 units of RBC (median Hb value 7.2 g/dL during hospitalization) and 19 PC (apheresis).
**Case 2** 47-years-old woman with upper urinary tract haematuria and hydronephrosis who arrived to our observation after being previously treated with 6 RBC units and 6 PC units. During hospitalization she received 8 RBC units (median Hb value 9.6 g/dL) and two PC units (apheresis) with a poor clinical response: therefore rFVIIa 90 mg/kg three times daily for 4 days was given.
**Results** CD41+ platelets were consistently shown after PC in case 1 who recovered from bleeding. In case 2, the absence of CD41+ platelets was proven immediately after two PC units consistently with the poor clinical response. This cytofluorimetric analysis could rapidly identify refractoriness to PC in this patient who could be immediately shifted to an intensive therapeutic regimen of rFVIIa.
**Conclusion** This rapid method should be strongly recommended during transfusion with PC in GT patients

## P071 HAEMOSTATIC EVALUATION AND ASSOCIATED CONDITIONS IN PATIENTS WITH SUBCONJUNCTIVAL HAEMORRHAGE

Imbimbo V.(1), Coppola A.(2), Loffredo M.(3), Russo A.(1), Sabatino D.(1), Abagnale I.(1), Di Palo M.(2), Marrone E.(2), Ingenito M.(4), Spina M.(1)
*(1) UOC Patologia Clinica, OORR Area Stabiese, ASL NA3 Sud, Castellammare di Stabia; (2) Università Federico II, Centro di Riferimento Regionale per le Emocoagulopatie, DACM, Napoli; (3) Dip. di Oftalmologia, Università Federico II, Napoli; (4) UO Citogenetica e Genetica Molecolare, ASL Salerno, Pagani*

**Introduction** Subconjunctival haemorrhage (SCH) is a frequent, clinically impressive manifestation, often occurring in the absence of trauma or local predisposing factors. Few data are available concerning haemostatic abnormalities and other systemic predisposing conditions in this setting.
**Methods** Haemostatic assessment, including PT, PTT, platelet count, bleeding time (BT, Ivy), FVIII:C and VWF:Ag, was carried out in 41 consecutive patients (17 women, 24 men; median age 62 yrs, range 31-83) with spontaneous SCH referred over a 6-mo period to the Emergency Care. Patients' clinical history was carefully recorded, with emphasis on drug intake and bleeding history (type 1 von Willebrand disease validated bleeding score).
**Results** Five patients (12%) were on antithrombotic treatment, 2 on aspirin and 3 on oral anticoagulation. Among the latter, INR above the therapeutic range was found in 2 cases. No patient had abnormal PTT, VWF:Ag or FVIII:C. Mild/moderate reduction of platelet count was detected in 4 patients, one also showing prolonged BT. Overall, mild abnormalities of BT (8-10 minutes) were shown in 5 patients (12%). Bleeding history revealed other mild bleeding symptoms (ecchymosis, epistaxis, menorrhagia) in nine patients (22%), including two with moderate

thrombocytopenia. In all cases, bleeding score was <3 in men or 5 in women. Eleven patients (27%) had a history of diabetes mellitus, 10 (24%) of arterial hypertension, 9 (22%) of chronic liver disease and one (2%) of chronic renal disease. No associated disease was detectable in 16 patients (39%).
**Conclusions** Laboratory assessment and bleeding score do not suggest a significant role for haemostatic abnormalities in the pathogenesis of spontaneous SCH. Our data confirm the possible contribution of some systemic diseases or drug intake, although in many cases no associated conditions is identifiable.

## P072 THE HAEMOPHILIA RER NETWORK: CHARACTERISTICS OF PATIENTS WITH INHIBITOR IN THE REGISTRY

Rivolta G.F.[(1)], Biasoli C.[(2)], Pedrazzi P.[(3)], Valdrè L.[(4)], Arbasi M.C.[(5)], Di Perna C.[(1)] D'Incà M.[(6)], Serino M.L.[(7)], Macchi S.[(8)], Pattacini C.[(1)], Tagliaferri A.[(1)]
*[(1)] Regional Reference Centre for Inherited Bleeding Disorders, University Hospital of Parma, Parma; [(2)] Haemophilia Centre, Bufalini Hospital, Cesena; [(3)] Haemophilia Centre, University Hospital, Modena; [(4)] Haemophilia Centre, Sant'Orsola University Hospital, Bologna; [(5)] Haemophilia Centre, G. da Saliceto Hospital, Piacenza; [(6)] Haemophilia Centre, Santa Maria Nuova Hospital, Reggio Emilia; [(7)] Haemophilia Centre, University Hospital, Ferrara; [(8)] Haemophilia Centre, S. Maria delle Croci Hospital, Ravenna*

Since 2003 the Health Authority of Emilia-Romagna Region (RER), funded a completely web-based registry (www.registroemofiliarer.it), with a shared database with the clinical record (xlEmofilia®) used in all RER Hemophilia Centres (HC). It was designed to monitor epidemiology and to improve the quality of health care. Great efforts have been made to ensure high quality of data collection.
The HC adopted strict guidelines to cover the organization of different periodic check-ups and to monitor the relevant clinical aspects of the disease.
We analyzed data about inhibitor: in 2009 data on 281 Haemophiliacs A (HA) and 80 B (HB) were available. 38 HA developed inhibitor; none in HB patients.
The overall incidence of inhibitor in HA is 13.5%: severe 26.9%; moderate 8%; and mild 4%.
25 patients developed inhibitor with rFVIII (HR=15; LR=10; transient=8) and 13 with pdFVIII concentrates (HR=8; LR=5; transient=3). The rate of inhibitors in pdFVIII-treated patients could be underestimated due to lack of suitable follow-up before 2003.
Since 2003 10 severe HA patients with HR inhibitors underwent to 15 ITI courses. Results are shown in the Table.

| Response | First-Line rFVIII | Second-Line pdFVIII | rFVIII+ Immunomodulation | VWF/FVIII |
|---|---|---|---|---|
| Complete | 3 | 2 (VWF/FVIII) | 1 | 1 |
| Partial | 1 | | | |
| Failure | 3 | 1 | | 1 |
| On-going | | | 2 | |

The use of a common clinical record and Registry greatly encouraged HC to collect data and to systematically organize the health care delivery to their patients; accuracy of data available for patients treated after 2003 has been improved by network.

## P073 LOW MOLECULAR WEIGHT HEPARIN MONITORING: VARIABILITY IN RELATION TO CALIBRATION AND ANTITHROMBIN PLASMA LEVEL

Testa S., Paoletti O., Bassi L., Cancellieri E., Alatri A., Stramezzi M., Cogrossi A.
*Haemostasis and Thrombosis Centre, Clinical Pathology Division, AO Istituti Ospitalieri, Cremona*

**Introduction** Anti-factor Xa activity (anti-Xa) measurement is the gold standard test for LMWH monitoring, even if a debate is ongoing about its usefulness, clinical impact and methodology assessment. Anyhow, anti-Xa monitoring is indicated for selected conditions (pregnancy, obesity and renal diseases).
**Aim of the study** was to evaluate:
1) calibration accuracy for 7 different commercial LMWH;
2) anti-Xa variability in relation to plasma antithrombin (AT) levels.

**Material and Methods** We tested 7 different commercially LMWH: calcium nadroparin (Seledie, Seleparina, Fraxiparina), bemiparin (Ivor), enoxaparin (Clexane), dalteparin (Fragmin) and parnaparin (Fluxum). We used a normal plasma pool to obtain LMWH levels ranging between 0.2 UI/mL and 2 UI/mL. Anti-Xa was performed on STA-R Roche, using an amidolytic method (STA Rotachrom Heparin), that requires the addition of FXa with or without AT. A specific calibration for each LMWH molecule was performed and compared with the commercial standard curve. Results were expressed as agreement between the two calibration curves and between expected and found heparin levels.
**Results** We observed a good correlation among the different calibration curves, without differences in respect to each molecule. The agreement between expected and found results was very good (r>0.99 in all cases). Anti-Xa resulted strictly dependent on AT levels, showing a reduction in anticoagulant effect with the decrease of AT levels (Table).

| AT levels (expected) % | AT levels (found) % | Anti-Xa 1.0 UI/mL | Anti-Xa 0.5 UI/mL |
|---|---|---|---|
| 106 | 106 | 1.13 | 0.56 |
| 53 | 54 | 0.82 | 0.42 |
| 26.5 | 26 | 0.58 | 0.28 |
| 13.2 | 13 | 0.36 | 0.14 |

**Conclusions** STA Rotachrom Heparin resulted well calibrated, without showing the necessity to redefine calibration curve for each molecule. We found a perfect agreement between anti-Xa activity and LMWH concentrations. Because of AT effect on final anticoagulant activity we suggest that AT should not be added *in vitro*, to better reflect the real individual anticoagulant regimen, avoiding the risk of inadequate dosages.

## P074 RENAL HEMATOMA (RH): AN UNUSUAL SITE OF SPONTANEOUS ORAL ANTICOAGULANT THERAPY (OAT) RELATED BLEEDING IN A VERY OLD PATIENT

Gori S., Masotti L., Cannistraro D., Bellizzi A., Mannucci A., Scotto F.P., Pampana A.
*UO Medicina Interna, Ospedale di Cecina, Cecina*

**Background** Major bleedings represent the most feared OAT complication. Case report of spontaneous warfarin related RH is described.

**Case Report** An 86-years old male taking warfarin for AF came to our attention for abrupt onset of dyspnoea and fatigue. Since three days he suffered for left sciatica and since few hours for left lumbar pain. He denied fever and haematuria. His history revealed previous CABG and prostatic cancer. Recent traumas were excluded. Physical examination showed pallor and sweating; systemic blood pressure (SBP) measured 75/50 mmHg. BGA was substantially normal. ECG revealed tachycardic atrial fibrillation and complete right branch block. Chest, abdominal x-rays and fast abdominal ultrasound seemed negative.
Results of laboratory assays showed haemoglobin 10.6 g/dL, creatinine 1.41 mg/dL, normal BNP and troponin, D-Dimer 336 ng/mL (n.v. 250). INR was 2.0. The patient was monitored by continuous ECG and he was treated with intravenous dopamine and digoxin with improvement in SBP and heart rate. In the next hours Hb levels fell to 8.9 g/dL and 6.8 g/dL. We start reversal-OAT by vitamin K1 and prothrombin complex concentrate (30 UI/kg) bringing INR to 1.2 together with two bags of red cells. We performed again abdominal ultrasound which revealed increased volume of left kidney. Abdominal CT confirmed a great haematoma of left kidney with extension to retroperitoneal space. We transferred the patient to surgical ward. He received clinical and CT monitoring which revealed bleeding cessation. Conservative treatment was chosen for avoiding post-surgical renal failure and haemodialysis. After two months CT control has excluded renal occult neoplasms.
**Conclusion** Thinking about RH as possibility in OAT patients when lumbar pain is present and major bleeding is suspected is warranted. In our case, urgent OAT-reversal has saved kidney, avoided surgical procedure and a possible next haemodialysis in a very old patient.

## *PIASTRINE: ALTERAZIONI QUALITATIVE O QUANTITATIVE*

### P075 THE MONOALLELIC ALA156VAL SUBSTITUTION IN THE GLYCOPROTEIN IB α (BOLZANO MUTATION) IS A FREQUENT CAUSE OF AUTOSOMAL DOMINANT THROMBO-CYTOPENIA IN THE ITALIAN POPULATION

Noris P.[1], Pecci A.[1], Grignani C.[1], Melazzini F.[1], Civaschi E.[1], Russo S.[2], Loffredo G.[3], Magrin S.[4], Ramenghi U.[5], Balduini C.[1]
*Dipartimento di Medicina Interna, Università di Pavia e Fondazione Policlinico San Matteo, Pavia; [2] UOC di Ematologia, Azienda Ospedaliero-Universitaria, Messina; [3] Dipartimento di Oncologia, Ospedale Pausillipon, Napoli; [4] Divisione di Ematologia e Trapianti, Azienda Ospedaliera Cervello, Palermo; [5] SCDU di Oncoematologia Pediatrica, Ospedale Infantile Regina Elena, Torino*

During the last decade we investigated 200 consecutive, unrelated patients who referred to our Institutions for inherited thrombocytopenia. The monoallelic 515C>T transition in GPIBA gene, which results in the Ala156Val substitution ("Bolzano" mutation) and prevents correct GPIbα-vWF interaction, was identified as the causative defect in 65 patients from 30 families with autosomal dominant thrombocytopenia. A biallelic Ala156Val substitution was formerly described in a patient with Bernard-Soulier syndrome and dysfunctional GPIb-IX-V. Patients' age ranged between 1 month and 76 years (median 29.12 y); the female/male ratio was 1.1.
62% of patients presented mild to moderate bleeding tendency, while 38% were asymptomatic. Because misdiagnosed with ITP, one female underwent splenectomy after failure of i.v. Ig and steroids, whereas 3 additional patients underwent courses of Ig or prednisone without clinical improvement. All subjects carrying the Ala156Val substitution had low platelet counts, but the degree of thrombocytopenia was quite variable (range 30-129x$10^9$/L, mean 81). Platelet size measured on peripheral blood films by computer-assisted image analysis was higher than normal in all cases (mean diameter 3.6 microns, range 2.9-5.1, mean value in healthy subjects 2.4). Because of platelet anysocytosis, automated analyzers did not report any value for MPV in about 50% of the population, while in the remainings the mean value was 14.8 fl (n.v. 9.1-12.3). Platelet aggregation induced by ristocetin 1.5 mg/mL was reduced in 4 out of 22 investigated patients but always normal after collagen 4-20 mcg/mL, ADP 5-20 mcM and ristocetin 3 mg/mL. Flow cytometry revealed a moderate reduction of GPIbα expression (61% of control) on platelet surface in 79% of patients, while the GPIbα/GPIIb-IIIa was reduced in all cases.
**Conclusions** The Ala156Val substitution in GPIbα is one of the most frequent causes of autosomal dominant thrombocytopenia in Italy and should be suspected in all patients with a low platelet count of unknown origin and platelet macrocytosis.

### P076 NEWS ON THROMBOCYTOPENIA AND THROMBOCYTOSIS FROM THE STUDY OF SARDINIAN GENETIC ISOLATES

Balduini C.[1], Cavallo P.[2], Vaccargiu S.[3], Parracciani D.[4], Serra D.[5], Pirastu M.[3]
*[1] Department of Internal Medicine, IRCCS Policlinico San Matteo, Pavia; [2] Department of Internal Medicine, University of Pavia, Pavia; [3] Shardna Life Sciences, Institute of Population Genetic, Sassari-Cagliari; [4] Genetic Park of Ogliastra, Cagliari; [5] Shardna Life Sciences, Cagliari*

The availability of clinical, laboratory and genetic data on 12.517 inhabitants of 10 villages in a secluded area of Sardinia (Ogliastra) allowed us to address the theme of the prevalence of thrombocytopenia in general population, a matter that has never been extensively investigated.
High density SNPs analysis and genealogical records demonstrated a high genetic differentiation among such villages.
We observed a platelet count lower than 150x$10^9$/L in 3.2% of females and 4.8% of males, with a mean value of 3.9% in the entire population. Thrombocytopenia was mild (100-150x$10^9$ platelets/L), asymptomatic and not associated with other cytopenias or overt disorders in most cases.
Its prevalence was quite different in different villages, with values ranging from 1.5% to 6.8%. Interestingly, it was negatively correlated with the prevalence of a mild form of thrombocytosis, which ranged from 0.9 to 4.5%. The analysis of platelet counts in different villages revealed that their distribution curves were roughly Gaussian, and that they were shifted to the left in the populations with the highest

prevalence of thrombocytopenia and the lowest of thrombocytosis, while they were shifted to the right in those with opposite characteristics.
Analysis of platelet counts in different classes of age revealed that platelet number progressively decreased during ageing. As a consequence, thrombocytopenia was nearly absent in young people and its prevalence regularly increased during the lifetime. The opposite occurred for thrombocytosis.
Given the high genetic differentiation among Ogliastra villages with "high" and "low" platelet counts and the substantial heritability of this quantitative trait (54%), we concluded that the propensity to present mild and transient thrombocytosis in the youth and to acquire mild thrombocytopenia during ageing are new genetic traits. Further investigation is required to ascertain whether this conclusion applies also to populations other than those of Ogliastra.

P077 **LINKAGE ANALYSIS TO THE THC2 LOCUS ON CHROMOSOME 10P11.2-12 IN FAMILIES WITH AUTOSOMAL DOMINANT THROMBOCYTOPENIAS AND SEARCH FOR THE CAUSATIVE GENE**

Noris P. [1], Castegnaro G.[2], Pippucci T.[3], Dezzani L.[1], Pecci A.[1], De Rocco D.[4], Loffredo G.[5], Savoia A.[4], Balduini C.L.[1], Seri M.[2]
[1] *University of Pavia and IRCCS Department of Internal Medicine, Pavia;* [2] *Medical Genetics Unit, Department of Gynaecology, Bologna;* [3] *Laboratory of Genetics, Department of Gynaecology, Bologna;* [4] *Institute for Maternal and Child Health, Laboratory of Genetics, Trieste;* [5] *Department of Oncology, Pausillipon Hospital, Naples*

Some years ago, we described two families with autosomal dominant thrombocytopenia, normal platelet size and reduced expression of glycoprotein Ia that did not fit the criteria for any known disorder. We excluded that mutations of coding regions of both ITGA2 and ITGB2 could be responsible for thrombocytopenia, but no gene localization was feasible because of the small size of both families. More recently, we collected five additional pedigrees with autosomal dominant thrombocytopenia and defective expression of glycoprotein Ia on probands platelet surface. As the clinical phenotype and platelet morphology of these subjects were similar to those described in two distinct families with a moderate autosomal dominant thrombocytopenia mapping on chromosome 10p11.2-12 (THC2 locus), all new pedigrees were analysed for linkage to the THC2 locus using polymorphic markers located in the critical region on chromosome 10p11.2-12. Two families showed a consistent linkage with THC2 locus (two point lod score values of 3.3 and 2.4 respectively at theta 0.00); in two smaller pedigrees a THC2 haplotype co-segregated in the affected individuals, while in the remaining family a linkage to the THC2 locus was excluded. Moreover, since the MASTL gene has been reported as responsible for THC2, we analysed the entire coding sequence of this gene in the affected members of the THC2-linked families, but we did not identify any mutation. We concluded that THC2 and autosomal dominant thrombocytopenia with GPIa deficiency are overlapping disorders and that they do not necessarily derive from MASTL mutations. Further, extensive sequencing of all candidate genes within the THC2 locus will unravel the aetiology of autosomal dominant thrombocytopenia with normal sized platelets in Italian patients.

P078 **PLATELET DYSFUNCTION AND GASTRIC ULCERATION MOST LIKELY DUE TO PHOSPHOLYPASE A2 DEFICIENCY: A CASE REPORT**

Pugliano M.[1], Lecchi A.[2], Podda G.M.[1], Zighetti L.M.[2], Cattaneo M.[1]
[1] *Dipartimento di Medicina, Chirurgia e Odontoiatria Azienda Ospedaliera San Paolo - Università degli Studi di Milano, Milano;* [2] *Dipartimento di Medicina e Specialità Mediche, IRCCS Fondazione Ospedale Maggiore, Mangiagalli e Regina Elena, Milano*

Two twin brothers, a girl (V.C.) and a boy (P.C.), with lifelong mucocutaneous bleedings and recurrent gastric ulcerations were studied. Many of their relatives were affected by gastric ulcerations, while their bleeding histories were not informative, because direct interviews were not possible. The association of bleeding diathesis and recurrent gastric ulcerations is suggestive of the presence of defects of the arachidonic acid (AA) pathway, inhibiting the production of prostanoids, which mediate platelet function and gastroprotection.
**Aim of the study** To investigate whether the bleeding diathesis of the two brothers is associated with abnormalities of the platelet AA pathway.
**Methods** Both patients were free of any medication known to affect platelet function for ≥10 days. The following laboratory measurements were performed in both patients: platelet count, platelet aggregation and ATP secretion in citrated platelet-rich plasma (PRP). Bleeding time, serum thromboxane B2 (TxB2), platelet content of adenine nucleotides, serotonin and fibrinogen were measured in V.C. only.
**Results** V.C. had normal platelet count and prolonged bleeding time (>12 minutes). Platelet aggregation and ATP secretion were reduced in response to ADP (2 and 4 miM), epinephrine (10 miM) or collagen (2 mig/mL), but normal in response to AA (0.5 mM). Serum TxB2 levels were severely reduced (63 pmol/$10^8$plt; normal range: 133-1,675). The platelet content of adenine nucleotides, serotonin and fibrinogen were normal. P.C. displayed similar defects of platelet aggregation and secretion.
**Conclusions** The severely decreased serum TxB2 levels measured in V.C. support the hypothesis that the patient is affected by a defect in the AA pathway. The normal aggregation response to exogenous AA suggests that phospholipase A2 (PLA2)-dependent release of AA from membrane phospholipids is responsible for the platelet defect. The same defect may be responsible for the pathogenesis of gastric ulcerations. Family studies and molecular characterization of the defect are ongoing.

P079 **AN ACQUIRED HAEMORRHAGIC DISORDER ASSOCIATED WITH A COMPLEX PHENOTYPE OF PLATELET DYSFUNCTION IN A PATIENT WITH CHRONIC MYELOMONOCYTIC LEUKAEMIA**

Lecchi A.[1], Artoni A.[1], Mantovani G.[2], Gelain F.[1], Motta G.[1]
[1] *Medicina Interna, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Centro Emofilia "A. Bianchi*

*Bonomi", Milano; [2] UO Endocrinologia e Diabetologia, Dipartimento di Scienze Mediche, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano*

A.T., a woman of 74 years, with no previous bleeding history, came to our observation for the recent occurrence of severe mucocutaneous bleedings. Coagulation tests and VWF were normal. She had mild thrombocytopenia and normocitic anemia, and was later diagnosed with chronic myelomonocytic leukemia. Platelet aggregation and ATP secretion induced by ADP, collagen and U46619 were found to be severely impaired on two separate occasions. CD41/61 and CD42b were normally expressed on her platelets (flow cytometry). The platelet contents of delta-granules (ADP and serotonin) and of α-granules (fibrinogen), and serum thromboxane B2 (TxB2) levels were markedly decreased (the patient was not taking non-steroidal anti-inflammatory drugs). The concentration of cyclic-AMP in resting platelets was higher than normal and dramatically increased after exposure of platelets to 1 mol/L prostaglandin E1 (PGE1).

| | | |
|---|---|---|
| **Platelet ADP (nmoles/$10^8$ plt)** | 0.31 | [1.3-2.88] |
| **Platelet serotonin (nmoles/$10^8$ plt)** | 0.1 | [0.19-0.4] |
| **Platelet fibrinogen (mg/$10^9$ plt)** | 0.011 | [0.03-0.19] |
| **TxB2 (pmoles/$10^8$ plt)** | 4.9 | [317-398] |
| **CD41/61 (MFI)** | 140 | [156-227] |
| **CD42b (MFI)** | 48 | [53-80] |
| **Cyclic-AMP baseline (pmoles/$10^9$ plt)** | 20.7 | [9.5±2.3] |
| **Cyclic-AMP after PGE1 (pmoles/$10^9$ plt)** | 473 | [3,623] |

The finding of high baseline levels of platelet cyclic-AMP and of their dramatic increase after platelet exposure to PGE1 suggest that the activity of Gs protein, which mediates the activation of adenylyl cyclase, is heightened in the patient's platelets. Indeed, western blot of platelet lysates revealed an increased expression of Gs, and real time PCR revealed increased Gs mRNA in the patient's platelets compared to normal. No mutations in the gene encoding for Gs were detected in the patient's genomic DNA. The patient displayed none of the clinical features associated with inherited or acquired Gs hyper-function. To our knowledge, this is the first description of selective, acquired platelet Gs hyperactivity, which, in association with other platelet abnormalities, contributes to a severe, acquired bleeding diathesis.

## P080 PLATELET MITOCHONDRIAL DYSFUNCTION IN PATIENTS WITH SEPTIC SHOCK

Lecchi A.[1], Artoni A.[1], Protti A.[2], Panigada M.[1], Motta G.[1], Josca M.[2]
*[1] Centro Emofilia e Trombosi "A. Bianchi Bonomi", Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano; [2] Anestesia e Rianimazione, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano*

Septic shock is a condition characterized by a systemic inflammatory response associated to organ dysfunction. Primary hemostasis is impaired in sepsis, and multiple causative mechanisms have been proposed. In this study we evaluated platelet function in patients with septic shock; in particular platelet mitochondrial potential was studied as a possible cause of platelet function impairment. We enrolled 21 consecutive patients admitted to our intensive care unit with diagnosis of septic shock, all patients were analysed within 24 hours from admission, and platelet function tests were performed. Platelet mitochondrial potential (Phi) was assessed by flow cytometry through JC1 staining. At admission platelet mitochondrial potential was reduced in septic patients in comparison to normal controls (2.6±1.4 vs. 3.4±0.3 p=0.06). At day one there was a good correlation between platelet mitochondrial impairment and the Sepsis-related Organ Failure Assessment (SOFA) score, an indicator of sepsis severity ($R^2$=0,39, p<0.001, n=18) with higher values indicating higher degree of severity. Platelets taken from patients with a SOFA score above the group median value (>9, n=8) had Phi values significantly lower than those taken from less severely ill patients (SOFA score ≤9, n=10) or healthy volunteers (1.6±0.6 vs. 3.3±1.4 vs. 3.4±0.3; p<0.01). At day one platelet aggregation and secretion were severely impaired, especially with strong aggregating agents as collagen (3/5 of patients with maximum platelet aggregation below 25%) and the maximum aggregation correlated to platelet mitochondrial potential ($R^2$=0,72, n=5, p=0.07). Intraplatelet ADP and serotonin content resulted low in around 60% of patients tested. Platelet GpIIb/IIIa and GpIb expression and Annexin V binding (flow cytometry) were within normal limits. In conclusion we demonstrated in a very well selected group of patients that during the early phase of a septic shock a mitochondrial dysfunction occurs, and this may have an impact on platelets dysfunction observed in this condition.

## *PIASTRINE: BIOCHIMICA E FISIOLOGIA*

## P081 THE INTEGRATED FUNCTION OF PLATELET RECEPTORS IN THE ADHESION AND ACTIVATION MECHANISMS

Cozzi M.R.[1], Battiston M.[1], Mazzucato M.[1], Jandrot-Perrus M.[2], Ruggeri Zaverio M.[3], De Marco L.[1]
*[1] Department of Laboratory Medicine, CRO National Cancer Institute I.R.C.C.S., Aviano; [1] Faculté Xavier-Bichat INSERM E348, Paris; [3] Department of Molecular and Experimental Medicine*

**Introduction** The activation of platelets leads to an increase in the concentration of cytosolic calcium ([Ca++]i) which is a key component of the signalling mechanism regulating platelet function. In this study, we have examined the relative contribution of GPIb-V-IX, GPVI and α2β1 in eliciting platelet cytosolic calcium responses during shear dependent platelet adhesion on immobilized collagen in the presence or absence of dVWFA1A2A3 domains under low and high flow conditions.
**Methods** We have used real-time video microscopy to study adhesion and calcium signalling in FLUO3-AM labelled platelets perfused over immobilized fibrillar collagen type I in the presence of dVWFA1A2A3. Adherent platelets within the 0.07-$mm^2$ optical field and individual [Ca++]i traces in single platelets perfused at high and low shear rates (3000 s-1 and 600 s-1) were analyzed.
**Results** We found that the presence of dVWFA1A2A3 affected significantly platelet adhesion and activation at 3000 s-1 but not at 600 s-1. By analyzing individual [Ca++]i traces in platelet perfused over collagen/dVWFA1A2A3 at 3000 s-1, we could identify four types of signals: Full, Reduced, Partial

and Transient, each starting with rapid rise in intracellular calcium but differing in form and intensity. Full and Reduced types were essentially abolished by treating the platelets with anti-α2β1 or anti-GPVI antibody. Experiments performed in platelets with either low or high α2β1 density revealed a decreased surface coverage by almost 50% in the former, accompanied by a significant decrease of Full types signals and an increase of the Reduced ones.
**Conclusion** Our results demonstrate that α2β1 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, reinforced by the activation through GPVI, leading to full platelet activation.

P082 **EVIDENCE OF MRP4-MEDIATED CYCLIC NUCLEOTIDES EFFLUX FROM PLATELETS**
Borgognone A., Trifirò E., Pulcinelli F.M.
*Medicina Sperimentale, Sapienza Università di Roma, Roma*

**Background/Aims** Cyclic nucleotides are potent inhibitory mediators in platelets, naturally produced by prostacyclin interaction with its receptor and NO stimulation on soluble guanylate cyclase. cAMP and cGMP can be transported by MRP4, an ABC transporter with broad substrate specificity. As MRP4 is highly expressed in platelets, we wished to investigate if such a transport exists in platelets and its influence on platelet function.
**Materials and Methods** All studies were performed in a population of drug-free healthy volunteers. Platelet function was assessed by ADP-induced aggregation in the presence of the cAMP elevating agent forskolin and the NO donor sodium nitroprusside. cAMP and cGMP effects on VASP phosphorylation were investigated by means of electrophoresis and western blot. In all experiments MRP4-mediated transport was inhibited by mk571.
**Result and Discussion** Forskolin or SNP pre-treatment gave dose-dependent inhibition of ADP-induced platelet aggregation, as expected. When using sub-threshold concentrations of these cAMP and cGMP elevating agents (forskolin 0,1 miM, sodium nitroprusside 4 miM), pre-incubation with mk571 completely abolished platelet aggregation, thus enhancing cyclic nucleotides inhibitory effects in platelets. mk571 enhancing effect on cAMP and cGMP-dependent platelet inhibition was confirmed by the study of VASP phosphorylation. In fact, VASP phosphorylation was markedly enhanced in platelets incubated with either forskolin or sodium nitroprusside in the presence of mk571 with respect to forskolin or sodium nitroprusside alone.
**Conclusions** Our data indicate that MRP4-mediated cyclic nucleotides efflux is a physiological way of cAMP and cGMP elimination in platelets, in addition to degradation by phosphodiestherases, while its inhibition enhances endothelium-dependent platelet function reduction. In the light of these findings, MRP4 inhibition could be a novel target of anti-platelet therapies.

P035 **PROSTAGLANDIN E2 AND EP3 ACTIVATION POTENTIATE HUMAN PLATELET RESPONSIVENESS**
Petrucci G.[(1)], Rocca B.[(1)], Rutella S.[(2)], Pocaterra D.[(1)], Lancellotti S.[(3)], De Cristofaro R.[(3)], Patrono C.[(1)]
*[(1)] Pharmacology, Catholic University School of Medicine Rome; [(2)] Haematology, Catholic University School of Medicine; IRCSS San Raffaele Pisana, Rome; [(3)] Internal Medicine, Catholic University School of Medicine Rome*

Activated human platelets synthesize prostaglandin (PG) E2, although at lower rates than thromboxane A2. PGE2 binds different receptors (EP1-4). EP3-deleted mice displayed lower platelet response to sub-threshold concentrations of agonists and less susceptibility to experimental thrombosis. While the role of PGE2 in inflammation is well known, its role in human platelets remains poorly characterized.
We investigated the effect of PGE2 on human platelet responsiveness. Platelets were incubated with increasing concentrations of PGE2 or different EP agonists, and stimulated by adenosine diphosphate (ADP), collagen or arachidonic acid (AA). Aggregation was studied by optical aggregometry, EP expression by immunohistochemistry in platelets and megakaryocytes, platelet VASP-P expression and microaggregates were investigated by flow cytometry.
PGE2 at nM concentrations (2-200 nM), dose-dependently increased the slope of the secondary wave of 8 miM ADP-induced platelet aggregation, with an EC50 of 25+6 nM (n=15), without affecting maximal aggregation. At lower (4 miM) ADP concentration, PGE2 reverted the reversible aggregation, with an EC50 of 29+10 nM (n=6). In aspirinated platelets, 200 nM PGE2 in part counteracted the effect of aspirin in abolishing the secondary wave of 8 μM ADP-induced aggregation. Moreover, PGE2 dose-dependently abolished shape change caused by 10 μg/mL collagen and 50 μg/mL AA, with an IC50 of 1,574 nM. The EP3 agonist, 11-deoxy-16,16-dimetyl PGE2 (11-dx) mimicked the PGE2 effect on the secondary wave of ADP-induced aggregation, with an EC50 58+1 nM (n=10). The EP2 agonist butaprost at nM concentration had opposing effects. By flow cytometry analysis, nM concentrations of 11-dx alone generated stable platelet microaggregates, induced the expression of P-selectin and counteracted the VASP phosphorylation induced by PGE1. By immunostains, EP3 and EP2 were observed in platelets and megakaryocytes.
PGE2, acting through EP3 potentiates human platelet response to common agonists, and can activate platelets even in the absence of other agonists. EP3 might represent a novel pharmacological target in atherothrombosis.

P084 **CALCIO INTRACELLULARE E ROIS MODULANO NELLE PIASTRINE UMANE LA FOSFORILAZIONE IN TIROSINA E LA RISPOSTA FUNZIONALE A BASSE DOSI DELL'ANALOGO DEL TROMBOSSANO U46619**
Minuz P.[(1)], Meneguzzi A.[(1)], Fumagalli L.[(2)], Degan M.[(1)], Tommasoli R.M.[(3)], Ferraro R.[(1)], Calabria S.[(1)], Berton G.[(2)]
*[(1)] Medicina Interna, Clinica Medica, Università di Verona, Verona; [(2)] Patologia Generale, Verona; [(3)] Scienze Morfologico-Biomediche, Biochimica Clinica, Verona*

Il trombossano (TX)A2 20-50 nmol/L induce fosforilazione in tirosina e l'attivazione di Rho/Rho-chinasi senza indurre attivazione dell'integrina αIIbβ3 e secrezione di ADP.
Abbiamo studiato il ruolo del calcio intracellulare (Ca-i) e

degli intermedi reattivi dell'ossigeno (ROIs) nell'attivazione piastrinica indotta da basse concentrazioni (50 nmol/L) di U46619 da solo o in associazione ad epinefrina (EPI, 10 mimol/L) attraverso le proteine G13 e Gz. L'espressione della forma attiva di αIIbβ3 indotta da U46619 pi EPI (26.1% ± 13.1, M±DS, n=5), misurata con citometria a flusso, era abolita dal chelante del calcio intracellulare BAPTA-AM 10 mimol/L (0,5% 0.3%, n=5), dallo scavenger dei ROIs EUK-134, 250 mimol/L, (14.4% ± 4.5, n=5) o dall'apocinina, 300 mimol/L, inibitore di NADPH-ossidasi (9.8±5.7, n=5). L'aggregazione piastrinica era inibita (>50%) da BAPTA-AM, apocinina e da EUK-134. Un transitorio aumento (10 s) del Ca-I (123.2±41.3 nmo/L, n=14), misurato in fluorimetria, era indotto da U46619 (63.7±29.3 nmol/L, n=8), mentre EPI non aveva alcun effetto. La combinazione di entrambi gli agonisti ha determinato un persistente aumento di Ca-i (187.1±72.6 nmol/L, n=14, tempo >30 s), non modificato da antiossidanti, inibitori di Rho-chinasi e di tirosino-chinasi. L'immunoblot con anticorpi anti-fosfotirosina e anti-fosfoproteina (n=3) ha dimostrato che la fosforilazione di Src indotta da U46619 dipende dall'aumento di Ca-i e ROIs. Tali segnali cooperano con Rho/Rho-chinasi nella fosforilazione della catena leggera della miosina (MLC), che era preventua da Y27632 30 mol/L, inibitore di Rho-chinasi, assieme a BAPTA-AM o EUK-134.
In conclusione, la fosforilazione in tirosina avviene a valle dell'aumento di Ca-i e della formazione di ROIs catalizzata da NADPH-ossidasi in piastrine umane stimolate con U46619 50 nmol/L. La fosforilazione in tirosina sinergizza con segnali mediati da Ca-i e Rho/Rho-chinasi nell'indurre aggregazione piastrinica in risposta ad agonisti deboli.

P085 **TRANSPORT OF SEROTONIN INTO HUMAN PLATELETS IS REGULATED BY TYROSINE PHOSPHORYLATION OF SERT AND ITS INTERACTION WITH INTEGRIN-αIIBβ3**

Pavanetto M., Borgo C., Busa G., Donella-Deana A., Deana R.
*Chimica Biologica, Università di Padova, Padova*

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in various neurological processes and pathologies including depression, obsessive-compulsive disorders, inflammation and hypertension. Serotonin is synthesized in the central nervous system (CNS) and in the gut enterochromaffin cells. 5-HT transport from blood stream into the platelets, and the reuptake following its release at the synaptic level, is mediated by a membrane transporter SERT displaying the same structural properties.
This study shows that 5-HT uptake by human platelets decreased upon cellular treatment with inhibitors of the tyrosine kinase Syk, a non-receptor type kinase involved in a wide variety of functions in normal and malignant tumour cells. The Syk inhibitors piceatannol and Syk-inhibitor II also caused a decrease of the monensin-induced 5-HT-efflux and imipramine binding to platelets. Tyr-phosphorylation of anti-SERT immuno-precipitates, obtained from membrane extracts, decreased upon platelet treatment with piceatannol.
It has been recently demonstrated that SERT forms complexes with an increasing number of membrane and enzymatic proteins, but it is not clear up to now the physiological significance of these interactions.
We have demonstrated an interaction between SERT and integrin-αIIb/β3 (GPIIbIIIa), a well-known receptor of fibrinogen and von Willebrand factor, proteins involved in platelet aggregation. Our conclusion is based on the findings that, on the one hand, 5-HT transport decreased by platelet treatment with integrin-αIIb/β3 antibodies and antagonists including integrilin, RGDS, decorsin and EDTA; and, on the other hand, that platelet aggregation is decreased in the presence of SERT inhibitors such as fluoxetine and imipramine. Experiments of coimmunoprecipitation of SERT with anti-integrin-αIIb/β3 antibody and viceversa are in progress.
It is concluded that also the Syk-mediated tyrosine phosphorylation of SERT (in addition to the SERT-phosphorylation mediated by other kinases) contributes to the regulation of 5-HT transport that seems also depending on the interaction of the transporter with integrin-αIIb/β3.

## *PIASTRINE: METODI DI STUDIO*

P086 **THE PLATELET COUNT OF PATIENTS WITH THROMBOCYTOPENIA MAY BE UNDERESTIMATED WHEN MEASURED IN ROUTINE LABORATORIES**

Podda G.M.[(1)], Pugliano M.[(1)], Carpani G.[(1)], Femia E.A.[(1)], Cattaneo M.[(1)]
[(1)] *Dipartimento di Medicina, Chirurgia e Odontoiatria, Azienda Ospedaliera San Paolo - Università degli Studi di Milano, Milano;* [(2)] *S.I.M.T, Azienda Ospedaliera San Paolo, Milano*

**Background** The accuracy of platelet count (PC) measurements is important for diagnosis and management of patients with thrombocytopenia. Only patients with PC<100,000/miL may be considered worthy of further diagnostic workup, while treatment is usually started when PC is lower than pre-defined cut-off values. Some normal individuals display pseudo-thrombocytopenia, a harmless condition, which is caused by a time-dependent, *in vitro* decrease of their PC in EDTA-anticoagulated blood, caused by antibodies inducing platelet agglutination. In most cases, this artefact is prevented by the use of alternative anticoagulants, such as Citrate-Tris-Pyridossalphosphate (CPT). We hypothesized that the same artefact could be responsible for spuriously low PC in patients with thrombocytopenia, because their PC is usually not immediately measured in routine laboratories.
**Aim of the study** To test the effect of time elapsed since blood sampling and of type of anticoagulant on PC of patients with thrombocytopenia.
**Methods** We enrolled 84 patients, in whom thrombocytopenia (<150,000/miL) of different etiologies had previously been diagnosed. PC was measured in EDTA- and CPT-anticoagulated samples immediately after blood drawing (T0) and 90 minutes after storage at room temperature (T90). The number of patients whose PC fell below pre-defined cut-off values at T90 was calculated.
**Results** In 16/84 blood samples in EDTA (19%) and in 4/84 in CPT (5%), PC decreased below clinically relevant cut-off values at T90. In particular, PC decreased from ≥100,000/miL to <100,000/miL in 7/84 (8%) and 1/84 (1%); from ≥50,000/miL to <50,000/miL in 5/84 (6%) and 2/84 (2%); from ≥20,000/miL to <20,000/miL in 4/84 (5%) and 1/84 (1%).

**Conclusion** PC in EDTA blood from some patients with thrombocytopenia spuriously decreased with time below clinically relevant cut-off values; like in pseudo-thrombocytopenia, this artefact occurred less frequently in CPT-anticoagulated samples. Spuriously low PC may lead to over-estimation of the bleeding risk and, consequently, over-treatment of patients with thrombocytopenia.

P087 **INCONSISTENCY OF DIFFERENT METHODS ASSESSING EX VIVO PLATELET FUNCTION - RELEVANCE FOR THE DETECTION OF ASPIRIN RESISTANCE**

Renda G., Zurro M., De Caterina R.
*Cardiologia, Università G. d'Annunzio, Chieti*

**Background** Assays to evaluate platelet function are often interchangeably used to assess resistance to aspirin (ASA).
**Design and Methods** We compared various platelet functional assays in 162 subjects: 85 not treated with any antiplatelet drug and 77 on chronic low-dose (100 mg/day) ASA. Subjects were evaluated for Platelet Function Analyzer (PFA) collagen/ADP (CADP)- and collagen/epinephrine (CEPI) Closure Times (CT), as well as light transmittance aggregometry (LTA) in response to ADP, collagen and arachidonic acid (AA) (this last in 47 ASA-treated patients). In 43 ASA-treated patients, serum thromboxane (TX)B2 was also measured.
**Results** In non-ASA-treated patients, CADP-CT and CEPI-CT correlated with each other (r=0.5, P=0.0001), but did not correlate with ADP- or collagen-LTA. In ASA-treated patients, CADP-CT was not different from untreated patients, while CEPI-CT was prolonged (CEPI-CT=241±75s vs. 160±58s, P<0.0001). ADP-LTA was unaffected by ASA, while collagen-LTA was reduced: %maxLTA=61±26% vs. 85±10%, P<0.0001. AA-LTA was almost completely suppressed: %maxLTA=5±13%. No correlation was observed between the different platelet functional assays. The percentage of patients featuring a normal platelet aggregation in spite of aspirin therapy varied in relation with the different assays: 4% if assessed by AA-LTA, 24% by ADP-LTA, 38% by collagen-LTA, 25% by CEPI-CT, 66% by CADP-CT. Interestingly, less than half (42%) of the patients were ASA-responders by all tests, and only 5.7% were ASA non-responders by all tests. Serum TXB2 was almost completely suppressed (8±17 ng/mL) in ASA-treated patients and neither correlated with AA-, ADP- and collagen-LTA nor with CADP-CT, but inversely correlated with CEPI-CT (r=–0.48, P=0.001).
**Conclusions** There is a high heterogeneity of results in tests evaluating inhibition of platelet function by aspirin and this results do not match with serum TXB2, the gold-standard biochemical index of COX-1 activity. Extreme caution should therefore be used in defining resistance to aspirin, since this is extremely dependent on the assay used.

P088 **ESTIMATED PERCENTAGE OF PLATELET INHIBITION BY ADP AND TRAP IN CORONARY ARTERY DISEASE PATIENTS ON DUAL ANTI-PLATELET TREATMENT**

Paniccia R., Marcucci R., Gori A.M., Antonucci E., Maggini N., Miranda M., Degl'Innocenti G., Fabbri A., Prisco D., Abbate R.
*Department of Medical and Surgical Critical Care, University of Florence, Florence*

Platelet function point-of-care systems in routine clinical practice do rapidly allow the identification of residual platelet reactivity (RPR) in coronary artery disease (CAD) patients on antiplatelet treatment.
The methodology of VerifyNow P2Y12 Assay (Accumetrics, USA), besides giving ADP/PGE1 aggregation results, expressed as P2Y12 Reaction Units (PRU), calculates also the percentage of inhibition (% INHIB) by using Thrombin Receptor Activating Peptide (iso-TRAP) as agonist of aggregation - expressed as BASE serving as the baseline. % INHIB is calculated as BASE-PRU/BASEx100. Aim of this study was to evaluate the percentage of inhibition by using three different methods: Multiplate electrode aggregometry (MEA) (Dynabyte, Germany; DASIT, Italy) and VerifyNow P2Y12 assay in whole blood and light transmission aggregation (LTA) on APACT-4004 aggregometer (LABiTec, Germany) in platelet-rich plasma. For MEA and LTA 10 mol/L ADP and 32 μmol/L TRAP-6 (sequence: SFLLRN) as agonists were used. The following estimate: (TRAP-6 ADP) aggregation/TRAP-6 aggregation x 100 = % inhibition was used. In high risk CAD patients on dual antiplatelet treatment the ADP and TRAP platelet aggregations were performed by using MEA (n=580) and LTA (n=240). Significant correlations were found between the percentages of inhibition of platelet function estimated by the three methods (MEA vs. VerifyNow, rho=0.64; MEA vs LTA, rho=0.62; LTA vs VerifyNow, rho=0.63; for all: p<0.0001) and between the estimated inhibitions and platelet aggregations induced by ADP (MEA: rho=–0.81; VerifyNow rho=–0.90; LTA: rho=–0.86; for all: p<0.0001).
These results show that the percentage of platelet inhibition may be estimated by VerifyNow P2Y12 Assay as well as by MEA and LTA. The clinical utility of estimated percentage of inhibition in affecting prognosis in CAD patients on dual antiplatelet treatment needs further studies.

## *PIASTRINOPENIE IMMUNI*

P089 **LOW-DOSE RITUXIMAB IN ADULT PATIENTS WITH PERSISTENT/CHRONIC PRIMARY IMMUNE THROMBOCYTOPENIA (PITP): A SINGLE CENTRE EXPERIENCE**

Biondo F., Santoro C., Baldacci E., Cafolla A., De Propris M.S., Guarini A., Leporace A.P., Mazzucconi M.G.
*Istituto di Biotecnologie cellulari ed Ematologia, Sapienza Università, Roma*

Standard dose of Rituximab (375 mg/m$^2$) for 4 weeks is effective in pITP patients; recent data support also the use of lower doses.
**Aim** To investigate the efficacy of low-dose Rituximab in patients with persistent/chronic pITP.
Ten adult pITP patients (5 males, 5 females; median age 40.5 years (range 19.8-63.8) were treated with Rituximab (weekly dose 100 mg i.v., for 4 consecutive weeks), at median time from diagnosis of 1.6 years (0.5-12.4). All patients had already received at least one line of therapy (median 2, range 1-3):

prednisone, dexamethasone, immunoglobulins, splenectomy. At the Rituximab start, median platelet count was $17x10^9$/L ($5\text{-}30x10^9$/L). Response definition: complete response (CR), platelet count $\geq 100x10^9$/L; partial response (PR), $>50<100x10^9$/L; minimal response (MR), $>30<=50x10^9$/L; no response (NR), $\leq 30x10^9$/L. After completing therapy, patients were evaluated after 1 and 3 months, and thereafter every 3 months. Peripheral CD20+Bcells were evaluated before treatment, 1 and 3 months after stopping therapy, and then every 3 months up to recovery.
One month after the end of Rituximab, seven responses (70%), 4 CR, 2 PR, 1 MR and 3 NR (30%) were observed. At the 3rd months evaluation, responses were 6 (60%), 4 CR, 2 PR, while NR were 2 (20%) and relapses were 2 (20%). Median overall follow-up was 5.5 months (3.3-19.7); median follow-up of responder patients was 5.5 months (3.3-16.3).
The median baseline value of peripheral blood CD20+Bcells was 289.5/miL (85-856). One month after completing treatment, all cases showed absence of circulating CD20+Bcells. At the last available control, all patients had still not recovered the baseline CD20+Bcells count.
At the last control, 6 patients (60%) were in persistent responses, 4 CR, 2 PR. The response seems to be independent from the post-therapy CD20+Bcells counts. No patient had to stop therapy because of severe side effects. Only one case of influenza-like illness was observed.

### P090 PLATELET MORPHOLOGY IN IMMUNE THROMBO-CYTOPENIC PURPURA (ITP)

Diquattro M.[(1)], Farruggia P.[(2)], Gagliano F.[(3)], Tagliavia A.M.[(1)], Marcatti M.[(1)], Palma B.[(1)]
*[(1)] Laboratorio di Patologia Clinica, A.R.N.A.S. Civico, Palermo; [(2)] Unità Operativa di Oncoematologia Pediatrica, A.R.N.A.S. Civico, Palermo; [(3)] U.O. di Clinica Pediatrica, Centro di Riferimento Emostasi e Trombosi in Età Pediatrica, A.R.N.A.S. Civico, Palermo*

**Introduction** Clinical picture in ITP is often asymptomatic-paucisymptomatic. Previous studies have described the presence of high platelet volume and platelet microparticles as hemostatic factor in thrombocytopenic patients. The platelet morphology in ITP was evaluated.
**Methods** 35 pediatric patients (18 male/17 female, mean age 8) with chronic (18) and acute (23) ITP were studied. Platelet morphology was evaluated using CDSapphire Abbott methods: CD61 immunoplatelet count, impedance count, PLT flow mode (0, 7a e 90) and fluorescence FL1-FL3 (CD61-CD41), a direct size measurement Axial Light Loss with PMP gate < 25 channel.
**Results** Immunological platelet count ($x10^9$/L) median 31,000, range 0.317-127,000, Mean Platelet Volume (MPV, fL) 12.1, range 5.5-17.8, Forward scatter (FSC, geomean) 514.4, range 254.2-1,155.0, Side Scatter (SSC, geomean) 319.14, range 164.97-559.00, platelet microparticles (PMP, %) 0.330, range 0.000-1.790. Immunological platelet counts inversely correlated with MPV: r –0.5936, (95% C.I. –0.7738 to –0.3245, p=0.0002). PMP inversely correlated with SSC: r –0.5936, (95% C.I. –0.7738 to –0.3245, p=0.0002), Immunological platelet counts did not correlate with PMP: r 0.3440 (95% C.I. 0.01218 to 0.6076, p=0.0430).
**Conclusion** Our data suggest that platelet destruction determines morphological changes in platelet population and that patients have heterogeneous platelet morphology (density and volume). As large platelets are a marker of an increased platelet production in conditions of rapid turnover, MPV can be used to provide insights into platelet regeneration in ITP. PMP are widely described in ITP [Nomura et al., 1995; Ahn et al., 2002; Yeon et al., 2002; Ahn, Jimenez & Horstman, 2003] and result from complement-mediated platelet lysis and fragmentation. From a clinical perspective, PMP associated with *in vivo* platelet activation have a procoagulant activity. Consensus Guidelines for diagnosis and therapy of ITP highlighted that treatment should be tailored to the individual patient, and so platelet quality could be take into account to evaluate therapeutic strategies.

## *BIOLOGIA CELLULARE DELL'EMOSTASI*

### P091 LMWH MODULATES THE EXPRESSION OF MARKERS OF HAEMOSTASIS AND ANGIOGENESIS IN ENDOTHELIAL CELLS

Favuzzi G., Chinni E., Colaizzo D., Pisanelli D., Cappucci F., Fischetti L., Vecchione G., Grandone E.
*Emostasi e trombosi, I.C.C.S. Casa Sollievo della Sofferenza, San Giovanni Rotondo*

**Introduction** LMWH may exert important actions on the vascular endothelium, which plays a key role in numerous physio-pathological processes, including hemostasis and angiogenesis.
The aim of the present study was to evaluate the effect of enoxaparin on the expression of haemostasis and angiogenesis in endothelial cells.
**Materials and Methods** Human Umbelical Vein Endothelial cells (HUVEC) were obtained from human umbilical cord obtained from at term pregnancies and spontaneous deliveries, using a modified method of Jaffe and coll. Confluent endothelial cell monolayers in 6-well plates were incubated with LMWH, at 0.01, 0.1, 1 and 10 IU/mL or saline (control cells) for 24 hours. RNA was obtained by means of Invitrogen Trizol, Reagent. Complementary DNA (cDNA) was prepared by means of RT-PCR. Quantity mRNA expression of TF, TFPI, VEGF, genes was evaluated by means of ABI 7700TM quantitative real time PCR system.
In order to evaluate the activity of heparins on a standard pro-inflammatory stimulus (i.e. LPS) the study was also conducted in the presence of 10 mig/mL LPS (Lipopolysaccharide endotoxin from Escherichia coli, Sigma) or saline (control cells), as previously reported by Vignoli et al Haematologica, 2006.
**Results** In HUVEC incubated with LPS TF and VEGF were respectively about 11- and 6-fold higher expressed than in control cells (Mann-Whitney test, $p<0.05$). In the presence of increasing concentrations of enoxaparin a reduction of about 8- fold in TF, 5- fold in VEGF and an increase of 2- fold in TFPI expression was observed (Mann-Whitney test, $p<0.05$).
**Conclusions** The increase of TF and VEGF in LPS- treated HUVEC is a consequence of an inflammatory stimulus-mediated effect. Enoxaparin replaces TF, TFPI and VEGF levels. It could be possible that enoxaparin play a role in the replacement of balance between haemostasis and angiogenesis in the endothelium.

P092 **L'ATTIVITÀ CITOPROTETTIVA DELLA PROTEINA C È CONDIZIONATA DAL LEGAME AL SUO RECETTORE ENDOTELIALE (EPCR) MA INDIPENDENTE DALLA SUA ATTIVAZIONE: UNO STUDIO *IN VITRO* CON CHIMERE DELLA PROTEINA C**

Pavani G.[(1)], Della Valle P.[(1)], Veschini L.[(2)], Ferrero E.[(2)], D'Angelo A.[(1)]
*[(1)] Servizio di Coagulazione & Unità Ricerca Trombosi, Istituto Scientifico Universitario San Raffaele, Milano; [(2)] Programma di Fisiopatologia Ossea, Istituto Scientifico Universitario San Raffaele, Milano*

Il sistema della Proteina C ricopre un ruolo chiave tra i processi coagulativi e quelli infiammatori. Anche se i meccanismi d'azione non sono ancora stati completamente chiariti, l'effetto protettivo della PC attivata (aPC) in modelli animali di endotossemia ha portato all'utilizzo di aPC ricombinante umana per il trattamento dei pazienti con sepsi severa.
Il clivaggio di PAR1 (Protease-Activated Receptor1) da parte della aPC legata all'EPCR ha effetti citoprotettivi e antiapoptotici, ma l'importanza biologica di questa osservazione è controversa poiché la trombina in grado di attivare PAR1 con un'efficienza molto maggiore rispetto alla PC (di circa 3 ordini di grandezza) e di promuovere un segnale proinfiammatorio. Di recente è stato dimostrato che l'occupazione di EPCR da parte della PC/aPC è in grado di convertire gli effetti della trombina in un segnale protettivo.
Gli effetti citoprotettivi di PC e aPC in presenza di trombina sono stati comparati in cellule endoteliali umane da cordone ombelicale (HUVEC). Saggi di permeabilità ed apoptosi hanno mostrato un effetto protettivo dose-dipendente dello zimogeno identico a quello ottenuto con concentrazioni equimolari di aPC. Quest'effetto scompariva in presenza di anticorpi bloccanti il legame PC-EPCR e il clivaggio di PAR1, mentre persisteva in presenza di anticorpi che prevenivano la formazione del complesso trombina-trombomodulina, necessario alla attivazione della PC.
Per esplorare l'interazione tra EPCR e dominio Gla della PC sono state utilizzate due chimere (attivata, Pt-aPC/ zimogeno, Pt-PC) in cui il dominio Gla stato sostituito con quello della protrombina. Nonostante un clivaggio significativo ($p<0.02$) e simile di PAR1 da parte di aPC e della chimera cataliticamente attiva, la coincubazione di quest'ultima con trombina non ha dimostrato effetti protettivi sulla barriera endoteliale.
I dati ottenuti confermano le osservazioni di Bae et al. e suggeriscono un effetto protettivo della PC sulle cellule endoteliali indipendente dall'attivazione del dominio catalitico, ma dipendente dal legame con EPCR.

## *BIOLOGIA VASCOLARE*

P093 **CAROTENOIDS ENHANCE NITRIC OXIDE BIOAVAILABILITY AND SUPPRESS TNF-α-INDUCED MONOCYTE ADHESION IN HUMAN ENDOTHELIAL CELLS**

Di Tomo P.[(1)], Canali R.[(2)], Ciavardelli D. [(1)], Di Silvestre S.[(1)], Azzini E.[(2)], Giardinelli A.[(1)], Di Pietro N.[(1)], Pipino C.[(1)], Virgili F.[(2)]
*[(1)] Department of Biomedical Sciences Aging Research Centre, Ce.S.I, Chieti; [(2)] INRAN - National Research Institute for food and nutrition, Rome*

Enhanced monocytes adhesion to the endothelium has been linked to cardiovascular disease due to endothelium activation, resulting in inflammation of blood vessels. Nitric Oxide (NO) plays vascular anti-inflammatory action by inhibiting vascular adhesion molecules expression. Epidemiological studies reported that diet-derived carotenoids might suppress and/or delay progression of cardiovascular disease, but their mechanism of action remains unclear.
We hypothesized that carotenoids may decrease vascular inflammation via increasing endothelial Nitric Oxide (NO) bioavailability.
Human Umbilical Vein Endothelial Cells (HUVEC) were stimulated with TNF-α (1 ng/mL) after a 24 hours pre-incubation with carotenoids (β-carotene [BC], Lycopene [Lyc] and β-cryptoxanthin [Cry], 0.5-2.5 μg/mL) or with vehicle alone. After 16 hours, vascular cell adhesion molecules (VCAM-1), intercellular cell adhesion molecules (ICAM-1) and E-Selectin protein levels (Western Blot) were assessed. The functional consequences of HUVEC treatment with carotenoids on human monocytoid cell (U937 line) were also evaluated by adhesion assay. In the same experimental conditions, NO production (DAF-2DA, FACS analysis) and Nuclear Factor-kB activation (NF-kB, EMSA) were assessed.
BC and Lyc (1 and 2.5 μM) reduced either adhesion molecules protein expression and U937 cell adhesion by attenuation of TNFα-induced NF-kB activation. At concentration of 2.5 μM, BC and Lyc time-dependently increased endothelial NO release. Cry did not significantly affect all parameters studied.
Taken together, these results provide the first evidence that β-carotene and Lycopene are able to increase endothelial NO bioavailability, inhibit adhesion molecule protein expression, monocytes-endothelial cells interaction, and TNF-alpha induced NF-kB activation. This suggests that the anti-inflammatory role of β-carotene and Lycopene may be mediated by increased NO bioavailability and possibly explains why carotenoids can prevent and/or delay cardiovascular disease.

P094 **INVOLVEMENT OF THE TP RECEPTOR IN TNF-α-INDUCED ENDOTHELIAL TISSUE FACTOR EXPRESSION**

Del Turco S.[(1)], Basta G.[(1)], Lazzerini G.[(1)], Chancharme L. [(2)], Lerond L.[(2)], De Caterina R.[(3)]
*[(1)] Fisiologia Clinica, CNR, Pisa; [(2)] Servier Institut de Recherches International; [(3)] Cardiology, Chair of Cardiology and Centre, Chieti*

**Background** Tissue factor (TF) expression is a key event in thrombosis, likely contributing to clinical events in vascular disease. Thromboxane (TX)A2, prostaglandin endoperoxides and F2-isoprostanes exert their effects through a TX-prostanoid (TP) receptor, also expressed in endothelial cells. We studied the involvement of the TP receptor on expression in human umbilical vein endothelial cells (HUVEC).
**Methods and Results** HUVEC pretreated for 1 h with the TP antagonist S18886 were stimulated with the TP receptor agonist U46619 or TNF-α for 6 hours. TF surface exposure

was assessed by an enzyme immunoassays, and TF-dependent procoagulant activity by the generation of Factor Xa. HUVEC exposed to U46619 featured a concentration-dependent increase in TF surface exposure and procoagulant activity. S18886 (1 mol/L)a TP receptor antagonist, significantly reduced U46619 (1 nM)-induced TF surface exposure (–32%±7%, P<0.001) and procoagulant activity (–44%±11%, P<0.001). Interestingly, the TP receptor antagonist also attenuated TF surface exposure (–26% ± 9%, P<0.001) and TF procoagulant activity (–31%±8%, P<0.001) after TNF-alpha (20 ng/mL) stimulation. Similar results were obtained using another TP antagonist, SQ 29,548. Both U46619- and TNF-α-induced TF expression were mediated by the increase of intracellular reactive oxygen species (ROS), and this was inhibited by S18886 (at 10 mol/L) (–44% ± 6% and –24% ± 5% P<0.001, respectively). S18886 also decreased the activation of the p47phox component of NAD(P)H oxidase, accounting for the reduced ROS production. Through pharmacological and gene silencing experiments, we found that S18886 reduced TF expression inhibiting JNK and ERK activation, as well as PKC activity. S18886-induced inhibition of TNF-α -induced TF expression mainly involved the inhibition of a TP receptor-dependent activation of ERK.
**Conclusions** The endothelial TP receptor mediates TF surface exposure and procoagulant activity stimulated both by TP agonists and TNF-alpha, thus expanding the therapeutic potential of TP receptor inhibition in atherothrombosis.

## P095 DOWNREGULATION OF TISSUE FACTOR EXPRESSION IN HUMAN MONONUCLEAR CELLS AND MACROPHAGES BY CONJUGATED LINOLEIC ACID ISOMERS

Cutrone A.[(1)], Napoleone E.[(1)], Cugino D.[(1)], Iacoviello L. [(1)], de Gaetano G.[(1)], Belton O.[(2)], Donati M.B.[(1)]
*[(1)] Laboratori di Ricerca, Università Cattolica, Campobasso; [(2)] School of Biomedical and Biomolecular Technology, University College, Dublin, Ireland*

**Background** Conjugated linoleic acid (CLA) belongs to a family of positional and geometric isomers of linoleic acid. CLA is a mixture of dietary fatty acids that exerts various beneficial effects such as decrease in cell proliferation, atherogenesis, diabetes and inflammation in animal models.
Tissue factor (TF), expressed by infiltrating inflammatory cells, can be detected in atherosclerotic plaques and its concentration contributes to the thrombogenicity associated with atheroma.
Aim: In this study we investigated the effect of the main isomers of CLA: cis-9, trans-11 (c9, t11), trans-10, cis-12 (t10, c12) and of the blend of isomers: 80% (c9, t11) and 20% (t10, c12) on TF expression in mononuclear cells (MNs) and macrophages (M).
**Methods** MNs from peripheral blood of healthy donors and Ms, obtained by spontaneous differentiation of blood monocytes in culture, were incubated with CLA isomers or blend with or without lipopolysaccharide (LPS) for 6 hours at 37°C. At the end of incubation, supernatants were drawn and cells were then disrupted by freezing and thawing and procoagulant activity was assessed by a one-stage clotting time. TF mRNA levels were assessed by real time RT-PCR.
**Results** CLA isomers and blend inhibited TF activity of LPS-stimulated MN in a dose dependent way. A decrease in TF mRNA levels was also detected. The decrease in TF activity was observed also when CLA isomers and blend were incubated with MNs exposed to IL-1β and TNF-α.
TF downregulation was also observed when CLA was tested on human macrophages. Interestingly, TF inhibition was accompanied by a decrease in TNF-α release, as assessed by ELISA.
**Conclusions** The downregulation of TF in macrophages by CLA isomers suggests a potential mechanism by which these substances may interfere with the formation and progression of atherosclerotic plaques and their cardiovascular complications.

## P096 ANGIO-MODULATION IN ENDOTHELIAL CELLS: AN UN-EXPECTED ROLE OF DESMOGLEIN-2 IN REGULATING ACTIN DYNAMICS AND ITS RELEVANCE TO ANGIOGENESIS DEREGULATION IN SYSTEMIC SCLEROSIS

Giusti B.[(1)], Margheri F.[(2)], Rossi L.[(1)], Lapini I.[(1)], Magi A.[(1)], Serratì S.[(2)], Chillà A.[(1)], Fibbi G.[(2)], Del Rosso M.[(2)], Abbate R.[(1)]
*[(1)] Dpt. Medical and Surgical Critical Care - Atherothrombotic Diseases Unit, AOU Careggi University of Florence, Florence; [(2)] Dpt. Experimental Pathology and Oncology, University of Florence, Florence*

Previous observations underlined the anti-angiogenic properties of endothelial cells of patients affected by the diffuse form of Systemic Sclerosis (SSc) leading to microvessel sufferance, capillary loss and then organ failure. In two previous studies we demonstrated that microvascular endothelial cells from SSc patients (SSc-MVECs), which largely over-express pro-angiogenic factors, also over-produce anti-angiogenic molecules and are defective for systems required to perform a suitable angiogenic program. Among the most striking difference between SSc-MVECs and normal MVECs (N-MVECs) was the down regulation of desmoglein-2 (DSG2). DSG2 function in normal and defective angiogenesis has not yet studied.
Aim of our study was to investigate by siRNA-dependent loss of function, the role of DSG2 in N-MVECs.
After silencing, DSG2 expression at mRNA level was reduced of about 14 fold. The amount of DSG2 protein upon the treatment is down-regulated to levels similar to those expressed by SSc-MVEC. Both Matrigel invasion in Boyden chamber assay and capillary morphogenesis were impaired, with a phenotype similar to that previously reported for SS-MVEC subjected to the same assays. To investigate the biological processes and pathways of the MVECs altered by the reduced expression of DSG2, Affymetrix Gene Expression Profiling of N-MVECs and N-MVECs after siRNA silencing of DSG2 gene expression (siDSG2-N-MVECs) was performed, showing the differential expression of 2,945 genes. After functional classification of the 2,945 genes, we observed a high number of functional terms and pathways implied in angiogenesis, blood vessel development, cytoskeleton organization and biogenesis. These observations were validated by molecular and functional evaluation of 7 genes (MACF1, DIAPH1, DIAPH2, ARPC3, RAC2, CDH5, and ITGB8) involved in cytoskeleton organization.
These data together with those of both impaired Matrigel

invasion and capillary morphogenesis suggested a crucial role of desmoglein-2 in regulating actin dynamics and its relevance to angiogenesis deregulation in SSc, an *in vivo* model of anti-angiogenesis.

## P097 ENDOTHELIAL PROGENITOR CELLS (EPCS), CIRCULATING ENDOTHELIAL CELLS (CECS) AND VASCULAR FUNCTION IN RENAL TRANSPLANT RECIPIENTS PATIENTS

Cesari F.[(1)], Fatini C.[(1)], Sticchi E.[(1)], Marcucci R.[(1)], Cioni G.[(1)], Salvadori M.[(2)], Zanazzi M.[(2)], Bertoni E.[(2)], Gensini G.F.[(1)], Abbate R.[(1)]
*[(1)] Department of Medical and Surgical Care, University of Florence, Florence; [(2)] Renal Unit, University of Florence, Florence*

**Introduction** Endothelial dysfunction contributes to accelerated atherosclerosis and few data about the number of endothelial progenitor cells (EPCs) and circulating endothelial cells (CECs) in renal transplant recipients (RTRs) are available.
**Aim** To assess the number of EPCs and CECs in a population of RTRs patients and to establish a relationship between these cells and endothelial function.
**Methods** In 87 stable renal transplant recipients we evaluated EPCs, CECs and endothelial function at least one year after renal transplantation. EPCs were defined as CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+, while CECs were defined as CD146+/CD31+/CD45–/CD61–. Endothelial function was assessed by using a finger plethysmograph (EndoPAT; Itamar Medical Ltd, Caesarea, Israel).
**Results** A significant higher number of CECs was detected in RTRs patients with a previous history of myocardial infarction and in relation with smoking habit [10 (0-47) vs. 7 (0-30) cells/106 events) p=0.04].
By dividing our patients populations into tertiles of reactivity hyperaemic index, a significant trend of increase for all the three types of EPCs was detected [CD34+/KDR+ 1$^{st}$ 0 (0-20) 2$^{nd}$ 3 (0-17) 3$^{rd}$ 7(0-27) cells/106 events p=0.036; CD133+/KDR+ 1$^{st}$ 0 (0-20) 2$^{nd}$ 3 (0-17) 3$^{rd}$ 7(0-30) cells/106 events p=0.036; CD34+/CD133+/KDR+ 1$^{st}$ 0 (0-17) 2$^{nd}$ 3 (0-17) 3$^{rd}$ 7 (0-27) cells/106 events p=0.049].
A significant correlation was observed between RHI and EPCs [CD34+/KDR+ and RHI r=0.21 p=0.04; CD133+/KDR+ and RHI r=0.20 p=0.04]. In relation with marker of renal insufficiency a significant and negative correlation was observed between parathyroid hormone levels (PTH) levels and EPCs [CD34+/KDR+ and PTH r=–0.40 p=0.005; CD133+/KDR+ and PTH r=–040 p=0.005; CD34+/CD133+/KDR+ and PTH r=–041 p=0.004].
**Conclusions** In RTRs patients, we documented a significant increase and a trend of decrease in EPCs and CECs number in relation with endothelial function. Moreover, the correlation between PTH levels and EPCs suggest a possible link between these cells and graft function.

## P098 EVALUATION OF MARKER OF ENDOTHELIAL DYSFUNCTION AND REGENERATION IN HIV-POSITIVE TREATMENT-NAIVE PATIENTS: A PILOT STUDY

Cesari F.[(1)], Cerini G.[(2)], Gori A.M.[(2)], Rogolino A.[(2)], Martinelli C.[(3)], Carocci A.[(3)], Corsi P.[(3)], Leoncini F.[(3)], Gensini G.F.[(1)], Abbate R.[(1)]
*[(1)] Department of Medical and Surgical Critical Care, Thrombosis Centre, University of Florence, Florence; [(2)] Department of Medical and Surgical Care, University of Florence, Florence; [(3)] Infectious Diseases Unit, Azienda Ospedaliero-Universitaria Careggi, Florence*

**Introduction** Over the last years, antiretroviral therapy has led to a dramatic decline in mortality and morbidity among HIV-positive patients. However, together with these beneficial effects, increased rate of cardiovascular disease has been reported in HIV patients.
**Aim** To evaluate markers of endothelial dysfunction and regeneration, such as circulating endothelial cells (CECs), von Willebrand factor (vWF), plasminogen activator inhibitor (PAI), tissue-type plasminogen activator (t-PA) and endothelial progenitor cells (EPCs) in a population of HIV+ treatment-nave patients.
**Methods** 18 HIV+ patients naïve for antiretroviral drugs (M 17, F 1) with a median age of 38 (22-65) years and 18 control subjects were enrolled in the study. vWF was measured by using miniVidas analyser (BioMérieux, Lyon, France), PAI and t-PA by an ELISA method. Circulating EPCs and CECs were defined as CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+, while CECs were defined as CD146+/CD31+/CD45–/CD61–.
**Results** HIV positive subjects showed a significant lower number of EPCs and a higher number of CECs with respect to the control population [CD34+/KDR+ 7 (0-23) cells/106 events vs. 17 (3-43) cells/106 events p=0.017; CD133+/KDR+ 7 (0-23) cells/106 events vs. 13 (7-43) cells/106 events p=0.013; CD146+/CD31+/CD45-/CD61 7 (0-23) cells/106 events vs. 3 (0-13) cells/106 events p=0.027]. Plasma levels of vWF, PAI and t-PA resulted to be elevated in 44.4%, 33.3%, and in 11.1% of the patients, respectively. In addition, in HIV+ patients, significant and positive correlations between CECs numbers and PAI and t-PA plasma levels were observed (r=0.45 p<0.05; r=0.46 p<0.05).
**Conclusions** Our data demonstrated the presence of an endothelial dysfunction, as documented by low EPCs number and high CECs number, in naïve HIV+ patients with respect to a control population. Moreover a positive correlation between CECs and markers of endothelial damage was observed. All these factors may suggest the presence of an adjunctive risk for the development of cardiovascular diseases in HIV+ patients.

## P099 CIGARETTE SMOKE-INDUCED IMBALANCE BETWEEN PGE2/PGI2 MODULATES ENDOTHELIAL TISSUE FACTOR: ROLE OF EP1 RECEPTOR AND SIRT-1

Barbieri S.S.[(1)], Zacchi E.[(1)], Amadio P.[(1)], Gianellini S.[(1)], Weksler B.B.[(2)], Tremoli E.[(3)]
*[(1)] Dept. Pharmacological Sciences, University of Milan, Milan; [(2)] Dept. Medicine/Hematology-Oncology, Weill Medical College of Cornell University, New York, USA; [(3)] Dept. Pharmacological Sciences, Monzino Cardiologic Centre IRCCS, Milan*

Cigarette smoke exposure increases the incidence of atherothrombotic disease, inducing expression of both cyclooxygenase-2 (COX-2), a key enzyme in the

inflammatory response, and Tissue Factor (TF), initiator of the coagulation cascade. We assessed whether and how the major products of COX-2 activity (PGE2 and PGI2) modulate TF induced by cigarette smoke in endothelial cells (EC).
We observed that an aqueous extract of cigarette smoke (TS) in association with inflammatory cytokine IL-1β altered the balance between PGE2/PGI2, increasing PGE2 and reducing PGI2 production, and induced TF (expression and activity) in EC *in vitro*. Inhibition of PGE synthase (PGES) activity by CAY10526, or by specific PGES siRNA, markedly diminished the amount of TF induced by TS/IL-1β. In contrast, treatment with exogenous PGE2 increased TF.
EC express three PGE2 receptors: EP1, EP2 and EP4. We showed that EP1 antagonists (AH6809 and SC19220) reduced TF induced by TS/IL-1β whereas an EP1 agonist (17-phenyl-trinor-PGE2,) increased TF. We excluded the involvement of other EP receptors because an EP4 antagonist (GW627368X) and EP2/EP4 agonists (misoprostol, butaprost) did not modify TF. The role of SIRT1, the NAD+-dependent protein deacetylase, in the regulation of TF was analyzed. Sirtinol, an SIRT1 deactivator, increased TF expression; in contrast, resveratrol, an SIRT1 activator, reduced TF induced by both TS/IL-1β and EP1 agonist.
Furthermore, carbacyclin, a stable PGI2 receptor (IP) agonist, prevented TF and reduced PGE2 production induced by TS/IL-1. Conversely, both the IP antagonist, CAY10441, and specific PGI2 synthase siRNA increased both TF and PGE2. Finally, we showed that carbacyclin prevented TF expression induced by both PGE2 and Sirtinol.
We conclude that cigarette smoke, by modulating the balance between PGE2/PGI2, increases expression and activity of TF by a pathway dependent on EP1/SIRT-1.

P100 **CIRCULATING ENDOTHELIAL AND APOPTOTIC MICROPARTICLES IN CYNOMOLGUS MONKEYS AFTER XENOGRAFT**

Radu C.[(1)], Spiezia L.[(1)], Campello E.[(1)], Ballin A.[(1)], Bulato C.[(1)], Gavasso S.[(1)], Cozzi E.[(2)], Simioni P.[(1)]
*[(1)] Cardiologic, Thoracic and Vascular Sciences, Università di Padova, Padova; [(2)] (Consortium for Research in Organ Transplantation) CORIT, Padova*

**Introduction** Activation of the clotting cascade, fibrin deposition and thrombosis are recognized as important elements of humoral rejection, which remains the major barrier to the long term survival when pig organs are transplanted into primates. Microparticles (MPs) are small vesicles shed from the surface of activated or apoptotic cells that have a procoagulant activity. In human, increased levels of circulating MP are associated with a prothrombotic state. We measured circulating MPs plasma levels in cynomolgus monkeys after porcine kidney transplantation.
**Materials and Methods** Blood samples were obtained from 10 healthy monkeys and 10 monkeys after xenotransplantation at the time of euthanasia. MPs were analyzed by flow cytometry (FC500 Cytomics, USA), with a gate defined by 1m beads (Megamix, Stago, France) and using FITC-Annexin V, PC5-anti-CD146, PE-antiCD62E (E-Selectin MP) and PE-anti-CD142 antibodies in order to identify apoptotic, endothelial and tissue factor bearing MPs, respectively.
**Results** Total MPs levels (mean±SD) were higher in transplanted monkeys than in healthy animals; the difference was not statistically significant (Students t-test p value 0.22). In particular, transplanted monkeys had significant higher plasma levels of both E-Selectin MPs (1,640±607 MP/L, p=0.028) and Annexin-V MPs (48,639±3,498 MP/L, p=0.041) than healthy monkeys (769±428 MP/L and 2,305±963 MP/L, respectively).
**Conclusion** Transplanted monkeys present increase circulating MPs plasma levels originated by activated endothelium (E-selectin) and apoptotic (Annexin-V) cells compared to healthy monkeys. The observed association underscores the possibility that MPs are involved in the prothrombotic status of xenotransplantation.

P101 **ROLE OF HUMAN PROTEIN C RECEPTOR (HEPCR) ON ACTIVATION OF PROTEIN C ON TRANSGENIC PORCINE AORTIC ENDOTHELIAL CELLS EXPRESSING HUMAN THROMBOMODULIN AND HEPCR**

Radu C.[(1)], Bulato C.[(1)], Gavasso S.[(1)], Spiezia L.[(1)], Dalla Valle F.[(1)], Boldrin M.[(2)], Galli C.[(3)], Cozzi E.[(2)], Simioni P.[(1)]
*[(1)] Cardiologic, Thoracic and Vascular Sciences, Università di Padova, Padova; [(2)] (Consortium for Research in Organ Transplantation) CORIT, Padova; [(3)] Laboratorio di Tecnologie della Riproduzione, AVANTEA srl, Cremona*

**Introduction** The protein C (PC) anticoagulant pathway is crucial in the control of haemostasis. PC activation is catalyzed on endothelium by a complex composed by thrombin, thrombomodulin (TM) and PC receptor (EPCR). The development of microvascular thrombosis in xenograft animals is probably due to the inability of pig anticoagulant proteins (TM, EPCR) to activate host circulating PC.
**Aim of the study** To study, *in vitro*, the role of hTM and hEPCR in the activation of human PC by transgenic porcine aortic endothelial cells (PAEC) expressing hTM and hEPCR.
**Materials and Methods** Confluent cells were incubated with human PC and thrombin in the presence of anti-hTM and/or anti-hEPCR antibodies.
**Results** The inhibition of PC activation in the presence of only anti-hTM or anti-hEPCR was 75% and 30% respectively, while in the presence of both antibodies the inhibition was 90%.
**Conclusions** This study suggests that both hTM and hEPCR, expressed on transgenic PAEC, could play a relevant role in the activation of PC. The development of genetically modified pigs that express inhibitors of coagulation pathway could overcome the problem of coagulation imbalance in xenotransplantation.

P102 **ENDOTHELIAL PROGENITOR CELLS MOBILIZATION AND INFLAMMATION AFTER CARDIAC REHABILITATION ON PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION AFTER ACUTE CORONARY SYNDROME**

Cesari F.[(1)], Marcussi R.[(1)], Sofi F. [1)], Luly S.[(1)], Burgisser C.[(2)], Abbate R.[(1)], Gensini G.F.[(1)], Fattirolli F.[(2)]
*[(1)] Department of Medical and Surgical Critical Care, Thrombosis Centre, University of Florence, Florence; [(2)] Unit of Gerontology and Geriatrics, Azienda Ospedaliero-Universitaria Careggi, Florence*

**Background** Few data regarding the effect of cardiac rehabilitation (CR) program after primary percutaneous coronary intervention (PCI) on EPCs are available. We performed this study in order to assess the variations of EPCs in relation to inflammatory markers in patients who performed a four weeks CR after PCI.
**Methods** 55 patients were admitted in a four weeks exercise-based CR program. The numbers of EPCs and the levels of NT-ProBNP and high sensitivity C-reactive protein (hs-CRP) were determined at the beginning (T1) and at the end (T2) of the CR program.
All patients performed a cardiopulmonary exercise test at T1 and at T2. Peripheral blood EPCs were defined as CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+. CRP and NT-ProBNP were measured by using a nephelometric and an immunometric method, respectively.
**Results** With regards to EPCs, we observed a significantly increase at T2 with respect to T1 [CD34+KDR+: 7 (0-27) vs. 13 (0-37) cells/106 events p=0.010; CD133+KDR+: 7 (0-27) vs. 10 (0-33) cells/106 events p=0.018; CD34+CD133+KDR+: 7 (0-27) vs. 10 (0-33) cells/106 events p=0.014]. As expected, a significantly increase at T2 was observed for cardiopulmonary parameters whereas hs-CRP and NT-ProBNP levels significantly decreased at T2 with respect to T1 values.
By diving our patients' populations in relation to the increase of EPCs, patients with an increase of EPCs were significantly younger with respect to the others showed significantly lower baseline levels of CRP and a better exercise tolerance with higher basal $VO_2$max.
**Conclusion** A four weeks exercise-based CR program after acute coronary syndrome and PCI, is able to determine an increase of EPCs number with a contemporary decrease of CRP and NT-ProBNP. However a different behaviour for EPCs can be detected among patients with regard to age, obesity, smoking habit, CRP levels and exercise tolerance.

## P103 MLPA-BASED COPY NUMBER VARIATION ANALYSIS IN PATIENTS WITH ABDOMINAL AORTIC ANEURYSM: IDENTIFICATION OF A NOVEL MUTATION IN THE HUMAN TGFβ-R1 GENE

Bianchi E.[(1)], Spirito R.[(2)], Colombo G.[(2)], Penco S.[(3)], Saccu C.[(2)], Tremoli E.[(1)], Camera M.[(1)]
*[(1)] Centro di Ricerche Farmacologiche, Centro Cardiologico Monzino IRCCS, Milano, [(2)] Università di Milano, Centro Cardiologico Monzino IRCCS, Milano; [(3)] Dip. di Medicina di Laboratori, Ospedale Niguarda Ca' Granda, Milano*

**Background** Abdominal aortic aneurysm (AAA) is a multifactorial condition associated with a strong genetic component. The transforming growth factor β (TGFβ) signalling pathway regulates several cell functions including proliferation, differentiation and vascular remodelling. TGFβ receptor 1 and 2 (TGFβR1 and TGFβR2) gene sequencing identified point mutations associated with AAA manifestation. Whether heterozygous exon deletions and duplications may also be associated with AAA is presently unknown.
**Aim** To screen TGFβR1 and TGFβR2 genes for genomic rearrangements, in a cohort of AAA patients, by using the Multiplex Ligation-dependent Probe Amplification (MLPA) technique.
**Methods** 50 patients undergoing elective open AAA repair referred to the Unit of Vascular Surgery of the Centro Cardiologico Monzino were enrolled. Genomic DNA was extracted from peripheral blood and MLPA analysis was performed by using an ordinary PCR thermal cycler for the hybridization and amplification steps, and a capillary sequencer for amplicons separation. TGFβR1 protein levels in tissue samples collected during surgery from aneurysmatic abdominal aortic wall were assessed by western blot.
**Results** MLPA identified a new mutation in TGFβR1, consisting of a heterozygous deletion of exon 1 and 2, encoding for the receptor extracellular domain, and partially of the promoter region, in one out of the 50 patients screened. This mutation does not affect the protein expression levels, which were comparable to those detected in AAA patients who did not carry the new mutation. No rearrangements were identified in the TGFβR2 gene.
**Conclusions** Application of the MLPA analysis to the screening of novel genomic rearrangements allowed the identification of a new heterozygous deletion in the TGFβR1 gene in a patient affected by AAA. Whether this mutation may affect the receptor functional activity, despite the apparently unaltered TGFβR1 protein levels, is currently matter of ongoing investigations.

## P104 RELATION OF ORAL ANTICOAGULANT TREATMENT TO CORONARY CALCIUM ASSESSED BY MULTISLICE SPIRAL COMPUTED TOMOGRAPHY

Quintavalla R.[(1)], Ferrini P.[(1)], Tassoni M.I.[(1)], Formisano D.[(1)]
*[(1)] Medicina Interna ad indirizzo Angiologico e Coagulativo - Centro Emostasi e Trombosi, Azienda Ospedaliero Universitaria di Parma, Parma; [(2)] Ufficio Statistica, Epidemiologia Clinica, Arcispedale Santa Maria Nuova, Reggio Emilia*

Vitamin K antagonists are widely used for the treatment and prophylaxis of thromboembolic diseases. Oral anticoagulant treatment (OAT) blocks the γ-carboxylation of the Gla proteins including the vitamin-K-dependent coagulation factors (II, VII, IX, X), the 3 anticoagulant proteins (C, S and Z) and the non-coagulation protein matrix Gla protein (MGP). MGP plays a dominant role in vascular calcium metabolism, although its mechanism of action is not completely understood. In the last decade many authors have hypothesized that OAT could be a risk factor for arterial calcification in man, via the impairment of MGP. Two studies have demonstrated that subjects on long term OAT have much more arterial and heart valve calcification than control population. Coronary artery calcification (CAC) is an independent predictor of cardiovascular disease and multisliced spiral computed tomography (MSCT) has been shown to be a valuable method for assessing CAC.
In this observational, retrospective study, we matched the database containing all coronary 64-MSCT (n=1,048) performed between 2007 and 2008 with the database of patients on OAT (n=3,857) and we found 71 patients who were on OAT at the moment of MSCT. These patients were compared to 76 patients without OAT. No differences were found in cardiovascular risk factors (smoke, diabetes, arterial hypertension, obesity, cholesterol level), age, weight or height between the two groups. By a range of measures patients on OAT demonstrated a highly variable increase in calcium

deposits. However, a non-parametric Mann-Whitney test of the square-root transformed data reveals no statistically significant difference between the two groups of patients (p=0,06). Further studies are suggested, as an augmented patient population is required to confirm our non-difference finding.

## *GENETICA, EMOSTASI E TROMBOSI*

### P105 **IMPACT OF CYP2C9 AND VKORC1 POLYMORPHISMS ON STABLE WARFARIN DOSE**

Cini M., Legnani C., Valdrè L., Guazzaloca G., Boggian O., Poggi M., Abate C., Cavallaroni K., Palareti G.
*Dept. Angiology and Blood Coagulation "Marino Golinelli", University Hospital S. Orsola-Malpighi, Bologna*

The optimum therapeutic dose of warfarin is affected by many demographic factors, but they do not fully account for interindividual variability in the drug response. Recently, polymorphisms in the gene for cytocrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex subunit 1 (VKORC1) have been shown to affect warfarin metabolism.
70 patients (39 males, mean age 70 years, range 22-92 years), referred to our centre for warfarin anticoagulation oral therapy monitoring, were genotyped for detection of two CYC2C9 (CYP2C9*2 and CYP2C9*3) and three VKORC1 (-1639G/A, 1173C/T, 3730G/A) single nucleotide polymorphism (SNPs). The frequencies of the CYP2C9*2 and CYP2C9*3 alleles were 0.114 and 0.150, respectively. Because the -1639G/A and 1173C/T VKORC1 SNPs have been shown to be in complete linkage disequilibrium, the same frequencies of their genotype variants were found in the evaluated population; we identified 34.3% wild-type subjects, 48.6% heterozygous and 17.1% homozygous variants. The frequencies of the 3730G/A VKORC1 genotypes were: 35.7% wild-type, 55.7% heterozygous and 8.6% homozygous.
The weekly warfarin dose was significantly higher in the CYP2C9 *1/*1 patients (mean 48.30 mg) than in patients with one variant allele (*1/*2 or *1/*3) (mean 26.77 mg, $p<0.001$) and two variant alleles (*2/*2 or *2/*3 or *3/*3) (mean 8.46 mg, $p<0.001$). For the -1639G/A and 1173C/T VKORC1 polymorphisms, the weekly warfarin dose significantly decreased from wild-type to mutant homozygous (wild-type: mean 54.11 mg; heterozygous: mean 32.73 mg, $p<0.001$; homozygous: mean 13.13 mg, $p<0.001$). On the contrary, for the 3730G/A genotype, the weekly warfarin dose significantly increased from wild-type to mutant homozygous (wild-type: mean 24.96 mg; heterozygous: mean 41.95 mg, $p<0.05$; homozygous: mean 51.67 mg, $p<0.05$).
In conclusion, the weekly warfarin dose is strongly associated with the CYP2C9 and the analyzed VKORC1 SNPs, but the 3730G/A polymorphism displayed an opposite gene-dose effect.

### P106 **THE EFFECTS OF ATORVASTATIN AND ROSUVASTATIN ON OXIDATIVE STRESS AND PLATELET ACTIVATION IN PATIENTS WITH HYPERCHOLESTEROLEMIA**

Santilli F.[(1)], Puccetti L.[(1)], Pasqui A.L.[(1)], Lattanzio S.[(1)], Liani R.[(1)], Ciani F.[(2)], Pastorelli M.[(3)], Ferrante E.[(1)], Ciabattoni G.[(1)], Auteri A.[(3)], Davì G.[(1)]
*[(1)] Centre of Excellence on Aging, G. D'Annunzio University of Chieti, Chieti; [(2)] Children Hospital Meyer, Florence; [(3)] Centre for Atherosclerosis Research, University of Siena, Siena*

**Objectives** We examined the time-dependent effects of atorvastatin and rosuvastatin on *in vivo* oxidative stress and platelet activation, to assess whether these phenomena are related to any pleiotropic effect of any statin or to their LDL-lowering effect. We also asked whether the presence of specific allele frequencies in carriers of to the 3UTR/lectin-like oxidized LDL receptor-1 (LOX-1) polymorphism may influence the effect of either statin.
**Methods** We included 60 hypercholesterolemic subjects, previously screened for LOX-1 3UTR polymorphism, randomized, according to genetic profile (15 T and 15 C carriers for each arm), to atorvastatin 20 mg/d or rosuvastatin 10 mg/d.
**Results** After 8 weeks, both therapies were associated with comparable, significant reductions in LDL cholesterol (41.1% and 43.5%, respectively), Apo B (31.7% vs. 29.1%), Apo B/Apo A ratio (37.1% vs. 31.8%), as well as plasma hs-CRP (9.3% vs. 13.2%), urinary 11-dehydro-thromboxane(TX)B2 (37.5% vs. 30.5%) and 8-iso-prostaglandin(PG)F2a (29.4% vs. 23.7%). The impact of rosuvastatin or atorvastatin on CRP, 8-iso-PGF2a, and 11-dehydro-TXB2 did not differ according to the LOX-1 haplotype. On multiple regression analyses, only CRP and LDL were independent predictors of 11-dehydro-TXB2, and only LDL was a significant predictor of 8-iso-PGF2a.
**Conclusions** Both atorvastatin and rosuvastatin cause comparable reductions of thromboxane-dependent platelet activation, lipid peroxidation and inflammation. The presence of 3UTR/LOX-1 polymorphism does not affect the changes induced by either statin.

### P107 **TYPE 2 DIABETES AND POLYMORPHISMS ON CHROMOSOME 9P21: A META-ANALYSIS**

Cugino D., Iacoviello L., Gianfagna F., Santimone I., de Gaetano G., Donati M.B., Di Castelnuovo A.
*Laboratori di Ricerca, Università Cattolica del Sacro Cuore, Campobasso*

**Background** Genome-Wide Association Studies identified variants on chromosome 9p21 associated with the risk of type 2 diabetes (T2D). We performed a meta-analysis of studies on the association of polymorphisms in chromosome 9p21 with T2D.
**Methods** Articles were retrieved screening electronic databases and cross references. Twenty-two publications were identified (8 in Caucasian and 14 in Asian populations), for a total of 38,455 T2D patients and 60,516 controls. Twenty-one studies investigated the role of the SNP rs10811661; in some studies three additional SNPs (rs564398, rs10757278, rs1333040) localized in the same region were genotyped. Population attributable risk (PAR) was computed as risk allele frequency*(OR-1)/OR, using the per-allele odds ratio (OR).
**Results** The risk allele (T) of rs10811661 was associated with T2D in most of the studies. In meta-analysis the overall per-allele OR was 1.24 (95% CI: 1.21-1.27; $P<0.0001$), with no difference according to ethnicity ($P=0.45$), and low heterogeneity ($P=0.040$) across studies partly explained by sample size (overall OR was slightly lower for studies with larger sample size, suggesting modest publication bias).

Modelling of inheritance suggests a codominant effect of the T allele. PAR of T2D related to this polymorphism is 15% for Caucasians and 13% for Asians. The overall odds ratio for the C allele of the SNP rs564398 was 1.10 (95% CI: 1.07-1.14; PAR=7%). The other SNPs showed negligible associations.
**Conclusion** This meta-analysis provides accurate and exhaustive estimates of the association of some genetic variants at chromosome 9p21 and type 2 diabetes. These findings clearly indicate a relatively small but irrefutable role of the T allele of the rs10811661 SNP in increasing by 21% to 27% the risk of T2D in a codominant way. The adjacency of this SNP to the CDKN2A/B genes suggest that the effect of this polymorphism on T2D risk may be related to the function of these two genes.

P108 **THROMBOPHILIC GENOTYPE IN CORONARY STENT THROMBOSIS?**

Lodigiani C.[(1)], Ferrazzi P.[(1)], Mendolicchio L.[(1)], Librè L.[(1)], Quaglia I.[(1)], Colombo A.[(1)], Mari D.[(2)], Rota L.L.[(1)]
*[(1)] Centro Trombosi, IRCCS Ist. Clinico Humanitas Milano-Rozzano; [(2)] Dipartimento di Scienze Mediche, Univ. di Milano - Unità di Geriatria, IRCCS Ist. Auxologico Italiano, Milano*

Thrombophilia is associated with venous thrombosis: recent data suggest a potential role in arterial thrombosis. The risk of stent thrombosis has been reduced by the widespread use of antiplatelets drugs: recent data suggest that the incidence of stent thrombosis is less than 1% and depends on several factors, such as type of stent, complexity of lesions and clinical aspects. The purpose of this study was to evaluate if a genetic thrombophilic assessment increases the risk of coronary stent thrombosis.
**Materials and Methods** We retrospectively studied 30 patients with history of stent thrombosis (M 28; F 2, mean age 63.5 years) and as control group 79 stable angina patients with stent and no evidence of thrombosis (M 63; F 15, mean age 53.6 years). All were assessed for cardiovascular risk factors, compliance to the antithrombotic therapy and genetic thrombophilia defects (G1691A FV mutation, G20210A FII mutation, C677T MTHFR polymorphism, T1040T TAFI polymorphism).
**Results** None of the polymorphisms and the evaluated cardiovascular risk factors showed a significant correlation with stent thrombosis. Age was statically correlated ($p<0.0001$); type II diabetes strongly correlated but didn't reach statistical significance and these patients were older. Among patients, at the onset of thrombosis 41.4% was on asa+ thyenopiridine, 20.7% on asa, 13.8% only on thyenopiridine, 20.7% with no antiplatelets therapy, 3.4% was in asa+ vitamin k antagonists. We couldn't evaluate the role of different antiplatelets therapy in the onset of stent thrombosis due to missing information in the control group, although the study was not designed for this purpose.
**Conclusions** Thrombophilia does not seem to be significantly involved in stent thrombosis.

## *FIBRINOLISI: BIOCHIMICA E FISIOLOGIA*

P109 **ELEVATED D-DIMER IN ASCITIC FLUID FROM CIRRHOTIC PATIENTS: WHAT IS THE SOURCE?**

Lami D.[(1)], Cellai A.P.[(2)], Antonucci E.[(1)], Paniccia R.[(1)], Maggini N.[(1)], Miranda M.[(1)], Natucci F.[(3)], Romanelli R.[(3)], Tostiguerra C.[(3)], Laffi G.[(3)], Abbate R.[(1)], Prisco D.[(1)]
*[(1)] Department of Medical and Surgical Critical Care, University of Florence, Florence; [(2)] Thrombosis Centre, Department of Heart and Vessels, Azienda Ospedaliero-Universitaria Careggi, Florence; [(3)] Department of Internal Medicine, University of Florence, Florence*

**Background** Ascitic fluid has been hypothesized to be the origin for hyperfibrinolysis observed in advanced liver disease.
**Objective** To determine whether a fibrinolytic activity is detectable in the ascitic fluid of patients with ascitic liver cirrhosis and whether such activity is correlated to blood fibrinolysis.
**Methods** We evaluated 12 patients with liver cirrhosis. Fibrinolytic system was studied both in plasma and ascitic fluid by Clot Lysis Time (CLT), D-dimer, tissue plasminogen activator (t-PA), plasminogen activator inhibitor (PAI-1), α2-antiplasm (α2AP), plasminogen (PLG) and thrombin activatable fibrinolysis inhibitor (TAFI). Thromboelastometry was performed by a 4-channel analyzer (ROTEM, Pentapharm, Germany).
**Results** We found that α2AP (%), PLG (%), TAFI (μg/mL) and fibrinogen (mg/dL) levels were significantly higher in plasma than in ascitic fluid (for all $p<0.0001$) from patients. For all these parameters plasma levels were lower in patients than in controls ($P<0.0001$). Instead, D-Dimer levels (ng/mL) were significantly lower in plasma than in ascitic fluid [2,360 (138-3,025) vs. 40,777 (3,448-60,351), $p<0.0001$], whereas similar concentrations were found for t-PA and PAI-1. D-dimer, PAI-1 and t-PA levels were significantly higher (at least $p=0.04$) and CLT shorter in plasma from patients than from healthy subjects ($p<0.004$). No fibrinolytic activity was detectable in ascitic fluid by CLT. By thromboelastometry no signal was detectable in ascitic fluid whereas in whole blood different parameters suggested an impaired coagulability in patients vs. controls without differences in the percentage of clot lysis.
**Conclusion**: Elevated levels of D-dimer are detectable both in plasma and in ascitic fluid from cirrhotic patients. The absence of detectable fibrinogen and the presence of some but not all fibrinolytic components in ascitic fluid suggest D-dimer may be produced in different sites and concentrated in ascitic fluid.

## *INFIAMMAZIONE E TROMBOSI*

P110 **SOLUBLE ENDOTHELIAL PROTEIN C RECEPTOR IS CORRELATED WITH LYMPHOCYTE CD4+ ABSOLUTE CELL COUNTS AND CHANGES OVER TIME IN HIV+, MARAVIROC-TREATED PATIENTS**

Faioni E.M.[(1)], Nozza S.[(2)], Tambussi G.[(2)], Fontana G.[(1)], Razzari C.[(1)], Galli L.[(2)], Spagnuolo V.[(2)], Cattaneo M.[(1)]
*[(1)] Dipartimento di Medicina, Chirurgia e Odontoiatria, Università degli Studi di Milano e Medicina III, Azienda Ospedaliera San Paolo, Milano; [(2)] Clinica delle Malattie Infettive, Ospedale San Raffaele, Centro San Luigi, Milano*

**Background** HIV infection is associated with disimmunity,

chronic inflammation and coagulation activation. Serum markers may be useful to understand the pathophysiology of the disease and to monitor the disease progression and/or response to therapy. The endothelial protein C receptor (EPCR) participates in protein C activation and in the regulation of inflammation; its soluble form is shed from the endothelium and found in plasma. Maraviroc (MVC) is a CCR5 antagonist with anti-inflammatory properties.
**Aim of the study** To evaluate changes of coagulation and inflammation markers in 96 stable disease HIV+ patients (82 men, 14 women) treated with MVC (56/96) or conventional therapy (CT), and to correlate them with features of the disease. The following were measured at baseline, 4, 8, 12 and 24 weeks: D-dimer, sEPCR, soluble thrombomodulin (sTM), interleukin-6 (IL-6) and hsPCR.
**Results** Patients in the 2 treatment groups did not differ for HIV disease characteristics. Markers of coagulation and inflammation were generally within their normal reference ranges, and did not differ between the two treatment groups at any time; sEPCR tended to decrease in both groups [median change per week (slope) in the MVC group: –0.70 (–1.53/+0.73); CT group: –0.23 (–1.05/+0.88); p=0.352]. CD4+ cells increased more rapidly in the MVC compared to the CT treatment group [MVC: 190/µL (100-261); CT: 133/µL (40-194); p=0.025]. Overall, changes at week 24 in sEPCR were correlated with baseline levels of CD4+ cell counts (r=–0.308, p=0.005) [MVC group: r=–0.613, p<0.0001; CT group: r=0.044, p=0.781].
**Conclusions** In stable HIV disease, markers of coagulation and inflammation are not useful to interpret the disease. Interestingly, sEPCR levels were inversely correlated with CD4+: this is the first report of a direct correlation between cellular immunity and the protein C system. Whether this is a functional correlation or just an epiphenomenon needs to be tested prospectively with clinical endpoints.

P111 **SOLUBLE CD40L IN MEDITERRANEAN SPOTTED FEVER: RELATION TO OXIDATIVE STRESS AND PLATELET ACTIVATION**

Ferrante E.[(1)], Lattanzio S.[(1)], Santilli F.[(1)], Vitale G.[(2)], Caruso R.[(2)], Davì V.[(2)], Di Rosa S.[(3)], Vazzana N.[(1)], Ciabattoni G.[(1)], Mansueto S.[(2)], Davì G.[(1)]
*[(1)] Centre of Excellence on Aging, G. d'Annunzio University Foundation, Chieti; [(2)] Department of Medicine, School of Medicine, Palermo; [(3)] Ospedale Villa Sofia Whitaker, Palermo*

**Introduction** In the present study, we tested the hypothesis that *Rickettsia Conorii* infection increased *in vivo* lipid peroxidation with generation of 8-iso-prostaglandin (PG)F2a and other biologically active iso-eicosanoids and that these compounds would in turn contribute to endothelial dysfunction and platelet activation in the setting of MSF. Moreover, consequent augmented release of CD40 ligand (CD40L) by activated platelets would be able to promote further inflammation and endothelial activation.
**materials and methods** We measured in 24 patients with MSF, in the acute stage and at follow-up (after day 21), plasma C-reactive protein (CRP), CD40L, asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, as a marker of endothelial dysfunction, and the urinary excretion rates of 8-iso-PGF2a and 11-dehydro-TXB2. Twenty healthy subjects were also studied as controls.
**Results** Plasma levels of CRP, sCD40L, ADMA and urinary metabolites excretion were significantly higher in MSF patients in the acute phase than at recovery (p<0.0001), or compared with healthy controls (p<0.0001).
When concentrations of ADMA of the entire sample at baseline were divided into quartiles, the excretion rate of 11-dehydro-TXB2 as well as plasma CD40L significantly increased from the first to the fourth quartile (by Kruskal-Wallis test; H, 15.9; p<0.001 an H, 16.7; p<0.001, respectively). Moreover, there was a direct correlation between time-related changes in plasma CD40L and changes in 11-dehydro-TXB2 excretion (Rho 0.47, p=0.033) or in 8-iso-PGF2a (Rho 0.45, p=0.028) throughout the observation period in the 24 patients.
**Conclusions** Thus, our study supports the idea that systemic inflammation, platelet activation and endothelial dysfunction are common features of Mediterranean Spotted Fever (MSF), with several feed-forward mechanisms sustaining enhanced sCD40L shedding and amplifying the relationship between inflammation and platelet activation.

P112 **SOLUBLE CD40 LIGAND AND ENDOTHELIAL DYSFUNCTION IN ASPIRIN-TREATED POLYCYTHEMIA VERA PATIENTS**

Lessiani G.[(1)], Dragani A.[(2)], Falco A.[(1)], Fioritoni F.[(2)], Santilli F.[(1)], Davì G.[(1)],
*[(1)] Centre of Excellence on Aging, G. d'Annunzio University Foundation, Chieti; [(2)] Department of Haematology, Pescara Hospital, Pescara*

Polycythemia vera (PV) is a chronic myeloproliferative disorder characterized by increased thrombotic risk and endothelial dysfunction.
Thirty-four patients with PV on aspirin 100 mg/die and 12 healthy volunteers were evaluated for CD40L plasma levels, flow-mediated dilation (FMD) and nitrate-mediated dilation (NMD).
PV patients showed significantly higher CD40L levels than controls (P<0.0001), and impaired FMD (P=0.008), with similar NMD response in the two groups (P=0.71). On multiple regression analysis FMD was the only predictor of CD40L (B=–0.43, P=0.01), independently of age, hematocrit, platelet count, risk factors or previous vascular events, hydroxyurea treatment, NMD.
Increased CD40L levels and endothelial dysfunction in aspirin-treated PV patients may constitute an aspirin-insensitive atherothrombotic mechanism.

## *LABORATORIO DI EMOSTASI E TROMBOSI*

P113 **DETECTION OF THE IMBALANCE OF PRO- VS. ANTI-COAGULANT FACTORS IN CIRRHOSIS BY A SIMPLE LABORATORY METHOD**

Chantarangkul V.[(1)], Tripodi A.[(1)], Primignani M.[(2)], Lemma L.[(1)], Dell'Era A.[(2)], Iannuzzi F.[(2)], Aghemo A.[(3)], Mannucci P.M.[(1)]
*[(1)] "Angelo Bianchi Bonomi" Hemophilia and Thrombosis Centre, Department of Internal Medicine, Department of Medicine and Medical Specialties, Milano; [(2)] Department of*

*Internal Medicine, Third Division of Gastroenterology, Milano; (3) Department of Internal Medicine, First Division of Gastroenterology, Milano*

Cirrhotic patients possess a pro- vs. anti-coagulant imbalance due to increased factor-VIII and decreased protein-C. This imbalance can be detected by thrombin-generation assays performed in the presence/absence of thrombomodulin (predicate-assay) that are not readily available in clinical laboratories. We sought to assess this hypercoagulability with a simpler thrombin-generation assay performed in the presence/absence of Protac, a snake venom that activates protein-C in a manner similar to thrombomodulin (new-assay). We analyzed blood from 105 cirrhotics and 105 healthy subjects (controls).
Results for the predicate- or the new-assay were expressed as ratio (with-to-without-thrombomodulin) or as Protac-induced-coagulation-inhibition (PICI%). By definition, high ratios or low PICI% translate into hypercoagulability. The median (range) PICI% was lower in patients [74% (31-97%)] than controls [93% (72-99%)] $p<0.001$ indicating that cirrhotics are resistant to the action of Protac. This resistance resulted in greater plasma hypercoagulability in patients of Child C than A-B. The hypercoagulability of Child C [63% (31-92%)] was similar to that observed for factor-V-Leiden patients [69% (15-80%)] $p=0.59$. The PICI% values were correlated with the levels of protein-C ($rho=0.728$, $p<0.001$) or factor-VIII ($rho=–0.517$, $p<0.001$). Finally the PICI% values were correlated with the predicate-assay ($rho=–0.580$, $p<0.001$).
In conclusion, the hypercoagulability of plasma from cirrhotics can be detected with the new-assay that compares favourably with the other markers of hypercoagulability (i.e, high factor-VIII and low protein-C) and with the predicate-assay based on thrombin-generation with/without thrombomodulin. Advantages of the new- over the predicate-assay are easy performance and standardized results. Prospective trials are needed to ascertain whether it is useful to predict thrombosis in cirrhotics.

## P114 CARBETOCIN INCREASE THROMBIN GENERATION AFTER CAESAREAN SECTION

Testa S.(1), Paoletti O.(1), Bassi L.(1), Alatri A.(1), Zimmermann A.(1), Cogrossi A.(2), Mammoliti D.(3), Riccardi A.(3), Cancellieri E.(1)
*(1) Clinical Pathology Division, Haemostasis and Thrombosis Centre, AO Istituti Ospitalieri, Cremona; (2) Clinical Pathology Division, AO Istituti Ospitalieri, Cremona; (3) Obstetrics and Gynaecology Division, AO Istituti Ospitalieri, Cremona*

**Background** To prevent post partum haemorrhage uterotonic prophylactic drugs are commonly used after caesarean section. Aim of the present pilot study is to evaluate thrombin generation(TG) after caesarean section (CS) in women treated with carbetocin and oxitocin, two different uterotonic agents.
**Materials and Methods** We enrolled 28 women, undergoing caesarean section, 14 treated with oxytocin and 14 treated with carbetocin. Patients, without previous bleeding or thrombotic events, were matched for age, weight, parity and race.
Blood samples for TG study and blood cell count were collected before delivery (T0), 1 hour (T1) and 24 hour (T2) after drug infusion.
Blood samples were immediate centrifuged and poor platelet plasma (PPP) stored at −80°C. Thrombin generation was performed with a commercial assay (Technothrombin TGA, Technoclone). TG measured lag time, peak, velocity, endogenous thrombin potential (ETP). The activation of coagulation was obtained with adding small amounts of tissue Factor and phospholipid. ETP results were expressed as nM-Thrombin/min.
**Results** No differences were observed in TG between two groups at T0. A significant increase in ETP at T1 was observed in the carbetocin group (ETP mean±DS= 3,810.8±661.95), than oxitocin treated patients (ETP mean±DS= 3,588.7±711.36) with $p<0.05$. T2 showed the persistent ETP increase in carbetocin group even if it didn't reach the statistical significance. Also other parameters like peak and velocity were significantly increased in the carbetocin compared with oxitocin group both at T1 and T2. No differences in bleeding were observed in the two groups.
**Conclusions** Our pilot study show a major ETP increase in patients treated with carbetocin, probably reflecting an important uterotonic action and its longer half-life in comparison with oxitocin. These properties, as recently reported, may support carbetocin use during delivery to prevent postpartum blood loss.

## P115 THROMBOPATH DETERMINATION DURING 1 YEAR-FOLLOW-UP IN ACUTE CORONARY SYNDROMES

Rogolino A.(1), Fedi S.(1), Marcucci R.(2), Fabbri A.(2), Cordisco A.(2), Giglioli C.(2), Valente S.(2), Abbate R.(2), Gensini G.F.(3), Gori A.M.(2)
*(1) Dpt. Heart and Vessels, Atherothrombotic Diseases Unit, AOU Careggi, Florence; (2) Dpt. Medical and Surgical Critical Care - Atherothrombotic Diseases Unit, AOU Careggi, University of Florence, Florence; (3) Dpt. Medical and Surgical Critical Care - Dpt. Heart and Vessels, AOU Careggi, University of Florence, Florence*

Acute coronary syndrome (ACS) is a state induced by thrombosis consequent to unstable atherosclerotic plaque rupture. The plaques procoagulant content triggers platelet and coagulation activation. After acute cardiovascular events occurrence, a hypercoagulable state has been documented, but few data on blood clotting activation durability are available.
Thrombopath (ThP, Instrumentation Laboratory) is a global assay sensitive to prothrombotic factors and activated protein C.
We aimed to evaluate the balance between anticoagulant and procoagulant factors, by using the ThP, during a follow-up of 12 months after an acute event in 115 (87 males/28 females) ACS patients undergoing percutaneous coronary intervention (PCI) with stent implantation on dual antiplatelet treatment.
ThP analysis was performed at the time of acute coronary events (T), after 1 (T1), 6 (T2) and 12 (T3) months. Altered ThP cut-off value was set at 77.31% protac-induced coagulation inhibition (PICI% mean values in 150 controls-2SD).
Baseline values (T0) of ThP were significantly lower in ACS patients than in controls [78.84 (40.82-94.11)% vs. 87.48 (72.50-97.65)%, $p<0.0001$].
PICI values significantly increased during the 12-month follow-up ($p<0.0001$). At T1 PICI values were slightly but not significantly higher than those observed at T [80.62 (51.00-

91.02)% vs. 78.84 (40.82-94.11)%]. After 6 and 12 months of follow-up a marked increased with respect to baseline PICI values was observed [T2: 82.42 (53.96-91.39)%; T3: 83.32 (67.07-91.77)%, p<0.0001 vs. T0].
At T altered ThP values were found in 46.1% of ACS patients. Similar figure was found at T1: altered ThP values were present in 41.7% of patients. Abnormal ThP values significantly (p<0.01) decreased to 25.2% at T2 and 21.7% at T3.
This study demonstrates that a marked unbalance of coagulation cascade persists 1 month after the acute coronary event. In spite of the progressive increase in ThP values during the follow-up a hypercoagulable state is yet present 6 and 12 months after the vascular events.

P116 **USE OF QUANTA LYZER ANALYZER FOR AUTOMATIZATION OF IMMUNOENZYMATIC ASSAYS FOR HAEMOSTASIS STUDY**

Morelli F., Novelli C., Stefanin C.
*Laboratorio di Ematologia - Trasfusionale, Ospedale di Legnano, Legnano*

**Background** The new business strategies in public hospitals (overall cost reduction, failure to replace staff on leave or termination for whatever reason) make problematic the implementation of certain hemostasis parameters of first and second level. The automatization of these tests becomes an important solution in terms of reducing the time spent by technical staff performing these tests while ensuring improved analytical performances in terms of reproducibility of results in relation to the methodology used.
**Purpose** To evaluate the analytical performance of the instrument Quanta Lyser manufactured by company GSG Robotix and distributed in Italy by the company INOVA (now acquired by Instrumentation Laboratory) in performing second-level parameters in the study of hemostasis.
**Materials and Methods** We evaluated the following commercial kits: Imuclone TAFI, Imubind TF, Imubind Total TFPI, Imubind Plasma PAI-1 and t-PA Imubind of American Diagnostica, Zymutest HIA IgGAM, Zymutest Protein Z and Zymuphen MP-Activity of Hyphen, Technozym ADAMTS-13 Activity, Antigen and INH of Technoclone, Enzygnost F1 +2 and Enzygnost TAT of Siemens, sICAM-1, CD62, sVCAM-1 and Human VEGF of DiaClone.
Results The methods were performed in both ways, in single analytical seat and together other tests inside the profiles created in the laboratory for: 1) diagnosis of specific diseases (e.g. TPP and HIT), 2) second level screening of thrombophilic states, or 3) research studies on specific parameters (microparticles. sICAM-1, CD62, VEGF and sVCAM-1). It was calculated the intra-assay CV and inter-assay using the controls included in the kits and a pool of plasmas prepared in the laboratory. The C.V. methods performed manually ranged between 6.5 and 11.8%, the CV methods performed on Quanta Lyzer ranged between 4.8 and 8.3%
**Conclusions** Excellent results have been obtained in the study of parameters of haemostasis providing a significant improvement in analytical performances in terms of reproducibility.

P117 **IDENTIFYING PROTEIN C PATHWAY-ASSOCIATED THROMBOPHILIA BY THE SCREENING ASSAY THROMBOPATH**

Imbimbo V.[(1)], Coppola A.[(2)], Di Capua M.[(2)], Coda R.[(1)], Russo A.[(1)], Tufano A.[(2)], Di Minno M.N.D.[(2)], Cerbone A.M.[(2)], Ingenito M.[(3)], Spina M.[(1)]
*[(1)] UOC Patologia Clinica, OORR Area Stabiese, ASL NA3 Sud, Castellammare di Stabia; [(2)] Università Federico II, Centro di Riferimento Regionale per le Emocoagulopatie - DACM, Napoli; [(3)] UO Citogenetica e Genetica Molecolare, ASL Salerno, Pagani*

**Introduction** Protein C (PC) pathway abnormalities (PC and protein S [PS] deficiencies, activated protein C resistance [APCR]/Factor V Leiden [FVL], Lupus anticoagulant [LAC]) are a major cause of hereditary or acquired thrombophilia. Running a full panel of protein C pathway tests for identifying these abnormalities is expensive, time consuming and, in some cases, unavailable. ThromboPath is an automated chromogenic assay measuring thrombin generation by using a Tissue Factor-based reagent, with and without endogenous protein C activation by Protac. The inadequacy of the natural anticoagulant activity is evaluated by the Protac-induced Coagulation Inhibition percentage (PiCI%). The optimal reference cut-off is usually determined at each user's site.
**Methods** ThromboPath (HemosIL, Instrumentation Laboratories) was evaluated in 80 subjects (51 women, 29 men) undergoing thrombophilia screening because of thrombotic events (venous, n=24; arterial, n=19), obstetric complications (n=8), or family history of thrombophilia and/or high thromboembolic risk conditions (n=29). All coagulation tests, including PC (chromogenic), PS (free, immunologic), APCR (coagulative), LAC (coagulative DRVVT and SCT screen and confirm) were carried by an ACL TOP analyzer. FVL was detected by a standard PCR-based method.
**Results** Twenty-three subjects revealed PC pathway abnormalities (2 PC and 3 PS deficiencies, 3 LAC, 15 APCR) and significantly lower PiCI% than subjects without such abnormalities (67.5±5.3 vs. 86.6±1.3, p<0.001). Thirty-eight subjects showed PiCI% values below the cut-off determined as 85.3% (mean −1SD of non-thrombophilic subjects). All but one subjects carrying PC pathway-related thrombophilia had PiCI% below this cut-off (a PS-deficient subject was falsely negative), vs. 16/57 non-thrombophilic subjects. In this population, TromboPath showed a 96% sensitivity and 72% specificity in identifying subjects with PC pathway-related thrombophilia.
**Conclusions** ThromboPath may represent an useful, cost-effective, highly sensitive screening assay for identifying subjects carrying PC pathway-related thrombophilia.

P118 **GLOBAL ASSAYS FOR MEASUREMENT OF THROMBOTIC RISK IN CHRONIC HEMODIALYSIS PATIENTS**

Papa M.L.[(1)], Capasso F.[(1)], Torre S.[(1)], Froncillo G.[(1)], Cavaliere E.[(1)], Russo V.[(1)], Sarti A.[(2)], Santoro G.[(1)], d'Eufemia M.D.[(3)], De Lucia D.[(3)]
*[(1)] UOC Laboratorio di Emostasi e Trombosi, Dipartimento Centrale Medicina di Laboratorio, PO San Giovanni Bosco, NA1 Centro, Napoli; [(2)] U.O. Nefrologia ed Emodialisi, Medicina, Scafati; [(3)] Seconda Università di Napoli, Azienda Ospedaliera, Napoli*

**Background** Haemodialytic treatment predisposes patients to thromboses in vascular access, in coronaric and cerebral districts Global haemostasis assays are the best methods for detecting prothrombotic states. Thromboelastometry (TEM), an indirect measure of thrombin and fibrin generation, may provide information for hypercoagulable conditions. TEM, performed by ROTEM, displays a velocity curve of clot formation with three parameters: MaxVel, t- MaxVel, AUC (Area Under Curve).
**Methods** 80 HD patients (55 males, 35 females), median age 5527.8 and 160 healthy individuals were studied. Prothrombin fragment 1+2 (F1+2), Thrombin- Antithrombin Complexes (TAT), D-Dimers were also determined.
**Results** The MaxVel, by INTEM test, median 20 mm*100/sec; range 14-31; was significantly higher compared with controls, median 14 mm*100/sec; range 11-18; p= 0.004. The AUC, median 6,478 mm*100/sec; range 5,276-7,674; was significantly increased compared with controls, median 5,724 mm*100/sec; range 4,763-6,523; p=0.004. The MaxVel, by EXTEM test, was higher, median 18 mm*100/sec; range 12-28; compared with controls, median 14 mm*100/sec; range 10-22; p=0.004. The AUC was raised in patients, median 6,300 mm*100/sec; range 5,270-7,566; compared to controls, median 5,701 mm*100/sec; range 4,819-6,627; p=0.003 .The F1+2 resulted increased in patients (1±1.2) vs. controls (0.7±0.1; p=0.001). The TAT was higher in patients (7±9.2) compared with controls (0.6±1.2; p= 0.000). The D-Dimers were significantly increased (467±205) compared to controls (191±21; p=0.000).
**Conclusions** A prothrombotic state in HD patients was identified. Prospective studies are necessary to clarify if ROTEM velocity parameters could reveal patients at higher thrombotic risk and provide a suitable prophylactic regimen.

## P119 ABNORMAL THROMBOPATH ASSAY IN HAEMODIALYSIS PATIENTS: A NEW MARKER OF THROMBOTIC RISK?

Papa M.L.[(1)], Migliorini O.[(2)], Capasso F.[(1)], Torre S.[(1)], Froncillo G.[(1)], Russo V.[(1)], Cavaliere E.[(1)], Palmiero G.[(3)], Sarti A.[(4)], Pone G.[(3)], d'Eufemia M.D.[(5)], Piccione A.[(3)]
*[(1)] UOC Laboratorio di Emostasi e Trombosi, Dipartimento Centrale Medicina di Laboratorio, PO San Giovanni Bosco, NA1 Centro, Napoli; [(2)] Linea Emostasi, Instrumentation Laboratori, Milano; [(3)] Emodialisi, Amb. Nefrologia ed Emodialisi, Napoli; [(4)] U.O. Nefrologia ed Emodialisi, Medicina, Scafati; [(5)] Seconda Università di Napoli, Azienda Ospedaliera, Napoli*

**Background** In hemodialysis patients abnormalities of protein C (PC) anticoagulant system are frequent. An assay, for determining globally the PC pathway components, would rationalize the use of many tests.
**Methods** 80 hemodialysis patients (55 males, 35 females) median age 55±2.78 and 80 healthy individuals were studied. A test to evaluate the functionality of the PC pathway was employed: the HemosIL ThromboPath (IL). This chromogenic assay is based on the ability of APC generated after activation of PC by snake venom (Protac) to reduce the thrombin generation. The results were expressed as the Protac-Induced Coagulation Inhibition percentage (PICI%). PC, PS, APC Resistance and Lupus Anticoagulant were determined simultaneously. The mutations of FV Leiden and of FIIG20210A were detected by the Xpert HemosIL FII and FV Assay, a rapid genotyping test. (IL).
**Results** The cut-off value for the PICI% in normal subjects was 83.93. The patients' results (71.39) were below the defined cut-off level for the PICI%. We found an influence of gender on cut- off value. Results were below the cut-off level for the PICI% in hemodialysis patients with any PC pathway abnormality but also in 32 patients without PC pathway abnormality.
**Conclusions** We have identified an elevated percentage of hemodialysis patients who had an abnormal Thrombopath test without any PC pathway abnormality. We might not exclude that unknown defects of PC pathway may decrease the PICI% or that Hemosil ThromboPath assay might be a marker of thrombotic risk. Patients with hypercoagulable states, or asymptomatic, undergoing treatments at high and moderate thrombotic risk, could be monitored with ThromboPath assay.

## P120 THROMBOPATH DETERMINATION IN A GROUP OF BLOOD DONORS AND IN PATIENTS WITH A HISTORY OF VENOUS THROMBOEMBOLISM

Zighetti M.L.[(1)], Leo L.[(2)], Vozzo N.P.[(2)], Carpani G.[(2)]
*[(1)] Dipartimento di Medicina Trasfusionale e di Ematologia, Azienda Ospedaliera della Provincia di Lodi, Lodi; [(2)] Dipartimento di Medicina Trasfusionale e di Ematologia, Azienda Ospedaliera San Paolo, Milano*

The HemosIL ThromboPath assay (ThP, Instrumentation Laboratory) is a chromogenic thrombin generation assay sensitive to Protein C (PC) pathway dysfunctions. It is based on the ability of endogenous APC, activated by a snake (Contortrix contortrix) venom extract (Protac), to reduce the thrombin generation induced by a tissue factor (TF) containing reagent. ThP assay is suitable for testing hereditary and acquired impairments in PC pathway (PC and PS deficiency, APCR/factor V Leiden, lupus anticoagulant), with an overall sensitivity of at least 90%. About 13-15% of the subjects without any PC pathway abnormality had a pathologic test result.
In keeping with the important issue of preemptive medicine offered by transfusion units to blood donors, usually all donors at our centre are screened for the presence of atherothrombotic risk factors (cholesterol, glycaemia, blood pressure and body mass index). In this study we determined the ThP assay in 65 blood donors as a possible biological marker of thrombophilia and in 31 patients with a history of thromboembolism (VTE) and ascertained abnormalities in PC pathway (19 PS and/or PC deficiency, 2 APCR/factor V Leiden heterozygotes, 10 LLAC). None of our blood donors had a history of present or past venous thrombosis. In patients affected by PC pathway abnormalities ThP values were significantly lower ($p<0.0001$) than in blood donors. Interestingly, a lower ThP value (below −1SD of controls mean) was found in 10.7% of blood donors.
Our data confirm that ThP is a global assay sensitive to PC pathway impairment and could be a useful tool in blood donors screening for the presence of venous thrombosis risk factors. Further studies are necessary to confirm these results.

## P121 VARIOUS SENSITIVITY TO APTT TESTS IN COAGULANT DEFECTS OF THE CONTACT FACTOR

**SYSTEM**

Consiglio P., De Michele S., Vitale G., Palma B.
*Patologia Clinica, ARNAS O. Civico, Palermo*

**Introduction** Recent studies have revalued the role of the contact system proteins in blood coagulation. Impairment in FXII, PK, HMWK, emphasized *in vitro* by a prolonged aPTT, is not associated with bleeding tendency thanks to other prothrombotic mechanisms such as RNA and polyphosphates. However, not all the commercially available aPTT reagents have the same sensitivity in detecting that deficiency. Only the use of low sensitivity reagents allows monitoring heparin during major surgical operations.
**Methods** We report the case of a woman admitted to the Gynaecology division of our hospital for an operation of ovarian cystis. At the admission, she presented coagulant tests showing a normal PT and a very prolonged aPTT. The familiar and personal anamnesis was negative for bleedings. The tests performed in our laboratory showed a normal aPTT (Actin FS Siemens). A different instrument (ACL TOP IL) and reagent (Synthasil IL) additionally showed a prolonged aPTT. The dosage of factors did not show any deficiency and the search for Lupus Anticoagulant was negative. Other commercially available aPTT reagents confirmed this double behaviour: aPTT in the range (ellagic acid) and prolonged aPTT (silica).
**Results** The analysis of the various reagents and the clinical history showed a different sensitivity of the silica activator with respect to ellagic acid/Kaolin, and suggested either a PK or a HMWK deficiency. The extension of the incubation time of the silica-containing reagents, necessary for maximizing the activation of Factor XII even in the absence of prekallicrein, led to a progressive reduction of coagulation time. This response seems to be a feature of prekallicrein deficiency, as reported in literature.
**Conclusions** The dosage of aPTT with the ellagic acid-containing reagent (ACTIN FS) allowed us to easily perform the surgical treatment and the anti-thrombotic profilaxis with heparin.

P122 **IS COAGULATION CASCADE BALANCE, ASSESSED BY THROMBOPATH, ASSOCIATED WITH PLATELET HYPER-REACTIVITY?**

Fedi S.[(1)], Rogolino A.[(2)], Gori A.M.[(1)], Marcucci R.[(1)], Paniccia R.[(1)], Miranda M.[(1)], Maggini N.[(1)], Antonucci E.[(1)], Gensini G.F.[(1)], Abbate R.[(1)]
[(1)] *Medical and Surgical Critical Care, University of Florence, Florence;* [(2)] *Heart and Vessels, Atherothrombotic Disease Unit, Az. Ospedaliero Universitaria, Careggi, Florence*

Hypercoagulability and platelet hyper-reactivity are involved in atherothrombotic diseases and are associated with an increased risk of the occurrence of adverse events in acute coronary syndrome (ACS) patients undergoing PCI with stent implantation. Thrombopath is a global assay sensitive to PC system and prothrombotic factors. We evaluated the association between coagulation cascade balance, assessed by Thrombopath, and platelet hyper-reactivity in 157 (118 males/39 females) ACS patients undergoing PCI with stent implantation on dual antiplatelet treatment.
Thrombopath analysis was performed on ACL Top (Instrumentation Laboratory). Platelet function was evaluated by VerifyNowAspirin (ARU) and VerifyNowP2Y12 (PRU) (Accumetrics). High-on treatment platelet reactivity (HPR) by VerifyNow was defined by the presence of PRU>=240 or ARU≥550. Altered Thrombopath (ThP) values was set at 77.31% protac-induced coagulation inhibition (PICI).
PICI values were significantly ($p<0.05$) lower in patients with HPR by VerifyNowP2Y12 than in patients without [76.63 (40.82-90.75) vs. 80.01 (45.60-94.12) PICI%]. Similarly, in patients with HPR by VerifyNowP2Y12 or HPR by VerifyNowAspirin we found significant ($p<0.05$) lower levels of PICI [76.45 (40.60-90.13) vs. 80.21 (45.64-97.15) PICI%].
Among the 62 ACS patients with HPR by VerifyNowP2Y12 (PRU ≥240) we found the occurrence of altered ThP values in 35(56.5%) patients, whereas in patients without HPR the percentage of patients with altered ThP values was 31.8% (27/85) ($p=0.031$). Altered ThP values were found in 10/18 (55.6%) ACS patients with HPR by VerifyNowAspirin (ARU ≥550) and in 63/139 (45.2%) patients without HPR.
ACS patients with HPR by VerifyNowP2Y12 and/or HPR by VerifyNowAspirin showed a significant ($p=0.010$) higher prevalence (58.3%, 42/72) of altered PICI values with respect to patients without HPR (36.5%, 35/85).
Our results indicate the association between unbalanced coagulation pathway and platelet hyper-reactivity in ACS patients. The unbalance between anticoagulant and procoagulant factors, assessed by Thrombopathή assay, may represent a mechanism underlying platelet activation.

P123 ***IN VITRO*** **UNDETECTABLE PT AND FIBRINOGEN (AND *IN VIVO*?)**

Pradella P.[(1)], Giacomello R.[(2)], Uxa L.[(1)], Florean M.[(1)]
[(1)] *Medicina Trasfusionale, A.O.U. Ospedali Riuniti di Trieste, Trieste;* [(2)] *Medicina di Laboratorio, A.O.U. S. Maria della Misericordia, Udine*

An 81 aged woman came to E.R. of Trieste University Hospital with a traumatic head injury. Blood cells count, liver enzymes and other parameters were normal, but with a photometric clot detection method PT and PT-derived Fibrinogen were undetectable, Fibrinogen-Clauss gave different results (157 to 357 mg/dL) and aPTT-Ratio was normal (0.96). When the instrument detection performance was improved, PT was normal and PT-derived Fibrinogen detectable, but Fibrinogen-Clauss was still very unsteady (352/294/558 mg/dL). On a mixing test with normal pool plasma, PT was corrected, PT-derived Fibrinogen was very low (80 mg/dL) and Fibrinogen-Clauss resulted 360 mg/dL. With a different optical analyzer, PT and aPTT yielded the same results, Fibrinogen-Clauss was 557 mg/dL with a very steep clot formation curve using 35 IU/mL Thrombin reagent, but it was 113 mg/dL with a normal curve using 15 IU/mL Thrombin reagent. With an electromechanical clot detection method, PT-INR and aPTT-Ratio were normal (0.88 and 0.96 respectively), Fibrinogen-Clauss was normal (400 mg/dL) but unsteady. By a nephelometric immunoassay, Fibrinogen-Antigen was 368 mg/dL. However in a few days our patient healed up perfectly; she declared that her sister had the same performance when she was referred to another Hospital for a check-up, nonetheless they never had any severe bleeding in

their life.
Samples from our patient's son and daughter were taken and resulted completely normal for coagulation tests.
We hypothetized:
1) a too fast Thrombin formation and/or Fibrinogen consumption, as shown by steep coagulation curves without a stable plateau;
2) an excessive thrombin formation, (in preliminary studies, however, G20210A mutation was absent and F1+2 were normal);
3) a dysfibrinogenemia, (to be studied).

Further studies for Endogenous Thrombin Potential about thrombin hypothesis and for genetical pattern about fibrinogen molecule are needed to clarify this case.

## *COAGULAZIONE INTRAVASCOLARE DISSEMINATA*

### P124 **IL SIGNIFICATO PROGNOSTICO DEI LIVELLI DI TROMBOMODULINA SOLUBILE IN PAZIENTI CON SEPSI SEVERA TRATTATI CON PROTEINA C ZIMOGENO**

Della Valle P.[(1)], Crivellari M.[(2)], Pavani G.[(1)], Bignami E.[(2)], Zangrillo A.[(2)], D'Angelo A.[(1)]
*[(1)] Servizio di Coagulazione & Unità Ricerca Trombosi, Istituto Scientifico Universitario San Raffaele, Milano; [(2)] Dipartimento di Anestesia e Terapia Intensiva, Istituto Scientifico Universitario San Raffaele, Milano*

La proteina C attivata, associata ad un aumentato rischio emorragico, è controindicata nella sepsi severa postchirurgica. Proteina C zimogeno (Ceprotin, Baxter) è stata somministrata (50 UI/kg in bolo + infusione continua di 3 UI/kg/h per 72 ore) a pazienti con sepsi severa (n=10) o shock settico (n=13) ricoverati in terapia intensiva cardiochirurgica (19 maschi, età 63±12 anni), con insufficienza multiorgano e score APACHE II di 25±5. Il livello mediano basale di proteina C (coagulativa) era del 45%, saliva a 73% a 6 ore dal bolo e risultava del 79% a 12 ore dalla fine dell'infusione, senza osservare aumento significativo dei livelli circolanti di proteina C attivata. Rispetto al dato basale, lo score DIC ISTH risultava ridotto a fine trattamento (p=0.008). La mortalità a 28 giorni è stata del 30.4% (7/23), inferiore all'attesa (p=0.046). Parametri plasmatici coagulativi ed infiammatori mostravano comportamenti diversi tra sopravvissuti e deceduti. I livelli di PT tendevano alla norma maggiormente nei sopravvissuti che nei deceduti (p=0.024); il d-dimero aumentava nei deceduti ma non nei sopravvissuti (p=0.008); l'antitrombina aumentava (p<0.001) ed interleuchina-6, interleuchina-8, interleuchina-10 ed e-selettina solubile si riducevano solo nei sopravvissuti (p≤0.03). I livelli di trombomodulina solubile, che non mostravano variazioni significative nel tempo, erano sempre più alti nei deceduti che nei sopravvissuti e mostravano una correlazione positiva con i livelli di EPCR solubile, misurati solo in una parte dei casi (p=0.01).
In questa serie di pazienti con sepsi severa trattati con proteina C zimogeno si evidenzia per la prima volta come aumentati livelli di trombomodulina solubile abbiano significato prognostico sfavorevole.

## *MICROANGIOPATIE TROMBOTICHE E ADAMTS13*

### P125 **ASSOCIATION BETWEEN OBESITY AND RISK OF THROMBOTIC THROMBOCYTOPENIC PURPURA: POSSIBLE ROLE OF THROMBOSPONDIN-1**

Lombardi A.M.[(1)], Dal Prà C.[(2)], Zanato V.[(2)], Bonamigo E.[(1)], Vettore S.[(1)], Berti De Marinis G.[(1)], Sartori M.T.[(3)], Saggiorato G.[(1)], Vettor R.[(2)], Fabris F.[(1)]
*[(1)] Dep. of Medical and Surgical Science, Internal Medicine, Padova; [(2)] Dep. of Medical and Surgical science, Internal Medicine 3, Padova; [(3)] Dep. of Cardiologic Thoracic and Vascular Science, Internal Medicine 2, Padova*

Thrombotic thrombocytopenic purpura (TTP) is an acquired or congenital dis-ease caused by protease ADAMTS13 deficiency. Some studies have described an association between obesity and the risk to develop TTP, with an Odds Ratio of 7,6. Obesity is associated with a chronic low-grade inflammation condition with increased levels of adipokines, such as leptin, interleukin-6 (IL-6), tumour necrosis factor (TNF)α and thrombospondin (TSP)1. ADAMTS13 contains eight thrombospondin repeat domains with structural analogy with TSP1.
We studied 49 obese patients and 26 lean normal subjects, who were characterized by weight, height, waist circumference, BMI and endocrine, metabolic and coagulative parameters. ADAMTS13 antigen and activity levels, ADAMTS13 autoantibodies, and TSP-1 levels were assessed.
There were no significant differences in ADAMTS13 antigen and activity levels between obese patients and controls. However, we found a significant titre of ADAMTS13 autoantibodies (21.3% in obese vs. 0% in lean subjects, p<0.01), even if without inhibitory activity towards the protease. No correlation was found between ADAMTS13 autoantibodies and BMI, HOMA-IR, PCR, IL-6, TNFα and leptin. Moreover TSP-1 levels were significantly higher in obese patients than in normal subjects (p<0.0001) and were inversely correlated with ADAMTS13 activity.
We hypothesize that the increased levels of TSP1 observed in obese subjects could induce the production of anti-TSP1 autoantibodies, which could cross-react with the protease as a consequence of the structural analogy between TSP1 and ADAMTS13. Although these antibodies have no inhibitory activity towards the protease, this property could be acquired because of somatic mutations in immunoglobulins.

### P126 **REFRACTORY/RELAPSED THROMBOTIC THROMBO-CYTOPENIC PURPURA: LONG TERM TREATMENT WITH CYCLOSPORINE**

Caimi T.M., Redaelli R., Mostarda G., Corno A.R., Perego L., Nosari A.M., Morra E.
*S.C. Ematologia - S.S. Emostasi e Trombosi, Ospedale Niguarda, Milano*

Patients (pts) with severe ADAMTS13 deficiency due to the presence of inhibitor are at high risk of recurrence. Recent reports suggest that immunosuppressive therapy with Cyclosporin (CSA) may be effective when associated to plasma exchange (PE) or given as single treatment in refractory/relapsed TTP. The duration of treatment and range

of CSA serum level remain to be established.
Based on the data from the literature reporting high rate of relapse after 6-months course of treatment with CSA and on our experience, we decided to prolong CSA treatment for at least one year to obtain a stable remission of disease and normalization of ADAMTS13 activity.
**Population** 3 refractory (2 pts with autoimmune disorder) and 6 multiple relapsed pts (2-10 relapses) with severe ADAMTS13 deficiency (<5%) and inhibitor.
Treatment and follow-up: CSA was administered orally at a dose of 3-5 mg/kg BW to obtain a plasmatic level within 150-250 ng/mL. In the 3 refractory pts CSA was added to PE and continued after PE interruption. A clinical and laboratory follow up was performed every month.
ADAMTS13 and inhibitor were carried out every 1-3 months.
**Results** No relapse was observed during treatment. The median of CSA duration therapy was 18 months (range 11-102).
ADAMTS13 activity returned rapidly to normal level in 6/9 pts; in 2/9 pts level was about 35% and in 1 remained <5%.
7 pts are still on treatment: in 5/7 CSA tapering is ongoing.
2 pts have interrupted the treatment after 1 year: 1 patient not responsive and 1 patient because of renal insufficiency due to autoimmune disease. Therapy was well-tolerated.
**Conclusion** Our results indicate that CSA is effective and safe as reported in literature. We expect that the prolongation of treatment will obtain a sustained remission with normal ADAMTS13 activity.

## *POCT IN EMOSTASI*

### P127 **COMPARISON OF METHODS FOR MONITORING RESIDUAL PLATELET REACTIVITY AFTER CLOPIDOGREL BY POINT-OF-CARE TESTS ON WHOLE BLOOD IN HIGH RISK CORONARY ARTERY DISEASE PATIENTS**

Paniccia R.[(1)], Antonucci E.[(2)], Maggini N.[(2)], Miranda M.[(2)], Lami D.[(2)], Gori A.M.[(2)], Marcucci R.[(2)], Giusti B.[(2)], Prisco D.[(2)], Abbate R.[(2)]
[(1)] *Department of Medical and Surgical Critical Care, Thrombosis Centre, University of Florence, Florence*; [(2)] *Department of Medical and Surgical Critical Care, University of Florence, Florence*

Cardiovascular events are more frequent in high risk coronary artery disease (CAD) patients on dual antiplatelet therapy with residual platelet reactivity (RPR) than in those showing inhibition of ADP-inducible platelet activation. In particular, post-interventional RPR is a clinically important entity confirming it as a risk factor for thromboischemic events. Multiple electrode platelet aggregometry (MEA) (Dynabyte, Germany; DASIT, Italy) on whole blood has been recently proposed as a rapid tool to evaluate RPR in high risk CAD patients on clopidogrel therapy. Aim of this study was to detect RPR in 801 high risk CAD patients on dual antiplatelet therapy comparing MEA with VerifyNow P2Y12 Assay (Accumetrics, USA) on whole blood and classical light transmission aggregation (LTA) by APACT-4004 aggregometer (LABiTec, Germany) on platelet-rich plasma. ADP (10 μmol/L) was employed as agonist for MEA and LTA.
The prevalence of RPR was 20.6% by MEA, 16.1% by LTA and 30.8% by VerifyNow. MEA showed a significant correlation (rho=0.62, p<0.0001) with VerifyNow and a moderate agreement (k=0.52, p<0.001) with 81.5% of concordant values. A significant correlation was found between MEA and LTA (rho=0.71, p<0.001) with a good agreement (k=0.63, p<0.001) and 88.8% of concordant values. MEA in relation to LTA showed a sensitivity of 80% and a specificity of 91%. MEA might represent a reliable method and valid alternative in comparison with other available platelet function assays. It might help to guide antiplatelet therapy and thus improve clinical outcome of high risk CAD patients.

### P128 **MONITORING OF PLATELET FUNCTION OF CORONARY ARTERY DISEASE PATIENTS ON ASPIRIN BY MULTIPLATE ELECTRODE AGGREGOMETRY ON WHOLE BLOOD**

Paniccia R., Antonucci E., Maggini N., Miranda M., Gori A.M., Sticchi E., Marcucci R., Prisco D., Abbate R
*Department of Medical and Surgical Critical Care, University of Florence, Florence*

Evidences indicate that coronary artery disease (CAD) patients with residual platelet reactivity (RPR) despite antiplatelet therapy may be at increased risk of ischemic vascular events. Recently, Multiplate electrode aggregometry (MEA) (Dynabyte, Germany; DASIT, Italy) has renewed the impedance platelet aggregometry in whole blood by using a point-of-care device and has been proposed as rapid tool to assess RPR.
Aim of this study was to compare Multiplate system with VerifyNow Aspirin Assay (Accumetrics, USA) and light transmission aggregometry (LTA) by using APACT-4004 aggregometer (LABiTec, Germany).
In 1,058 CAD patients receiving ASA platelet function was measured with MEA and LTA, both induced by 1 mmol/L arachidonic acid (AA), and VerifyNow Aspirin Assay. MEA was quantified as AU and area under curve (AUC) of arbitrary units (AU*min) and in house CVs were 5.1% in controls and 7.1% in CAD patients. MEA correlated with LTA (rho=0.50, p<0.0001) and VerifyNow Aspirin Assay (rho=0.38, p<0.001).
By using a cut-off value of 18 AUC for MEA, of 20% for LTA and 550 Aspirin Reaction Units (ARU) for VerifyNow system to identify CAD patients with RPR, the prevalence of RPR was: 23.3% for MEA, 22.0% for LTA and 12.5% for VerifyNow system. MEA moderately agreed with LTA (k=0.50, p<0.0001, CI: 0.44-0.56) and fairly with VerifyNow Aspirin Assay (k=0.26, p<0.001, CI: 0.16-0.30).
Further studies using these different POC systems are necessary to evaluate their comparability and usefulness in the clinical practice.

### P129 **THROMBOELASTOMETRIC ASSAY FOR MONITORING CLOTTING ALTERATIONS IN A RAT MODEL OF POLYMICROBIAL INFECTION INDUCING SEPSIS**

Adembri C., Paniccia R., Miranda M., Donati A., Cosenza L., Antonucci E., Maggini N., Selmi V., De Gaudio A., Abbate R.
*Department of Medical and Surgical Critical Care, University of Florence, Florence*

The mechanisms underlying the activation of inflammation and coagulation during sepsis, although playing a major role in causing microvascular dysfunction and organ failure, have been not fully clarified yet. To investigate clotting alterations following sepsis, we induced sepsis in the rat by using the cecal ligation and puncture (CLP), a model of polymicrobial infection that mimics human sepsis. Ten rats underwent CLP, and 8 rats, receiving laparotomy only, served as control (CTRL). At two different time points after CLP/laparotomy (3 hrs and 7 hrs) a transcardiac puncture was performed and blood samples were collected. Prothrombin time (PT) was measured by using point-of-care coagulometer. Thromboelastometric evaluation (by rotational thromboelastometry, ROTEM, Tem International, Germany) was performed to test the extrinsic (EXTEM) and the intrinsic (INTEM) activities, by measuring clotting time (CT), clotting formation time (CFT) and maximal clot firmness (MCF) for each test. PT (INR) was significantly prolonged in CLP rats at both 3 and 7 hrs as compared to CTRL rats (2.8±0.6 and 2.8±0.5 respectively vs. 1.8±0.1 $p<0.05$). The EXTEM test showed a CFT prolonged by 29 and 18% at 3 and 7 hrs respectively ($p<.05$ vs CTRL), while the MCF was decreased by 12 and 24% at the same experimental time points as compared to CTRL ($p<0.05$ vs CTRL). Finally, the INTEM test was prolonged in both CT and CFT by 29 and 16%, together with an MCF reduction by 36%, but at 3 hrs only ($p<0.05$ vs CTRL). These data suggest that sepsis induces a significant and early, but transient, hypocoagulative state due to the activation of both intrinsic and extrinsic pathways. These alterations may be induced at least by the consumption of coagulation factors during sepsis.

## *TROMBOFILIE E RISCHIO TROMBOTICO*

### P130 **SELECTION CRITERIA OF PATIENTS WITH VENOUS THROMBOEMBOLISM FOR LABORATORY INVESTIGATION OF INHERITED THROMBOPHILIA**

Rossi E., Za T., Ciminello A., Betti S., Leone G., De Stefano V.
*Institute of Hematology, Catholic University, Rome*

Laboratory investigation for inherited thrombophilia is warranted in young patients, especially those with severe venous thromboembolism (VTE) occurred spontaneously or recurrently. Investigation of older patients is discouraged, especially when events are mild or provoked. Such policy could miss some carriers, leaving undiagnosed their kindreds.
We analyzed the files of 1,835 patients referred to our Thrombosis Centre (1996-2009). The median age at the first VTE was 37 years (range 0-89); 736 were males (40.1%). Patients were stratified according to family history of VTE, age of first VTE (<45 years), type of first VTE (defined severe for proximal DVT and/or pulmonary embolism and mild for distal DVT or superficial vein thrombosis), circumstances of first VTE (unprovoked or provoked), history of recurrent VTE.
Multiple regression was carried out labelling as dependent variable diagnosis of overall thrombophilia or severe thrombophilia (AT, PC, PS deficiency, homozygous or multiple defects, n=211) or mild thrombophilia (heterozygous factor V Leiden or prothrombin G20210A, n=415). Diagnosis of overall thrombophilia was associated with family history ($p=0.005$), severity of VTE ($p=0.008$) and recurrent events ($p<0.0001$); the aforementioned criteria were all absent only in 8% of patients with thrombophilia. Among patients with thrombophilia 30% had clinical onset >45 years, 62% had a first provoked VTE, and 11% had both. Severe thrombophilia was associated with family history ($p=0.02$), first unprovoked VTE ($p=0.015$) and recurrent events ($p=0.04$). Mild thrombophilia was associated with family history ($p=0.05$), severity of VTE ($p=0.03$) and recurrent events ($p<0.0001$).
In conclusion, family history, clinical severity and recurrence of VTE are strong predictors of inherited thrombophilia, and at least one of these parameters is present in more than 90% of cases. Selection of the patients to be investigated according only to age and/or circumstances of the first VTE could miss diagnosis of thrombophilia in a relevant number of cases.

### P131 **ENDOTHELIAL AND PLATELET CIRCULATING MICROPARTICLES IN CARRIERS FOR FACTOR V LEIDEN**

Campello E., Spiezia L., Radu C., Ballin A., Bon M., Zerbinati P., Gavasso S., Simioni P.
*Cardiologic, Thoracic and Vascular Sciences, Università di Padova, Padova*

**Background** Microparticles (MPs) are small (diameter <1 um) membrane-bound vesicles released from the surface of different type cells, during membrane activation or apoptosis. Throughout different mechanisms, MPs participate in haemostasis and play an important role in thrombosis. The utility of measuring MPs as a diagnostic and prognostic marker of hypercoagulability is currently a matter of investigation. For evaluate the possible role of MPs in the pathogenesis of thromboembolic events, a population at risk for developing thrombosis were studied.
**Aim of the study** To investigate the endothelial (EMPs) and platelet (PMPs) derived MPs plasma levels in a cohort of heterozygous and homozygous carriers for Factor V (FV) Leiden mutation.
**Material and Methods** After informed consent 10 healthy subjects (M=5, F=5; mean age=51 years), 10 heterozygous (M=4, F=3; mean age=44 years) and 10 homozygous (M=3, F=7; mean age=42 years) for FV Leiden were enrolled. Using flow-cytometric techniques (FC500, Beckman Coulter, USA), levels of circulating platelet (CD61+) and activated endothelial (CD62E+) derived MPs were detected.
Results Circulating MP (mean standard deviation, SD) were significantly higher in the FVL cohort (heterozygous 72,938±5,923 MP/uL, homozygous 72,295±3,385 MP/uL) than in the control group (59,188±5,142 MP/uL, Students t Test $p=0.039$). In particular, homozygous and heterozygous subjects had higher EMPs plasma levels (9,840±1,856 MP/uL and 5,965±1,534 MP/uL) than controls (3,331±1,084 MP/uL, Students t Test $p=0.046$ and 0.028 respectively). On the contrary, healthy subjects had higher PMPs (1,589±825 MP/uL) than FVL cohort patients (heterozygous 600±119 MP/uL, $p=0.006$; homozygous 1,134±303 MP/uL, p=ns).
**Conclusions** The increase of MPs levels in carriers for FVL mutation could play an important role in the pathogenesis of the prothrombotic state. The most important contribute could be played by the EMPs. The predictive value of circulating

MP in subjects at risk for thrombosis needs to be clarified with long-term prospective studies.

P132 **RISK OF RECURRENT VENOUS THROMBOSIS IN HOMOZYGOUS AND DOUBLE HETEROZYGOUS CARRIERS OF FACTOR V LEIDEN AND PROTHROMBIN G20210A**

Tormene D., Ferri V., Carraro S., Gavasso S., Simioni P.
*Department of Cardiologic, Thoracic and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua, Italy*

**Background** Whether individuals with homozygous or double heterozygous factor V Leiden and/or prothrombin G20210A have an increased risk of recurrent venous thrombosis is uncertain.
**Methods and Results** We performed a retrospective analysis in our family cohort of 1,905 individuals with factor V Leiden or/and G20210A prothrombin variant. Both probands and relatives (first and second-degree) were evaluated. Subjects with recurrent venous thromboembolism were compared with subjects with only one VTE episode and noncarriers.
The final cohort of patients with VTE was of 246 subjects. One-hundred and eighty-eight (48% women) were heterozygous carriers of factor V Leiden or prothrombin G20210A, 37 (58% women) were homozygous or double heterozygous carriers of the two mutations and 21 (48% women) were the noncarriers.
Twenty-one idiopathic thrombotic events (59%, n=22/37) occurred in the group of homozygous and double-heterozygous carriers of both mutations, eighty-nine (47%, n=89/188) occurred in the group of heterozygous carriers of both mutations and 11 (52%, n=11/21) in the noncarriers group. Fifteen recurrent VTE (all idiopathic) occurred in the first group, thirty-three (31 idiopathic) in the second group and 1 (idiopathic) in the noncarriers group. Odds ratios of recurrent venous thromboembolism in subjects who were homozygous or double-heterozygous carriers of the two mutations or who were heterozygous carriers of both mutations were 7.5 (95% CI; 1.13 to 49.7) and 4.08 (95% CI; 0.62 to 27.0) respectively compared with family members noncarriers of the mutations.
**Conclusions** These data suggest an increased risk for recurrent VTE both in homozygous ad double heterozygous than in single heterozygous carriers of factor V Leiden and prothrombin G20210A compared with family member noncarriers of thrombophilia.

P133 **PROTHROMBIN ACTIVITY AND ANTIGEN IN CARRIERS OF PROTHROMBIN G20210A MUTATION**

Mazzucconi M.G., Baldacci E., Biondo F., Cafolla A., Chistolini A., Dragoni F., Leporace A., Pignoloni P., Torelli F.
*Biotecnologie Cellulari ed Ematologia, Roma*

Patients with prothrombin G20210A mutation have higher prothrombin levels than normal subjects. Relationship of prothrombin activity (FII:C) with the related antigen (FII:Ag) is poorly understood. It is unclear if FII:Ag high levels correlate with FII:C levels in mutated patients.
**Aim of study** Evaluation of FII:C, FII:Ag and prothrombin fragments 1+2(F1+2) levels in patients with mutation when compared to a normal population.
We studied patients with prothrombin G20210A mutation and a personal/family history of venous/arterial thromboembolic events.
Subjects without mutation and no personal/family history of venous/arterial thromboembolisms, age and sex matched, were the controls. In patients and controls, all other markers of congenital and acquired thrombophilia were excluded. Normal ranges: FII:C, 60-120 U/dL; FII:Ag, 50-150 U/dL; F1+2, 0.4-1.1 nmol/L.
We studied 63 subjects with prothrombin mutation, all heterozygous (Group A). Sixty-three healthy subjects were the control group (Group B). In Group A mean value of FII:C was 122.6 U/dL (SD=18.90), of FII:Ag was 141.8 U/dL (SD=48.20), of F1+2 was 0.55 nmol/L (SD=0.11).
In Group B mean value of FII:C was 105.7 U/dL (SD=12), of FII:Ag was 98.6 U/dL (SD= 22), of F1+2 was 0.59 nmol/L (SD=0.14). Mean ratio between values of activity and antigen was 0.92 (SD=0.20) in Group A and 1.11 (SD=0.20) in Group B. In Group A, 34/63 (54%) patients showed almost one previous thromboembolic event.
In comparison with Group B, Group A patients showed either higher levels of FII:Ag ($p<0.000$) and of FII:C ($p=0<000$), or a lower ratio FII:C/FII:Ag ($p<0.000$).
In patients of Group A, with and without previous thromboembolic events, no significant differences concerning levels of FII:C, FII:Ag, and F1+2 were recorded.
These data suggest that the prothrombin G20210A mutation induces a procoagulant effect. In carriers of mutation, we did not find any correlation between FII:C and FII:Ag levels with the occurrence of thrombotic events.

## *HIT*

P134 **D-DIMER LEVELS IN HEPARIN-INDUCED THROMBOCYTOPENIA PATIENTS**

Scarparo P., Tezza F., Duner E., Allemand E., Plebani M., Fabris F.
*Dep. of Medical and Surgical science, Internal Medicine, Padova*

Heparin-induced thrombocytopenia (HIT) is a severe complication of heparin therapy, and is mediated by autoantibodies against Heparin/PF4 complex. HIT is characterized by platelet activation, aggregation and thrombin generation, leading to a prothrombotic state. Therefore we have measured D-Dimer levels in patients with HIT and HITT (HIT + thrombotic complications) and in controls to evaluate the diagnostic value in this prothrombotic conditions.
**Patients** Diagnosis of HIT was performed in 34 patients (14 HIT and 20 HITT) by mean of a 4T score >3 and the presence of IgG against H/PF4 complex assayed by a commercial ELISA test (OD>1.0) and HIPA functional test.
**Controls** 85 hospitalized patients with suspected HIT not confirmed by the presence of anti-H/PF4 IgG (negative group); 20 patients studied after cardiosurgery with IgG against H/PF4 without thrombocytopenia or thrombosis (Ab+ group).
The D-Dimer levels were measured using a quantitative automated latex agglutination test (normal value <250 ug/L).
**Results** The mean values of D-Dimer are higher in HIT-HITT

patients (3,099+3,100 ug/L) than in Ab+ group (1,035+408 ug/L), and in negative group (mean 1,628+2,000 ug/L) with a p-value of 0.0055 and 0.0034 respectively. Since we did not find between HIT and HITT we can conclude that D-Dimer level doesn't depend on the thrombotic events, but it's probably due to the prothrombotic state present in this syndrome. D-Dimer levels were increased also in control group, because the patients are hospitalized for different pathological settings, such as cardiovascular disease, stroke, tumours or surgery with thrombocytopenia as additional risk factor.
Using a ROC curve (HIT-HITT versus controls), we obtain a cut off value of 1,300 ug/L, so patients with D-Dimer lower than this level are less likely to have HIT syndrome, with a sensivity of 70% and a specificity of 68%. The negative predictive value for this cut off is 88.5%.

P135 **PRELIMINARY EVALUATION OF A NEW IMMUNO-TURBIDIMETRIC ASSAY FOR HIT DIAGNOSIS**

Morelli B., Novelli C., Grassi A.C., Stefanin C.
*Centro Immunotrasfusionale, A.O. Ospedale Civile di Legnano, Legnano*

**Background** HIT (Heparin-induced thrombocytopenia) is a serious antibody-mediated complication of heparin therapy that occurs in approximately 0.5-5% of patients treated with heparin. It confers significant risk of thrombosis and devastating outcomes.
Diagnosis of HIT is based on clinical criteria and laboratory assays. Several assays are available for HIT diagnosis, functional or immunological. Even if functional assays represent the gold standard, immunological assays are spreadly used in most laboratories; therefore the latest methods present some drawbacks.
**Aim of the study** We have evaluated a new kit produced by Instrumentation Laboratory (HemosIL HIT-Ab-PF4-H), which is an immunological total assay. The results have been compared with those obtained with the kit in use in our laboratory (Zymutest HIA IgGAM produced by Hyphen Biomed).
**Materials and Methods** 55 patients with Acute Coronary Syndrome (ACS) or who underwent CardioPulmonary Bypass (CPB) surgery were enrolled; inclusion criteria were heparin administration (at least for 5 days) and severe subsequent thrombocytopenia.
**Results** We have analyzed 104 samples, obtained from 55 patients. Considering the possible use of this kit as exclusion of the HIT complication we have compared the two assays in line with this approach using two different cut-off levels. With the first cut-off considered the agreement was 80%; with the second cut-off the agreement was 84%. The choice of the cut-off has to be confirmed in future clinical studies.
**Conclusions** The evaluated kit presents many advantages: it is a quantitative assay, it is possible to run a single sample, it is very fast to execute, it doesn't require specialized technicians and it can be performed even in emergency.

P136 **LA GENERAZIONE DI TROMBINA: UN TEST UTILE PER L'INDIVIDUZIONE DI ANTICORPI PATOGENETICI NEL SOSPETTO DI PIASTRINOPENIA DA EPARINA**

Pavani G.[(1)], Della Valle P.[(1)], Sampietro F.[(1)], Pattarini E.[(2)], D'Angelo A.[(1)]
[(1)] *Servizio di Coagulazione & Unità Ricerca Trombosi, Istituto Scientifico Universitario San Raffaele, Milano;* [(2)] *Laboraf S.p.A., Istituto Scientifico Universitario San Raffaele, Milano*

La piastrinopenia da eparina di tipo II (HIT) e diagnosticata sulla base di criteri clinici e laboratoristici. La ricerca degli anticorpi della HIT (HIT-Abs) è fatta con metodi immunochimici (altamente sensibili) o funzionali (più specifici), che svolgono un ruolo fondamentale nell'evitare una prolungata anticoagulazione per i pazienti con piastrinopenie di altra origine. Recentemente, Tardy-Poncet *et al* hanno descritto l'utilizzo del test di generazione della trombina (TGT) per lidentificazione di HIT-Abs patogenetici (JTH 2009).
In un TGT modificato, abbiamo testato per la presenza di HIT-Abs plasmi di 46 pazienti consecutivi con sospetto di HIT. Misture di piastrine da donatore (c.f. 180-200x$10^9$/L) e plasma campione sono incubate con fattore tissutale (c.f. 0.5 pmol, Thrombinoscope, Maastricht, the Netherlands) in presenza di eparina non frazionata (ENF, c.f. 0, 0.2 and 1 UI/mL) o eparina a basso peso molecolare (stesse c.f.) con o senza l'aggiunta di trombomodulina (TM, c.f. 5 nmol) e fondaparinux (c.f. 200 ng/mL).
Dei 46 pazienti, 20 risultavano negativi al test ELISA (HPIA Asserachrom, Stago, Asnieres sur Siere, France), e solo 6 risultavano positivi tanto a questo che al test funzionale HIPA (Greinacher et al, TH 1991). In assenza di ENF (o EBPM), i pazienti +/+/+ (positivi per HPIA, HIPA e TGT) mostravano maggiori valori di Picco tanto con che senza TM e fondaparinux ($p \leq 0.008$), e maggiori valori di ETP in presenza di TM e fondaparinux ($p=0.01$). In ogni circostanza, l'aggiunta di ENF (0.2 UI/mL) riduceva di più del 50% i valori di Picco ed ETP nei pazienti –/–/– e +/–/– ($p<0.001$), ma non modificava significativamente entrambi i parametri nei pazienti +/+/+ ($p>0.58$).
Il test, che può utilizzare anche siero campione scomplementato, appare di grande utilità per l'individuazione di HIT-Abs patogenetici.

## *SINDROME DA ANTICORPI ANTIFOSFOLIPIDI*

P137 **CLINICAL RELEVANCE OF NITRIC OXIDE METABOLITES AND NITRATIVE STRESS IN THROMBOTIC PRIMARY ANTIPHOSPHOLIPID SYNDROME**

Ames P.R.J.[(1)], Batuca J.R.[(2)], Ciampa A.[(3)], Iannaccone L.[(4)], Brancaccio V.[(5)], Delgado A.J.[(6)]
[(1)] *Haematology/Rheumatology, Airedale DGH, Steeton, United Kingdom;* [(2)] *Pharmacology, New University of Lisbon, Lisbon, Portugal;* [(3)] *Coagulation Unit, Moscati Hospital, Avellino;* [(4)] *Chemical Pathology, Cardarelli Hospital, Naples;* [(5)] *Ex Coagulation Unit, Ex Cardarelli Hospital, Naples;* [(6)] *Pharmacology, New University of Lisbon, Lisbon, Portugal*

To assess the clinical relevance of nitric oxide (NO) and nitrotyrosine (marker of nitrative stress) in thrombotic primary antiphospholipid syndrome (PAPS) we investigated 46 thrombotic PAPS patients, 21 persistent asymptomatic carriers

of antiphospholipid antibodies (PCaPL), 38 inherited thrombophilia (IT) patients, 33 patients with systemic lupus erythematosus (SLE) and 29 healthy controls (CTR).
IgG anticardiolipin (aCL), IgG anti-β-2-glycoprotein I (2GPI) crude nitrotyrosine (NT) (indicator of nitrative stress) and high sensitivity C-reactive protein (CRP) were measured by immune-assays. Plasma nitrite ($NO_2^-$), nitrate ($NO_{3-}$) and total antioxidant capacity (TAC) were measured by colorimetric assays.
Plasma $NO_2^-$ was lowest in PAPS ($p<0.0001$) and $NO_3^-$ highest in SLE ($p<0.0001$) whereas NT was highest in PAPS and SLE ($p=0.01$). In PAPS IgG aCL titre and thrombosis number negatively predicted $NO_2^-$ ($p=0.03$ and $p=0.001$ respectively), arterial thrombosis and smoking positively predicted $NO_3^-$ ($p=0.05$ and $p=0.005$ respectively) and CRP positively predicted NT ($p=0.004$). In the PCaPL group IgG 2GPI almost negatively predicted $NO_2^-$ ($p=0.07$) whereas IgG aCL negatively predicted $NO_3^-$ ($p=0.03$) and a trend was seen for IgG 2GPI ($p=0.06$). In the SLE group IgG aCL negatively predicted $NO_2^-$ ($p=0.03$) and $NO_3^-$ ($p=0.02$).
PAPS is characterised by decreased $NO_3^-$ in relation to thrombosis type and number as well as to aPL titres. Low grade nitrative stress and low grade inflammation are linked phenomena in PAPS and may have implications for thrombosis and atherosclerosis.

## P138 **MORTALITY PREDICTORS IN PRIMARY ANTIPHOSPHOLIPID SYNDROME: A SINGLE CENTRE SURVEY**

Ames P.R.J.[(1)], Iannaccone L.[(2)], Ciampa A.[(3)], Brancaccio V.[(4)]
*[(1)] Haematology/Rheumatology, Airedale DGH, Steeton, United Kingdom; [(2)] Chemical Pathology, Cardarelli Hospital, Naples; [(3)] Coagulation Unit, Moscati Hospital, Avellino; [(4)] Pharmacology, New University of Lisbon, Lisbon, Portugal*

We analysed baseline factors that predicted mortality in deceased patients with primary thrombotic antiphospholipid syndrome (PAPS) (n=11, age 55±9 yrs), surviving PAPS (n=68, age 47±14 yrs) and persistent carriers of antiphospholipid antibodies in the absence of any underlying autoimmune disorder (PCaPL) (n=29, age 50±16 yrs) followed up for an average of 21±13 years.
IgG aCL (GPL) was 328±344 in deceased PAPS, 105±146 in surviving PAPS and 51±67 in PCaPL ($p<0.0001$); aPTT ratio was 2.17±0.46 in surviving PAPS 1.92±0.65 in surviving PAPS and 1.79±1.01 in PCaPL ($p=0.02$); DRVVT ratio was 2.30±0.80 in deceased PAPS, 1.65±0.43 in surviving PAPS and 1.33±0.32 in PCaPL ($p<0.0001$); fibrinogen (mg/dL) was 385±70 in deceased PAPS, 335±49 in surviving PAPS and 312±63 in PCaPL ($p=0.02$); homocysteine (mmol/L) was 16.3±7.1 in deceased PAPS, 13.4±11 in surviving PAPS and 9.6±5.9 in PCaPL ($p=0.01$); platelets (x $10^9$ L) were 293±105 in deceased PAPS, 202±86 in surviving PAPS and 141±77 in PCaPL ($p<0.0001$). KCT ratio and von Willebrand factor concentration were not different across groups. IgG aCL, aPTT, DRVVT, FNG, HC and PLT were entered in a multiple regression model as independent variables with mortality as the dependent: baseline IgG aCL, DRVVT and PLT were independent predictors of mortality.
Efforts of future research should be aimed at decreasing antiphospholipid antibody titres.

## P139 **MIGRAINE, ANTIPHOSPHOLIPIS AND ANTIPHOSPHOLIPID SYNDROME**

Cavestro C.[(1)], Bazzan M.[(2)], Micca G.[(3)], Molinari F.[(3)], Di Pietrantonj C.[(4)], Aloi R.[(5)], Iannini R.[(6)], Roccatello D.[(2)]
*[(1)] Centro Cefalee, Ospedale S. Lazzaro, Alba; [(2)] CMID, Centro Multidisciplinare, Osp. Giovanni Bosco, Torino; [(3)] Lab analisi, Osp. S. Lazzaro, Alba; [(4)] SeREMI, Servizio Regionale di ASL Alessandria, Alessandria; [(5)] Medicina del lavoro, Osp. S. Lazzaro, Alba; [(6)] Serv. Trasfusionale, Osp. S. Lazzaro, Alba*

Migraine is a severe multifactorial disorder, characterized by a severe disabling troubling headache. In the last few years an increased risk of stroke has been described in young migraineurs, especially if suffering from migraine with aura. In the last decades a lot of co-morbidities have also been associated to migraine. Looking for co-morbidities, we conducted a case-control study on prevalence of antiphospholipids in migraineurs and in an age- and sex-matched control group. The study lasted from September 2008 up to august 2009. We examined all consecutive patients visited in the outpatients Headache Centre on our Hospital; controls were volunteers employed in our Hospital, not suffering from headache. Diagnosis of migraine was done on the basis of the last international classification; we respected international criteria for diagnosis of antiphospholipid positivity and antiphospholipid syndrome. In one year we recruited 284 patients (225 women and 59 men) and 225 controls (174 women and 51 men); among migraineurs, 203 had migraine without aura, and 81 with aura. In 33 patients and 7 controls a mid-high antiphospholipid positivity was confirmed [OR 4.08 (CI 1.77 to 9.39), p=0.0004]; among these patients, 2 had positivity for LAC, ACA and antiβ2glicoproteine antibodies, and 1 patient showed positivity for both LAC and antiβ2glicoprotein antibodies. None of the controls had more than one positivity. Among those with confirmed mid-high positivity, 12 patients (4%) and 1 control (0,004%) had a thrombotic or obstetric event, and diagnosis of antiphospholipid syndrome was done. In conclusion, our data show that migraine and antiphospholipid syndrome are co-morbid and that migraine could be consider a main symptom of antiphospholipid syndrome, such as antiphospholipids should be consider in screening migraine. This study had Regional Health Project financial support.

## P140 **FUNDING RESEARCH IN ANTIPHOSPHOLIPID SYNDROME AND NOT ONLY: A NOVEL MODEL**

Ames P.R.J.[(1)], Gentile F.[(2)]
*[(1)] Haematology/Rheumatology, Airedale DGH, Steeton, United Kingdom; [(2)] R&D CAF SIALS, Naples*

The Italian Tax System is quite bureaucratic and incomprehensive to the uninitiated, but accountancy has still jobs to offers: oftentimes accountants join forces or offer services under the umbrella of Centro di Assistenza Fiscale (CAF). With a bit of brainstorming a CAF was transformed into a Solidal CAF (SCAF) that has charitable issues at its very core. In essence, regardless of the number of employees, companies and organisations may freely subscribe to the SCAF accepting the solidarity clause and support the charitable causes of the SCAF. In a nutshell every time a CAF

submits a tax return to the Italian Revenue Agency (IRA) the latter pays back 15 euro to a CAF. Of these 15 euro a normal CAF returns to those companies who subscribed to it a proportion of the 15 euro and keeps the rest for its expenses.
In observance to the solidarity clause, companies who subscribe to SCAF must release 0.50 euro for each tax return to a charitable entity of their choice whereas SCAF will release 3% of its annual profit for its own charitable causes: research in autoimmune vascular diseases and support of an Orphanage in Eritrea.
In its first year SCAF has made approximately 60,000 euro of charitable funds. Considering that the number of Italians that might transmit their tax return through SCAF is almost 40 million there are almost 2 million of research funds to be made. Support this initiative: transform IRA payments into charity for the upcoming APS Foundation Lets get IRA to pay for our research.

## *TERAPIA ANTITROMBOTICA: PROBLEMI CLINICI E DI LABORATORIO*

### P141 PREDICTION OF OPTIMAL WARFARIN MAINTENANCE DOSE USING ADVANCED ARTIFICIAL NEURAL NETWORKS

Grossi E.[(1)], Podda G.M.[(2)], Verri A.[(1)], Pugliano M.[(2)], Gabba S.[(1)], Carpani G.[(3)], Buscema M.[(4)], Cattaneo M.[(2)]
*[(1)] Ricerca e Sviluppo, Centro Diagnostico Italiano, Milano; [(2)] Dipartimento di Medicina, Chirurgia e Odontoiatria, Università degli Studi di Milano, Medicina III, Azienda Ospedaliera San Paolo, Milano; [(3)] S.I.M.T., Azienda Ospedaliera San Paolo, Milano; [(4)] Centro di Ricerca Semeion, Roma*

**Background** The individual response to vitamin K antagonists (VKA) is highly variable, being influenced by genetic variants of enzymes that are involved in drug and vitamin K metabolism (CYP2C9 and VKORC1) and interference by clinical variables. Currently, the dose of VKA is adjusted based on measurements of the prothrombin time. In the last years, mathematical algorithms were developed for estimating the appropriate VKA dose, based on different mathematical approaches working on clinical and genetic data. Artificial Neural Networks (ANN) are computerized algorithms resembling interactive processes of human brain, which allow to study very complex non-linear phenomena like biological systems.
**Aim** To evaluate the performance of new generation ANN on a large data base of patients on chronic VKA treatment.
**Methods** Clinical and genetic data from 380 patients (184 men; 196 women) treated with a VKA (warfarin) average weekly maintenance dose of 23.67mg (11.42 SD) were used to create a dose algorithm. Forty-nine variables, including demographic, clinical and genetic data (5 CYP2C9 and 3 VKORC1 genetic variants) were entered into the ANN Twist system, which can select fundamental variables during their evolution in search for the best predictive model. The final model, based on 24 variables expressed a functional approximation of the actual dose within a validation protocol based on a tripartite division of the data set (training, testing, validation).
**Results** In the validation cohort, the pharmacogenetic algorithm reached high accuracy, with an average absolute error of 5.4 mg/week. The most accurate prediction was achieved in the subset of patients requiring ≤21 mg warfarin, 80% being correctly identified by the algorithm.
**Conclusion** Our results suggest that ANN can be applied successfully for VKA maintenance dose prediction and represent a robust basis for a prospective multicentre clinical trial of the efficacy of genetically informed dose estimation for patients who require VKA.

### P142 A MULTICENTRE SURVEY OF THE QUALITY OF THERAPEUTIC MONITORING OF ANTITHROMBOTIC THERAPY IN ANTICOAGULATION CENTRES IN ITALY: THE FCSA EXPERIENCE

Tosetto A.[(1)], Manotti C.[(2)], FCSA on behalf of Antithrombotic Centres, Federation of Italy
*[(1)] Centro Emostasi e Trombosi, Divisione di Ematologia, Vicenza; [(2)] Centro Terapia Anticoagulante Orale, Presidio Ospedaliero Fidenza San Secondo, Fidenza*

**Background** Despite anticoagulation with vitamin K antagonist (VKA) is a widely used antithrombotic therapy, few data are available on the effectiveness of monitoring in Antithrombotic Centres (AC). In this study, we aimed at evaluating the quality of VKA anticoagulation in ACs affiliated to the Italian Federation of Antithrombotic Clinics (FCSA).
**Methods** We asked each FCSA AC to provide data of all visits from January 1st, 2009 to December 31, 2009. Records contained information about date of visit, INR at time of visit, indication for VKA therapy, therapeutic INR range. Databases were merged, and for each patient percent of visits within therapeutic range and time in therapeutic range (TIR) were computed. The main outcome measure was the within AC patient median TIR.
**Results** 116 Centres agreed to participate in the study, for a total of 1,355,909 visits, from 84,680 patients, with a median age of 76 years. The median length of VKA therapy was 8 months. The median number of visits was 16 per patient per year, with a median time to control (TTC) of 16.6 days. In all patients, the median TIR was 68%; in a multivariate regression model, TIR was increased in males and in older subjects, while TIR decreased in patients with a diagnosis of VTE or of prosthetic valves or with VKA therapy duration ≤ one month ($p<0.001$ for all variables). Within Centres, the only factor explaining a worse mean TIR was the presence of an increased proportion of patients with a duration of VKA therapy ≤ one month.
**Conclusions** This study demonstrates that pooling data from individual Centres is feasible, allowing large-scale analysis of follow-up data from patients on VKA therapy. The project is the basis for an ongoing, nation-wide quality assessment program aimed at improving clinical outcomes in patients using VKA antithrombotic treatment.

### P143 NATURAL HISTORY OF PLATELET REACTIVITY IN ACUTE CORONARY SYNDROME PATIENTS ON DUAL ANTIPLATELET TREATMENT: THE OCCURRENCE OF HIGH ON-TREATMENT PLATELET REACTIVITY IN THE SUBACUTE PHASE OF THE DISEASE

Marcucci R., Gori A.M., Paniccia R., Giusti B., Fabbri A.,

Maggini N., Antonucci E., Miranda M., Gensini G.F., Abbate R.
*Medical and Surgical Critical Area, University of Florence, Florence*

**Background** Meta-analysis of antiplatelet therapy demonstrated a progressive decrease of aspirins clinical efficacy, particularly after two years of treatment. Scarce data are available on a progressive decrease in the entity of platelet inhibition by aspirin, whereas no data are available on the durability of platelet inhibition by clopidogrel.
**Purpose** We sought to evaluate the entity of platelet inhibition induced by aspirin and clopidogrel during a 1 month- (T1) and 6 (T2) month- follow-up in 195 (148 M/ 47 F) patients with acute coronary syndrome undergoing PCI with stent implantation.
**Methods** Platelet function was evaluated by VerifyNow Aspirin (ARU) and P2Y12 (PRU). All patients received 600 mg clopidogrel loading dose followed by 75 mg daily and aspirin 100 mg daily for 6 months. HPR was defined in presence of ARU ≥550 and PRU ≥240. Patients with HPR at the time of the acute event (T0) were excluded from the analysis.
**Results** Among the 167 patients with ARU<550 at T0 [T0=mean±sd: 439±60; T1=451±71; T2=463±84], we found the occurrence of HPR in 18 patients (9.2%) at T1 ($p<0.05$ vs. T0) and in 24 patients (12.3%) at T2 ($p<0.05$ vs. T0).
Similarly, among the 114 patients with PRU<240 at T0 [T0=mean±sd: 154±55; T1=212±90; T2=239±86], we found the occurrence of HPR in 31 patients (15.8%) at T1 ($p<0.05$ vs. T0) and in 44 patients (22.5%) at T2 ($p<0.05$ vs. T0).
**Conclusions** In the framework of a study aimed to evaluate the durability of HPR, unexpectedly we have found that in a percentage of ACS patients ranging from 10 to 20%, the entity of platelet inhibition by different stimuli progressively decreases. Adherence to therapy is the first issue to be evaluated. Nevertheless, further studies are needed to explore the possible clinical meaningful of this phenomenon.

## P144 PLATELET CYCLOOXYGENASE INHIBITION BY LOW-DOSE ASPIRIN IS NOT REFLECTED CONSISTENTLY BY PLATELET FUNCTION ASSAYS. IMPLICATIONS FOR ASPIRIN RESISTANCE

Santilli F.[(1)], Rocca B.[(2)], De Cristofaro R.[(2)], Lattanzio S.[(1)], Pietrangelo L.[(1)], Habib A.[(3)], Pettinella C.[(1)], Recchiuti A.[(1)], Ferrante E.[(1)], Ciabattoni G.[(1)], Davì G.[(1)], Patrono C.[(2)]
*[(1)] Centre of Excellence on Aging, G. d'Annunzio University Foundation, Chieti; [(2)] Department of Pharmacology, Catholic University School of Medicine, Rome; [(3)] American University of Beirut, Beirut, Lebanon*

**Purpose** Functional assays of the antiplatelet effects of low-dose aspirin variably reflect the thromboxane-dependent component of platelet aggregation. We assessed the thromboxane-dependence of biochemical and functional indexes used to monitor the effect of low-dose aspirin, and the inter- and intra-subject variability on aspirin and after its withdrawal.
**Methods** Forty-eight healthy volunteers were randomized to receive aspirin 100 mg/od for one to eight weeks.
**Results** Serum thromboxane (TX)B2 was evenly suppressed by ≥99%. Urinary 11-dehydro-TXB2, arachidonic acid-induced aggregation and Verify-Now-Aspirin showed a stable, incomplete inhibition (65, 80 and 35%, respectively). Adenosine-diphosphate- and collagen-induced-aggregation were highly variable, poorly aspirin-sensitive, with an apparent time-dependent reversal. Inhibition of TXB2 was non-linearly related to aggregation inhibition. Platelet function largely recovered by day 3 post-aspirin, independently of treatment duration. No statistically significant differences in thromboxane-related indexes were observed between functional measurements below or above arbitrary thresholds currently used to define responder or resistant subjects. With any functional assay, occasionally resistant subjects were always responder on previous or subsequent determinations.
**Conclusion** Platelet cyclooxygenase activity, as reflected by serum TXB2, is uniformly and persistently suppressed by low-dose aspirin in healthy subjects. However, the effect of aspirin is variably and randomly detected by functional assays, potentially leading to misclassification of responder and resistant phenotype due to their poor reproducibility. The non-linear relationship between inhibition of TXB2 production versus platelet function has important clinical implications.

## P145 A SEVERE CASE OF WARFARIN-CANRENOATE INTERACTION: A ROLE FOR GENETIC PREDISPOSITION?

Giusti B.[(1)], Maggini V.[(2)], Pugi A.[(2)], Coletta D.[(3)], Vietri M.[(2)], Lapi F.[(2)], Mugelli A.[(2)], Vannacci A.[(2)], Prisco D.[(1)], Abbate R.[(1)]
*[(1)] Dpt. Medical and Surgical Critical Care - Atherothrombotic Diseases Unit, AOU Careggi, University of Florence, Florence; [(2)] Dpt. Preclinical and Clinical Pharmacology, University of Florence, Florence; [(3)] General Practioner, AUSL 11, Empoli*

Interindividual variability in responses to warfarin is attributed to dietary vitamin K, drug interactions, demographic and genetic factors. Many drugs are known to modify international normalized ratio (INR) values in warfarin-treated patients both for pharmacodynamic and pharmacokinetic interactions.
We report an unusual case of interaction between potassium canrenoate (PC) and warfarin in a 77-year-old Caucasian female with atrial fibrillation, heart failure (NYHA class II) and hypertension. During the previous 5 years, the patient had been effectively anticoagulated with warfarin 35.0 mg/wk with a very stable INR. Two weeks following the initiation of PC 50 mg/die to control a slight hypokalaemia, she suddenly developed an extensive facial and neck haematoma after a mild lesion of the internal jowl, followed by analogous limb lesions as consequences of mild traumas. Blood examinations revealed a marked INR increase (10.8), requiring warfarin discontinuation and vitamin K administration. Subsequent titrations after warfarin reintroduction eventually resulted in a stable INR within the therapeutic range with a final regimen of 22.5 mg/wk. Any other possible factor able to interfere with warfarin therapy was excluded. She resulted wild-type homozygote for both CYP2C9*2 and *3 polymorphisms and -1639AA homozygote (warfarin sensitive, low dose required) for VKORC1 (rs9923231).
Subjects with this VKORC1 genotype may be more susceptible to drug-drug interactions which alter pharmacokinetics and pharmacodynamics of warfarin. Both PC and warfarin are highly protein-bound to albumin. Therefore, it is possible that the addition of PC potentiates the anticoagulant effect of warfarin by protein-binding

displacement of warfarin from albumin. Moreover, warfarin and PC could compete for metabolism via CYP3A with consequent decreased clearance of the warfarin. To our knowledge, an interaction between warfarin and potassium canrenoate has not been previously reported. While further research should be done to confirm this interaction, practitioners should be made aware of this possibility.

P146 **HIGH ON-TREATMENT PLATELET REACTIVITY IN ACUTE CORONARY SYNDROME PATIENTS: AN ACUTE-PHASE REACTION?**

Marcucci R.[(1)], Gori A.M.[(2)], Giusti B.[(2)], Paniccia R.[(2)], Fabbri A.[(2)], Cordisco A.[(2)], Giglioli C.[(3)], Valente S.[(3)], Abbate R.[(2)], Gensini G.F.[(1)]

*[(1)] Department of Medical and Surgical critical area, University of Florence, Florence; [(2)] Medical and Surgical Critical Area, University of Florence, Florence; [(3)] Heart and Vessel, AOU Careggi, Florence Medical and Surgical Critical Area, University of Florence, Florence*

**Background** High-on treatment Platelet Reactivity (HPR) is associated with an increased risk of stent thrombosis and cardiovascular death in acute coronary syndrome (ACS) patients undergoing PCI with stent implantation. Advanced age, diabetes, inflammation are clinical characteristics associated with a higher risk of high-on treatment platelet reactivity. No data are available on the natural history of this phenomenon.
**Purpose** We sought to evaluate the durability of HPR at the time of the acute event (T0) with a follow-up of 1 (T1) and 6 (T2) months in 195 (148 M/ 47 F) patients with acute coronary syndrome undergoing PCI with stent implantation.
**Methods** Platelet function was evaluated by VerifyNow Aspirin (ARU) and P2Y12 (PRU). All patients received 600 mg clopidogrel loading dose followed by 75 mg daily and aspirin 100 mg daily for 6 months. HPR was defined in presence of ARU≥550 and PRU≥240.
**Results** HPR by ARU [T0=mean±sd: 575±29; T1=439±51; T2=441±82] significantly decreased from 10% (19/195) at T0 to 4% (8/195) at T1 ($p<0.01$ vs. T0) and T2 ($p<0.01$ vs. T0).
Similarly, HPR by PRU [T0=mean±sd: 306±47; T1=214±80; T2=206±94] significantly decreased from 39.7% (77/195) at T0 to 30.7% (60/195) at T1 ($p<0.05$ vs. T0) and 26.1% (51/195) at T2 ($p<0.05$ vs. T0).
No clinical characteristic was found significantly associated with the persistence of HPR by different stimuli at T1 and T2.
**Conclusions** This is the first study aimed to evaluate the durability of HPR in ACS patients.
We show that the entity of platelet reactivity progressively decreases from the acute event to 6 month-follow up in patients with HPR. These results suggest the need of an antiplatelet treatment tailored on the timing from the acute coronary syndrome.

P147 **CAROTID VERSUS CORONARY STENT REVASCULARIZATION: COMPARISON OF THE PLATELET ACTIVATION PROFILE**

Brambilla M.[(1)], Montorsi P.[(2)], Tirloni E.[(1)], Trabattoni D.[(3)], Ghulam Ali S.[(3)], Canzano P.[(1)], Bartorelli A.[(2)], Tremoli E.[(1)], Camera M.[(1)]

*[(1)] Centro di Ricerche Farmacologiche per lo Studio e la Prevenzione delle Malattie Cardiovascolari, Centro Cardiologico Monzino IRCCS, Milano; [(2)] Dipartimento di Scienze Cardiovascolari, Centro Cardiologico Monzino IRCCS, Milano; [(3)] Università di Milano, Centro Cardiologico Monzino IRCCS, Milano*

Platelet activation occurs in both coronary and carotid artery stenting as a result of vessel wall damage and subendothelium exposure. The dual antiplatelet regimen has a significant impact on reducing stent thrombosis and adverse outcomes. Whether differences exist in the degree of platelet activation among stent-treated coronary and carotid vessel is not known.
**Aim** To compare platelet activation in patients who underwent carotid versus coronary revascularization.
**Methods** 20 patients with high-grade carotid artery stenosis and 20 stable angina patients who underwent revascularization with bare metal stent implantation were studied. To assess platelet function, blood was withdrawn 1 month (T1) after stenting procedure and 2 months after thienopyridine discontinuation (T2). Platelet activation markers (PAC1, CD62 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.
**Results** At T1, CD62 and PAC1 positive (+) platelets were comparable in carotid and coronary-treated patients. By contrast, TF+ platelets as well as TF+ MPA were 3 fold higher in coronary vs. carotid-treated patients, both under resting conditions and upon ADP stimulation ($p<0.001$). At T2, the platelet activation profile was comparable to that observed at T1, with TF+ platelets and TF+ MPA being still significantly higher (2-3 fold) in coronary vs. carotid-treated patients, both under resting conditions and upon ADP stimulation ($p<0.01$). No significant differences in the platelet activation markers expression were observed between T1 and T2 both in carotid- as well as in coronary-treated patients.
**Conclusion** Significant higher levels of TF+ platelets and TF+ MPA were observed in peripheral blood of coronary patients who underwent revascularization with stent implantation compared to patients with carotid artery stenting, both 1 month after stenting and 2 months after thienopyridine discontinuation. This prothrombotic platelet phenotype may have implications for thrombotic complications in coronary patients.

P148 **RISK FACTORS ASSOCIATED WITH BLEEDING IN VERY OLD PATIENTS ON VKA TREATMENT FOR VENOUS THROMBEMBOLISM: RESULTS FROM A PROSPECTIVE COLLABORATIVE STUDY. ON BEHALF OF THE AD HOC STUDY GROUP OF FCSA**

Poli D.[(1)], Antonucci E.[(2)], Cappelli R.[(3)], Lucherini E.[(4)], Orlandini F.[(5)], Pini F.[(6)], Piana A.[(7)], Porcu A.[(8)], Ruocco L.[(9)], Tiraferri E.[(10)]

*[(1)] Department of Heart and Vessels Thrombosis Centre, Azienda Ospedaliero-Universitaria Careggi, Florence; [(2)] Department of Medical and Surgical Critical Care, University of Florence, Florence; [(3)] Thrombosis Centre, Hospital of Siena, Siena; [(4)] Angiology Unit, Civic Hospital, Ravenna; [(5)] Dept Internal Medicine 1, Civic Hospital S. Andrea, La Spezia; [(6)] Thrombosis Centre, Civic Hospital Villa Marina, Piombino; [(7)] Dept of Internal Medicine, University of Genova, Genova; [(8)] Thrombosis Centre, ASL n. 8 Cagliari, Cagliari; [(9)] Thrombosis Centre, Cisanello Hospital, Pisa; [(10)] Thrombosis Centre, Hospital of Rimini, Rimini*

The increasing number of very old patients on treatment with vitamin K antagonists (VKAs) requires a better knowledge of the risks associated with this treatment in elderly. We performed a prospective collaborative study among Centres affiliated to FCSA to assess the adverse events of VKAs in patients who started treatment after 80 years of age. Patients ≥80 years were prospectively followed-up from the start of treatment. Quality of anticoagulation and adverse events occurring during follow-up were recorded. The total number of patients recruited was 4,067, 1,052 patients for venous thromboembolism (VTE) (females 63%; follow-up 1,951 patient/years; mean time of follow-up 1.85 years). 187 (18%) patients were on long-term VKA for recurrent VTE.
The total quality of anticoagulation measured as time spent within, above and below the international normalized ratio therapeutic range was 59%, 14% and 27%, respectively [IQR for time in therapeutic range (TTR) 45-72].
During follow-up 47 major bleeding events (2.40x100 patient/years) were recorded, 20 (42.5%) in the first 90 days of treatment. The univariate and multivariate analysis for risk factors associated with bleeding risk are reported in the Table.

| | OR | 95% CI | p value | OR | 95% CI | p value |
|---|---|---|---|---|---|---|
| **Age** | 1.0 | 0.9-1.1 | 1.0 | | | |
| **Male gender** | 1.1 | 0.6-1.9 | 0.8 | | | |
| **Hypertension** | 1.5 | 0.8-2.8 | 0.2 | | | |
| **Previous bleed** | 2.7 | 0.8-9.3 | 0.1 | | | |
| **Active Cancer** | 2.1 | 1.0-4.4 | 0.06 | 2.2 | 1.0-4.7 | 0.05 |
| **History of falls** | 3.3 | 1.2-8.8 | 0.02 | 3.5 | 1.3-9.5 | 0.01 |
| **TTR** | 1.0 | 1.0-1.1 | 0.5 | | | |
| **Renal failure** | 1.6 | 0.7-4.0 | 0.3 | | | |

In conclusion, among very old patients on VKA treatment for VTE the rate of bleeding events is 2.40x100 patient/years. History of falls and active cancer are independently associated with bleeding risk.

## P149 IMPROVING VITAMIN K ANTAGONIST (VKA) MANAGEMENT THROUGH DAILY LOW DOSE VITAMIN K SUPPLEMENTATION

Paoletti O., Zimmermann A., Cancellieri E., Alatri A., Bassi L., Testa S.
*Haemostasis and Thrombosis Centre, Clinical Pathology Division, AO Istituti Ospitalieri, Cremona*

**Introduction** VKA therapy is a lifesaving treatment, used in the primary and secondary prevention of both arterial and venous thrombosis. It is characterized by a high intra/inter-individual variability due to drug interactions, genetic factors, intercurrent diseases and dietary vitamin K intake. As shown in literature, instability of anticoagulation may be associated with a low dietary intake of vitamin K. As a consequence of INR variability an increase of bleeding and thrombotic complications is observed.
**Case Report** A 79-year-old man, without significant previous diseases, was admitted to the cardiological division of Cremona Hospital, because of the onset of atrial fibrillation, Warfarin treatment was immediately started. 5 days after discharge the patient was re-admitted with epistaxis and overanticoagulation, showing a PT INR=12, immediately normalized with concentrated prothrombin complex and vitamin K.
One month after restarting anticoagulation patient condition was as follow: very low quality treatment (time in the therapeutic range =5%), high frequency of INR monitoring (2-3 times/week), very low mean warfarin posology (1.75 mg/week). Within possible causes of high variability, only the concomitant treatment with amiodarone could only partially explain the high sensitivity to AVK. To control the high INR variability, we decided to trait the patient with a supplementation of low dose of vitamin K (50 mcg/die). Rapidly we observed a stabilization of INR levels, with an improvement of quality treatment: time in the therapeutic range reached the 65%; frequency of INR monitoring was reduced to 1 control each 2-3/week, warfarin posology was stabilized at 3.75-5 mg/week.
**Discussion** We observed that supplementation with a low dose of oral vitamin K contributes to improve anticoagulant stability, with a consequent reduction of both hemorrhagic and thrombotic risks.
Probably, in a next future, new anticoagulant drugs may find an indication to improve and simplify anticoagulation management of very AVK unstable patients.

## P150 ANTICOAGULATED PATIENTS: HOW THE POPULATION IS CHANGING

Testa S., Paoletti O., Zimmermann A., Alatri A., Bassi L., Cancellieri E.
*Patologia Clinica, Centro Emostasi e Trombosi, A.O. Istituti Ospitalieri Cremona, Cremona*

**Introduction** In the last 20 years we observed a dramatically increase of vitamin K antagonists (VKA) treatments. At Cremona Anticoagulation Clinic (AC), the number of patients increased significantly and in the last few years we observed a differentiation in therapeutic drug choices.
**Aims** Evaluate:
1) principal characteristics of anticoagulated population;
2) type of anticoagulant drug used;
3) suitability of our net supported program to manage patients on other anticoagulant drugs (LMWH, Fondaparinux).

**Material and Method** We used our centralized net supported program (TAOnet Roche) to collect laboratory and clinical data. In details we registered clinical information and PT INR (venous or capillary) for AVK patients and blood cell count and renal function for LMWH and fondaparinux patients. Anti Xa activity was performed in critical patients.
**Results** Main characteristics are shown in the Table.

| | 2003 | 2010 | Δ% |
|---|---|---|---|
| **Pts (n°)** | 2.123 | 4.352 | +105 |
| **Atrial Fibrillation (n°)** | 913 | 2.258 | +147.3 |
| **Venous Thromboembolism (n°)** | 362 | 543 | +50.0 |
| **Cardiac prosthesis (n°)** | 441 | 470 | +6.6 |
| **Others (n°)** | 407 | 981 | +141 |
| **Warfarin (n°)** | 1799 | 3946 | +119.3 |
| **Acenocumarol (n°)** | 316 | 245 | –22.5 |
| **LMWH (n°)** | 8 | 157 | +1,862.5 |
| **Fondaparinux (n°)** | 0 | 4 | n.e. |
| **PT INR (n°)** | 31.845 | 65.280 | +105 |
| **aXa activity (n°)** | 32 | 359 | +1,021.9 |
| **Blood cell count (n°)** | 96 | 1.932 | +1,912.5 |
| **Creatinine** | 57 | 892 | 1,464.9 |

Our net program showed some important limitations because posology, therapeutic indications, type and necessity of laboratory monitoring is different for each molecule.

**Conclusion** This evaluation gave us some important indications:

1) the population of anticoagulated patients has changed in the last few years and probably will rapidly change in the next future through the introduction of new antithrombotic drugs;
2) our program gave us the possibility to register clinical and laboratory data, but it should be significantly improved in relation to the different molecules.

Thereby it's hoped a clinical-lab management program suitable for each type of anticoagulant.

P151 **QUALITY OF LIFE EVALUATION IN ANTICOAGULATED PATIENTS**

Sottilotta G.[(1)], Genna E.[(1)], Latella C.[(1)], Oriana V.[(1)], Piromalli A.[(1)], Romeo E.[(1)], Trapani Lombardo V.[(1)]
*[(1)] Centro Emofilia - Servizio Emostasi e Trombosi, Azienda Ospedaliera Bianchi-Melacrino-Morelli, Reggio Calabria; [(2)] Psicoterapia, Istituto Tolman, Palermo*

**Introduction** Oral anticoagulant therapy (OAT) has a variable therapeutic response as a result of comorbidities, drug and food interactions; patients on OAT need monitoring by periodic blood sampling. The purpose of this study was to determine whether management of OAT has effects on patient's quality of life (QoL).

**Materials and Methods** 150 patients (males 86, females 64, age 25-85, average 64.8) with more than 2 years of OAT were investigated during a 8-months period. A 12 item-questionnaire was expressly designed and focused on symptoms and perceptions, habits of life, physical ability, psychological distress, social/usual activities and work ability; it also included satisfaction or dissatisfaction with medical attention.

**Results** We evidenced that OAT does not have a negative impact in QoL and it does not adversely affect the psychological well-being if compared to the time before the start of OAT. Some patients perceived the disease and OAT as a problem for the continuous sampling and concerns arising, but others were rather indifferent to these issues, because of the development of a pattern of adaptive behaviour. The analysis of the social area showed homogeneity about the continuation of their work and leisure activities, without any influence from the disease. Regarding future expectations: 62% of subjects reported difficulty in conducting a normal life, because of concern about a worsening of the disease, while the remaining 38% thought that their life could get better.

**Conclusions** Psychotherapeutic interventions in patients on OAT could improve the QoL, allowing the perception of a happy and successful life. A patient with a good compliance of management of OAT can achieve and maintain the international normalized ratio (INR) within the appropriate target range, better than patients with worst psychological impact, reducing significantly the risk of thrombotic or hemorrhagic complications, and thus hospitalizations and healthcare costs resulting from them.

P152 **LONG-TERM FONDAPARINUX IN AN ELDERLY PATIENT WITH RECURRENT PORTAL VEIN THROMBOSIS AND LIVER CIRRHOSIS**

Coppola A.[(1)], Conca P.[(1)], Tufano A.[(1)], Guida A.[(1)], Di Capua M.[(1)], Russolillo A.[(1)], Cerbone A.M.[(1)], Mancini M.[(2)]
*[(1)] Centro di Riferimento Regionale per le Emocoagulopatie DACM, Università Federico II, Napoli; [(2)] Dip. di Scienze Radiologiche, Università Federico II, Napoli*

Portal vein thrombosis (PVT) is frequently associated with liver cirrhosis. Antithrombotic treatment in these patients should balance the concomitant bleeding risk due to thrombocytopenia and portal hypertension with oesophageal varices and congestive gastropathy.

We report the case of a 72 yr-old woman with a history of liver cirrhosis, who presented persistent dull abdominal pain. She was on low-dose oral anticoagulation (INR target 2.0) because of PVT diagnosed ten months earlier. At that time enoxaparin 100 IU/kg daily was given, in association with antithrombin concentrate supplementation (30 IU/kg every 48-72 hrs) because of her severe acquired deficiency (35%). Complete portal vein recanalization was detected after three weeks. Enoxaparin and antithrombin concentrate treatment was tapered off and, taking into account her thrombocytopenia (55,000/mmc) and portal hypertension (F1-F2 oesophageal varices and congestive gastropathy), low-dose warfarin was started. On admission, ultrasound and computed tomography scan revealed recurrence of PVT, with an almost occlusive thrombus not involving portal branches. INR was 1.89, therefore low-dose enoxaparin was started, again in association with antithrombin concentrate. Two weeks later, the need for hospital administration of antithrombin and the previous difficult management of oral anticoagulation (with evidence of PVT recurrence, although subtherapeutic INR could not be ruled out) led to continue treatment with fondaparinux 2.5 mg daily long-term. Portal vein was completely recanalized at ultrasound assessment one month later. Treatment is still ongoing, in the absence of bleeding complications or thrombotic recurrence after 8 months.

Balancing bleeding risk and the need of antithrombotic treatment or prophylaxis in patients with liver cirrhosis and PVT is a difficult task. Fondaparinux has been shown as a safe and effective option in this patient.

## *FARMACI ANTIPIASTRINICI*

P153 **FIBRIN RESISTANCE TO LYSIS IN CORONARY ARTERY DISEASE PATIENTS AND PLATELET HYPER-REACTIVITY IN CORONARY ARTERY DISEASE PATIENTS**

Fiorillo C.[(1)], Gori A.M.[(2)], Marcucci R.[(2)], Olimpieri B.[(2)], Becatti M.[(1)], Prisco D.[(2)], Nassi P.[(1)], Giglioli C.[(3)], Abbate R.[(2)], Gensini G.F.[(2)]
*[(1)] Biochemical Sciences, University of Florence, Florence; [(2)] Medical and Surgical Critical Care, University of Florence, [(3)] Heart and Vessels, Atherothrombotic Disease Unit, Az. Ospedaliero Universitaria Careggi, Florence*

Altered properties of the plasma clot architecture, as evidenced by decreased clot permeability, are demonstrated in patients with coronary artery disease (CAD) who underwent to

percutaneous coronary intervention (PCI), but scarce information is available on fibrin resistance to lysis in CAD. Platelet hyper-reactivity in patients on dual antiplatelet treatment represents a risk factor for the occurrence of adverse cardiovascular events.
Aim of the present study was to ascertain whether fibrin resistance to lysis occurs in CAD patients on dual antiplatelet therapy and its relationship with platelet hyper-reactivity. We studied 57 CAD patients (18F/39M) on dual antiplatelet therapy 6 months after PCI and 33 controls (10F/23M) of equivalent age. Fibrinogen was purified from citrated plasma and exposed to thrombin. Plasmin-mediated cleavage of fibrin β chain was assessed hourly over a 6-hour period by polyacrylamide gel electrophoresis and fibrin band intensity was measured by densitometry of stained gels. Residual platelet reactivity (RPR-aggregation by collagen ≥56%.) was assessed in platelet-rich plasma stimulated by 2 μg/mL collagen.
After 6 hours in all controls degradation of the fibrin β chain occurred, whereas it was not observed in 27 (47.6%) CAD patients. A significant decline in fibrin band intensity was observed in 29 (50.9%) CAD patients.
Degradation of the fibrin β chain was not significantly different between patients STEMI (14/28, 50%) and non-STEMI (15/29, 51.8%) patients and between patients with and without traditional risk factors. The decline in fibrin band intensity was significantly ($p<0.05$) different between patients with and without RPR by collagen: the degradation of fibrin did not occur in 14/20 (70%) patients with RPR by collagen and in 15/37 (39.4%) patients without.
Persistence of fibrin β chain occurs in CAD patients and is related to platelet hyper-reactivity, suggesting a new pathophysiological mechanism underlying thrombus formation.

P154 **SERUM THROMBOXANE B2 (TXB2) IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS (MPN) TREATED WITH LOW-DOSE ASPIRIN (ASA): PRELIMINARY DATA**

Vettore S.[(1)], Tezza F.[(2)], Candeo N.[(2)], Bonamigo E.[(2)], Fabris F.[(2)], Randi M.L.[(2)]
*[(1)] Dip. Scienze Mediche e Chirurgiche, Medicina Interna CLOPD, Padova; [(2)] Dip. Scienze Mediche e Chirurgiche, Medicina Interna, Padova*

ASA reduces thrombosis in MPN patients. However, some patients are ASA-resistant.
We studied 64 MPN (mean age 60±14 y, mean platelet 614±323x$10^9$/L) treated with ASA (100 mg/die) (MPN-ASA), 10 untreated MPN patients (mean age 58±15 y, mean platelet 585±120x$10^9$/L) (MPN-basal) and 27 healthy subjects, platelets 256±120x$10^9$/L (normal). In 10 of these controls the study was performed also after ASA 100 mg/day (normal ASA). We performed aggregation under Arachidonic Acid (AA) (1 mM) with Born method. TxB2 was evaluated using an ELISA kit (Cayman Chemical, USA). TxB2 values were expressed as serum TxB2 content (pg/mL) and as ratio between serum TxB2 and platelet number (pg/10-8 plts).
Statistical analysis was performed with student T test to compare the mean values expressed as mean±standard error.
Platelet counts of MPN were higher ($p=0.01$) than controls. 18 (29%) MPN were ASA-resistant to AA, while all the controls had a normal aggregation. Serum and platelet TxB2 in normal were respectively 22,054±4,578 and 8,751±1,847, in MPN-basal 44,033±18,906 and 6,156±2,585, in normal-ASA 1,144±162 and 421±60, in MPN-ASA 11,059±1,936 and 2,349±649. No difference was observed comparing serum and platelets TxB2 in controls and MPN patients without ASA while, in treated cases, serum TxB2 was higher in MPN ($p=0.04$) than in normal, but the difference disappeared considering platelet TxB2.
ASA resistance is as frequent in MPN as in normal subjects (30%) on the basis of the data of the literature. The small number of our controls does not permit to drive definitive conclusions. The high TxB2 levels found in MPN seems related to the high platelet number as suggested by the platelet TxB2 levels. Our data confirm previous results in urine-TxB2.

P155 **WHOLE BLOOD PLATELET AGGREGATION: SCREENING TEST FOR MONITORING ANTIPLATELET THERAPY**

Diquattro M.[(1)], Calabrò G.M.[(1)], De Michele S.[(1)], Bellomare D.[(2)], Tagliavia A.M.[(1)], Palma B.[(1)]
*[(1)] Diagnostica di Laboratorio, Unità Operativa Patologia Clinica, Palermo; [(2)] Medicina, Unità Operativa Cardiologia, Palermo*

The goal of antiplatelet therapy is to prevent ischemic events, but in some patients events occur despite chronic treatment with standard doses of antiplatelet agents. Variability has been found in patient responses to aspirin and clopidogrel. The availability of platelet function tests has led to interest in whether such tests can be used to guide antiplatelet therapy. We evaluated variable platelet response to aspirin and clopidogrel.
**Methods** Measurements were performed in 32 patients with stable coronary artery disease (CAD), treated with antiplatelet therapy (ASA 100 mg and clopidogrel 75 mg daily), from the Unit of Cardiology of the CIVIC Hospital, Palermo, Italy. Platelet count and platelet parameters were determined using XE2100 analyser (Sysmex, Kobe, Japan). Platelet aggregation was determined, within 1 h from blood collection, in citrate-anticoagulated blood by the Multiplate analyser (Dynabyte Medical, Munich, Germany), using arachidonic acid 0.5 μM and ADP 6 mM. We applied the reference limits from 40 healthy control subjects (ADP 6 mM induced aggregation: median 52, range 32-71 AUC, Arachidonic acid 0.5 μM - induced aggregation median 48, range 22-72 AUC), as criteria for differentiation between responders and non responders.
**Results** PLTi (impedance platelet count) median 230.0x$10^9$/L, range 121.0-531.0x$10^9$/L, PLTo (optical platelet count) median 233.0x$10^9$/L, range 129.0-458.0x$10^9$/L, MPV (mean platelet volume) median 10.6 fL, range 9.4-12.2 fL, IPF (Immature Platelet Fraction) median 2.2%, range 0.6-8.3%. ADPAUC: median 28, range 2-65 ($p<0.0001$ vs. control); ARA-AUC: median 6.5, range 2-37($p<0.0001$ vs. control). Of the 32 patients, 18 (56%) were clopidogrel good responders, 30 (93%) were ASA good responders.
**Conclusions** Whole blood platelet aggregation, simple and standardized test, could be use to identify poor responders to antiplatelet treatment, and select ADP poor responders for more complex tests, as the Vasodilator-Stimulated Phosphoprotein

Assay (VASP) to verify inhibition of P2Y12 receptors.

P156 **ABCIXIMAB-ASSOCIATED THROMBOCYTOPENIA**

Albisinni R., Casillo R., Caianiello C., Pinto D., Malgeri U., Durante-Mangoni E., Utili R., Siciliano S., Di Giacomo A.
*Cardiologia, AORN Monaldi, Napoli*

Thrombocytopenia is a possible complication of treatment with glycoprotein (GP) IIb/IIIa antagonists during percutaneous coronary interventions. Its incidence ranges from 0.5% to 5.6%. We report 4 cases, 3 men and one woman, of thrombocytopenia in patients treated with abciximab. The mean age was 67 years. The abciximab was administered at a dose of 0.25 mg/kg bolus followed by an infusion of 0.125 to 10 mig/kg per minute for 12 hours. All patients received 150 mg/d aspirin within 24 hours of the procedure and 100 mg/d thereafter. A 300-mg clopidogrel was administered before procedure. Heparin was administered at the start of the procedure at a dose of 70 U/kg, and stopped at the end of the procedure. The platelet count in all patients before procedure was superior to 200,000/$mm^3$. Platelet monitoring was performed every 6 hours for the first 24 hours and then every 24 hours until discharge.
In all patients thrombocytopenia occurred within 24 hours from the beginning of treatment and then it slowly progressed: the lowest value observed was 1,000/mm3 in two patients and 6,000 and 10,000 in the other two, respectively. Two patients had minor bleeding complications: one presented spots on the arms; the second had a large haematoma at the site of femoral arterial puncture; the other two patients had no bleeding complications. The peripheral blood smear demonstrated extensive platelet clumping. All patients were treated with metil-prednisolone at the dose of 40 mg/d for three days and then 20 mg/d for 4 days, and all of them had an increase of platelet count at a normal value within 7 days from the begin of corticosteroid therapy. Abciximab-induced thrombocytopenia could be immune-mediated. Steroid treatment may represent a valid therapeutic option in these patients.

## *MANIFESTAZIONI TROMBOTICHE ED EMORRAGICHE NELLE NEOPLASIE EMATOLOGICHE*

P157 **NEW TET2 GENE MUTATIONS IN PATIENTS WITH MYLOPROLIFERATIVE NEOPLASM AND SPLANCHNIC VEIN THROMBOSIS**

Colaizzo D.[(1)], Tiscia G.L.[(1)], D'Andrea G.[(1)], Vecchione G.[(1)], Pisanelli D.[(1)], Vergura P.[(1)], Grandone E.[(1)], Margaglione M.[(1,2)]
[(1)] *Laboratorio di Emostasi e Trombosi, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo;* [(2)] *Scienze Biomediche-Genetica Medica, Università degli Studi di Foggia, Foggia*

**Background** TET2 mutations have been identified in a high proportion of patients with JAK2V617F-positive JAK2V617F-negative myeloproliferative neoplasms (MPN). MPN, whether overt or latent, represents a main intrinsic factor for the development of thrombosis in the portal, mesenteric, or hepatic areas. The aim of the present study was to investigate for mutations in the TET2 gene locus in patients with splanchnic venous thrombosis.
**Materials and Methods** Twenty-three unrelated non-cirrhotic patients with the JAK2 V617F mutation and presenting with a splanchnic venous thrombosis diagnosed and followed-up at the Gastroenterology Unit of the "A. Cardarelli" Hospital, Naples were enrolled. All coding regions of the TET2 gene and intron/exon boundaries were investigated using sense and antisense oligonucleotide designed on the basis of the known sequence of the TET2 gene locus (NM_001127208). Then, amplified DNA fragments were subjected to direct cycle sequence analysis using an ABI PRISM 3100 Genetic Analyzer sequencer (PE Biosystems, USA).
**Results** Gene sequencing of the TET2 gene locus showed previously unreported heterozygous deletions, in exon 3 [F572fsX7 (c.2102delT; #1) and Q642fsX57 (c.2311delA; #2)], and in exon 11 [E1555fsX22 (c.5050delAG; #3)]. Only a patient (#3) carried an overt MPN while others developed a MPN years after a splanchnic occurred.
**Discussion e Conclusions** Both TET2 and JAK2 mutations are observed in a range of phenotypically different diseases, the former being an early event and the latter a subsequent acquisition in MPN. It does not exclude that an additional genetic hit needs for overt MPN developing. Considering that because a portion of patients presenting with splanchnic venous thrombosis did not suffer from an overt myeloproliferative disorder, present findings suggest that, independently of the JAK2 V617F mutation, screening for TET2 mutations may be useful to recognize patients who should be carefully observed for the subsequent development of an overt disease.

P158 **THROMBOTIC EVENTS IN ESSENTIAL THROMBOCYTEMIA (ET): PREVALENCE AND PROGNOSTIC FACTORS IN 218 UNSELECTED PATIENTS MONITORED IN CLINICAL PRACTICE**

Rupoli S.[(1)], Mulattieri S.[(1)], Albani L.[(1)], Picardi P.[(1)], Scortechini A.R.[(1)], Da Lio L.[(2)], Berardinelli E.[(1)], Tomassetti S.[(1)], Canafoglia L.[(1)], Leoni P.[(1)]
[(1)] *Dipartimento di Scienze Mediche e Chirurgiche Clinica di Ematologia, Università Politecnica delle Marche, Ancona;* [(2)] *Laboratorio Analisi, Dipartimento di Scienze Mediche e Chirurgiche, Ospedali Riuniti Umberto I, Ancona*

**Background and aim of the study** Thromboembolic disease (TE) is a major cause of morbidity and mortality in Essential Thrombocytemia (ET). Recently JAK2V617 mutational status and allele burden seem to add prognostic significance to standard risk factors. The aim of the study was to evaluate such factors in a series of patients with ET.
**Methods** Patients were recruited from the Ancona Hematology Clinic database for ET. Standard statistical methods were applied to test significance of associations between thrombosis and other variables.
**Results** A total of 218 cases were studied, of whom 27 had a past history of thrombosis.
Of the 110 patients analysed for the presence of JAK2V617F, 49 (44.5%) were positive. Quantitative analysis was performed in 42 cases: 6 (14.3%) were homozygotic and 36 (85.7%) heterozygotic.
At diagnosis, a total of 17 patients (7.8%) had TE. During

follow-up, TE was recorded in 25 (11.5%). Gender, age, hemoglobin, platelets, leukocyte count, splenomegaly, increased cellularity of bone marrow, bone marrow fibrosis and common vascular risk factors did not affect the probability of thrombotic events. On univariate analysis a significant increase of thrombosis as presenting manifestation was registered in patients with a history of remote thrombosis (OR= 10.0, 95% CI 4.1-24.5; p<0.001) and in those with JAK2V617F mutation (OR=3.73, 95% CI 1.06-13.05; p=0.032). Subjects with both JAK2 mutation and a history of remote thrombosis have a OR of 24.44 (95% CI 3.07-194.66; p=0.01).
During follow-up, vascular events were predicted only by a history of remote thrombosis (OR=2,3, 95% CI 1.01-5.35; p=0.05).
**Conclusions** The present analysis suggests that the thrombotic risk is higher in the JAK2V617F positive patients and is further increased by a past history of thrombosis. The opportunity of combine such criteria must be defined in prospective trials.

P159 **USE OF RECOMBINANT FACTOR VII WITH OMOLOGOUS PLATELETS TRANSFUSION IN INTRACRANIAL HEMORRAGES OF ACUTE PROMYELOCYTIC LEUKEMIA**
Santi R.M., Contino L., Primon V., Baraldi A., Pini M., Levis A.
*Ematologia, Azienda Ospedaliera, Alessandria*

Intracranial hemorrhage (ICH) is the most common hemorrhagic complication in acute promyelocytic leukaemia, (APL) which accounts for approximately 6580% of the bleeding sites and accounted for 41% of deaths in patients with APL.
Growth of hematoma occurs in 38% of cases of intracerebral hemorrhage within the first 24 hours after the onset of symptoms.
We report one successful treatment of intracranial hemorrage in a newly APL diagnosed patient using Recombinant Factor VII (rFVIIa) combined with homologous platelets transfusion.
**Case report** A 47-year old man was admitted to our haematology unit in July 2009 with a diagnosis of APL. Modest signs of consumptive coagulopathy were present: INR: 1,4 APTT ratio 1,0, fibrinogen: 187 mg/dL, PLT 11,000/mL. He was in coma (Glasgow Coma Scale of 13). Computed tomography (CT) scan disclosed two lesions in mesencephalic and occipital area of the brain and petechial haemorrhages in the choroid plexus.
Considering the emergency of the initial clinical picture, we decided to start transfusion therapy immediately with plasma to maintain the fibrinogen above 100 mg/dL and homologous platelets to maintain the platelet level above 50,000/mL. rFVIIa boluses of 2 mg were administered suddenly after the platelets transfusions for three consecutive days.
Standard therapy was done with ATRA and idarubicine.
The anti-haemorrhagic therapy quickly brought the bleeding under control; as a result, a followed ANGIOTAC done 12 days later showed complete resorption of intracranial hemorrages.
Clinically the patient showed an improvement in status, he was alert and oriented.
**Conclusion** Anecdotal cases of rFVIIa therapy successful in APL have been reported. We think that the administration of rFVIIa improves response to antihaemorragic therapies, activating platelets binding to tissue factor. Thus the quick mode of action should induce to choice the rFactor VIIa as first-line therapy in the mortality high risk events.

## *TUMORI E TROMBOSI*

P160 **RECURRENT VENOUS THROMBOEMBOLISM IN A PATIENT WITH NEURINOMA OF THE FIFTH RIGHT CRANIAL NERVE (SUSPECTED TYPE-2 NEUROFIBROMATOSIS)**
Quintavalla R., Carolla G., Ferrini P.M., Rossetti A., Degliantoni G.
*Medicina Interna ad indirizzo angiologico e coagulativo-Centro Emostasi e Trombosi, Azienda Ospedaliero-Universitaria, Parma*

The case history refers to a 44 years old male (L.M) in VKA therapy for unprovoked deep vein thrombosis of the right leg. In anamnesis febrile seizures during infancy, AMI. 40-years-old brother with neurinoma of the V cranial nerve, spinal and peripheral neurinoma. During 2009 L.M. was hospitalized in neurology department for walking instability and increasing disequilibrium. Brain RMN: voluminous expanding lesion localized in posterior cranial fossa characterized by a cystic-kind component and by a solid cranial component, image compatibility with neurinoma of the V right cranial nerve. PET: neo-formations near the right suprarenal, left psoas muscles and the right trapezium, VIII right costa and left iliac bone. Result of the biopsy of the trapezium: neurinoma.
Due to neurosurgery, VKA was suspended and a vena cava filter placed. A week later: vena cava trombosis cranially extending to the filter, common and right-external iliac vein trombosis.
**Therapy** Unfractionated heparin followed by LMWH.
**Neurosurgery** Endoscopic ventriculostomy and puncture of the tumoral cist.
**Criteria for the diagnosys of type-2 neurofibromatosis** Family-history + 2 NF2 associated lesions (neurinomas, meningiomas, neurofibromas or cataract).
**Correlations with VTE** The few cases reported in literature describe an association between type-2 neurofribromatosis and arterial thrombosis. The hypothesis is that the mutation of the merlin protein, a membrane-cytoskeletal scaffolding protein coded by the gene NF2, which controls most of the cellular proliferation processes, may advantage iperplasia and vascular occlusion. This mutation may also cause venous thromboembolysm, considering the even more consistent evidence between cardiovascular risk factors and venous thrombosis.
**Conclusions** Neurofibromatosis could be identified as a condition with high risk of thrombotic complications, both arterial and venous. Positioning of IVC filter has to be evaluated with attention and the absolute indication to initiate oral anticoagulant therapy as soon as possible must be kept in mind.

P161 **HYPERCOAGULABLE STATE IN SMALL CELL LUNG CANCER WITH ECTOPIC ACTH SECRETION**

**RESULTING IN CUSHING SYNDROME**

Barbar S.[(1)], Casonato A.[(1)], Scaroni C.[(2)], Fadin M.[(1)], Daidone V.[(1)], Saggiorato G.[(1)], Sartori M.T.[(1)]
[(1)] *Department of Cardiologic, Thoracic and Vascular Sciences, Second Chair Internal Medicine, Padua;* [(2)] *Department of Medical and Surgical Care Endocrinology Unit Padua*

Small cell lung cancer (SCLC) is a highly malignant tumour not infrequently associated with ectopic neuropeptide secretion, especially antidiuretic hormone (ADH) resulting in the syndrome of inappropriate secretion of antidiuretic hormone (SIADH), and adreno-corticotrophin hormone (ACTH) resulting in Cushing's syndrome (CS). Available studies suggest that CS features high-glucocorticoid secretion and an associated hypercoagulable state often involving an increase in von Willebrand factor (VWF) and factor VIII (FVIII) leading to a high risk of venous thromboembolism.
We describe a 73 year-old man admitted for mental confusion and weakness, associated with a severe decrease in serum sodium (101 mmol/L) and osmolarity (219 mOsm/kg) consistent with SIADH. A PET-TC showed mediastinal, right hilar and sub-carinal lymphadenopathy; an endobronchial biopsy confirmed small cell lung cancer. The patient was treated with demeclocycline for SIADH and chemotherapy.
Five months later, he presented with muscle weakness, severe hypokaliemia (1.7 mmol/L) and diabetes mellitus. Ectopic ACTH syndrome was confirmed by biochemical test findings (ACTH 126 ng/L). Highly increased serum and urinary free cortisol levels (1,117 nmol/L and 10,506 nmol/24 hours, respectively) were associated with shortened aPTT (22.9 sec), marked increase in FVIII (302.3%) and VWF levels (VWF:Ag 749.5%, VWF:CB 691%), and elevated values of factor IX and PAI-1. Second line chemotherapy and s.c. octreotide were given, leading to a progressive reduction in ACTH and cortisol levels. According to hormonal improvement, FVIII and VWF values decreased. Prophylaxis with enoxaparin effectively prevented thrombotic complications. Despite paraneoplastic syndromes control by specific therapy, the patient died for SCLC progression four months later.
This case shows two very peculiar and rare clinical aspects: first, the uncommon sequential presentation of two paraneoplastic disorders in the same SCLC, namely SIADH and CS; secondly, the onset of a severe hypercoagulable state related to ectopic cortisol secretion, confirming the risk of CS to develop a prothrombotic condition.

P162 **INHIBITION OF THE RENIN-ANGIOTENSIN SYSTEM DOWNREGULATES TISSUE FACTOR EXPRESSION IN HUMAN BREAST CARCINOMA CELLS**

Napoleone E., Cutrone A., Cugino D., Iacoviello L., de Gaetano G., Donati M.B., Lorenzet R.
*Laboratori di Ricerca, Università Cattolica, Campobasso*

**Background** The renin-angiotensin system (RAS) promotes angiogenesis and growth of neoplastic cells. Angiotensin-converting enzyme (ACE) inhibitors and blockade of the angiotensin II (AngII) receptor AT1 may protect against cancer, thus suggesting new treatment strategies of malignancies.
Tissue factor (TF), for its involvement in tumour growth, angiogenesis, and metastasis is considered a hallmark of cancer progression.
**Aim** Having shown that RAS system is involved in modulation of TF expression in human stimulated monocytes (Napoleone et al. CircRes 2000), we evaluated whether RAS blockade modulates TF constitutive expression by the metastatic breast carcinoma MDA-MB-231 cell-line.
**Materials and Methods** MDA-MB-231 were incubated with the different reagents at 37C. TF activity was assessed by one stage clotting time, TF and VEGF antigens by ELISA, and TF mRNA levels by real time PCR. Angiotensin receptor (AT1) was detected by flow-cytometry and angiotensin-II levels were measured by EIA.
**Results** Both the strong constitutive TF activity and antigen expressed by MDA-MB-231 were significantly reduced in a dose-dependent manner by captopril and enalapril. AT1 was present on MDA-MB-231 membrane and losartan, a competitive inhibitor of AT1, reduced TF activity and antigen to a degree similar as that exerted by ACE inhibitors. Moreover, captopril and losartan downregulated the strong constitutive expression of TF mRNA. Similar results were observed when an anti-AT1 antibody was used instead of losartan. Abs against angiotensin-II, which was present in the cell conditioned medium, could reduce TF activity and antigen.
In addition, concomitantly with TF inhibition, captopril and losartan decreased the constitutive VEGF expression by MDA-MB-231, and an anti-VEGF MoAb downregulated TF activity.
**Conclusions** These results could, at least in part, contribute to explain the supposed effects of ACE inhibitors and AT1 receptor antagonists in some types of malignancy, and offer new clues to support their use for tumour control.

P163 **THROMBIN GENERATION (TG) OF ACUTE PROMYELOCYTIC LEUKEMIA (APL) CELLS IS DIFFERENTLY AFFECTED BY ARSENIC TRIOXIDE (ATO) AND ALL-TRANS RETINOIC ACID (ATRA)**

Diani E., Marchetti M., Russo L., Balducci D.
*Division of Immunohematology and Transfusion Medicine & Hemostasis and Thrombosis Centre, Department of Oncology-Hematology, Ospedali Riuniti di Bergamo, Bergamo*

APL is characterized by life-threatening coagulopathy, resulting from deregulated activation of coagulation by blast cell procoagulant activities [tissue factor (TF) and cancer procoagulant (CP)]. Both ATRA and ATO effectively cure APL by inducing differentiation, and reducing blast procoagulant activity. Little is known whether this procoagulant activity reduction significantly affects APL-TG capacity. The calibrated automated thrombogram (CAT) assay was used to evaluate the TG of NB4-APL cell after treatment with 0.1 μM ATO, 1 μM ATRA, or their combination. The TG potential was evaluated in normal pooled plasma (NPP) and, in order to evaluate TF contribution, in NPP + anti-TF-Ab or in FVII-deficient plasma (FVII-DP). Levels of TF, CP, thrombomodulin (TM), and differentiation, proliferation, apoptosis/necrosis were also evaluated. The results show that ATRA and ATRA/ATO treatment significantly reduced NB4-TG in both NPP and FVII-DP, whereas ATO gives only a slight reduction. Compared to NPP, NB4-TG performed in

NPP after incubation with anti TF-Ab, or in FVII-DP were significantly lower, but still measurable, indicating a major role for TF in this system. The residual TG activity, measured in both experimental conditions, suggested also the contribution of FVII-independent procoagulants (i.e. CP, phospholipids). Both ATRA and ATO treatments determined a decrease in TF and CP levels (significantly greater in ATRA-treated cells), whereas an increase of the anticoagulant TM was observed only in presence of ATRA. Significant correlations were found between TG parameters and TF, CP, the rate of cell proliferation and differentiation.
In conclusion, by the CAT assay, we could demonstrate that APL procoagulant activities reduction and anticoagulant induction by ATRA and ATO translates into a significant inhibition of APL cell TG capacity. In future, CAT might possibly help to define the *in vivo* role of different TG phenotypes in the outcome of APL-associated coagulopathy and in patient prognosis.

## P164 MARKERS OF COAGULATION, FIBRINOLYSIS AND ANGIOGENESIS IN WOMEN WITH BREAST CANCER

Conti L.[(1)], Faioni E.M.[(2)], Razzari C.[(2)], Fontana G.[(2)], Antenucci A.[(1)], Mandoj C.[(1)], Temperilli F.[(1)], Cattaneo M.[(2)]
*[(1)] UOSD Patologia Clinica, Istituto Nazionale Tumori Regina Elena, Roma; [(2)] Dipartimento di Medicina, Chirurgia e Odontoiatria, Università degli Studi di Milano e Azienda Ospedaliera Medicina III, Milano*

**Background** Breast cancer is associated with procoagulant changes, angiogenesis and matrix remodelling, mediated by the host and the tumour. Procoagulant responses are associated with tumour progression and metastasis. Circulating physiologic anticoagulants are decreased in cancer patients, while the receptors thrombomodulin and endothelial protein C receptor (EPCR) are downregulated and/or shed from the endothelium due to inflammatory mediators and thrombin generation. The soluble receptors sEPCR and sTM may be considered markers of endothelial activation by different mechanisms. Circulating sEPCR functions as a procoagulant, and was shown to be associated with venous and arterial thrombosis. We recently reported that some breast cancers express EPCR. Aim of the study. To correlate a panel of plasma or serum biological markers of coagulation and inflammation with TNM and age at presentation in patients with breast cancer.
**Methods** Thrombin-antithrombin complexes (TAT), prothrombin fragment F1+2 (F1+2), plasminogen activator inhibitor-1 (PAI-1), vascular endothelial growth factor (VEGF), tissue factor (TF) and sEPCR were measured in 80 patients at diagnosis of breast cancer (90% ductal). A control group of 30 women was also studied.
**Results** All markers were significantly increased in cancer patients, compared with controls ($p<0.001$). No significant differences were observed when markers were compared between women with (n=14) or without (n=66) metastatic cancer, N0 (n=44) vs. N1+2 (n=32) or T1 (n=35) vs T2 or T3 (n=41). No correlation was observed with age at presentation.
**Conclusions** Circulating markers of coagulation, inflammation and angiogenesis in women with breast cancer were markedly increased compared to normal women, but did not correlate with breast cancer basic features (TNM), or with the women's age at presentation. They possibly represent a general response of the host to the tumour. As in other cases, they might be more useful prospectively to identify thrombosis prone patients rather than being prognostically related to the cancer.

## P165 CHARACTERIZATION OF THE THROMBIN GENERATION POTENTIAL OF TUMOR CELLS

Marchetti M.[(1)], Diani E.[(1)], ten Cate H.[(2)], Falanga A.[(2)],
*[(1)] Division of Immunohematology and Transfusion Medicine & Hemostasis and Thrombosis Centre, Department of Oncology-Hematology, Ospedali Riuniti di Bergamo, Bergamo; [(2)] Department of Internal Medicine, Cardiovascular Research Institute Maastricht, Maastricht, University Maastricht, The Netherlands*

Thrombin is a multifunctional serine protease that regulates the behaviour of many cells, including tumour cells. Tumour cells can induce thrombin generation (TG) and fibrin formation by different mechanisms, including the direct expression of procoagulant proteins. The calibrated automated thrombogram (CAT) was used to characterize the TG activity of different human tumour cell lines: i.e. MDA.MB231 and MCF7 (breast), H69 (small cell lung), NB4, HEL, and K562 (myelogenous leukemia). The endothelial HMEC-1 and embryonic kidney HEK-293 cells were used as non tumour cells. CAT was performed in normal pool plasma (NPP), FVII-, FXII- and FIX- deficient plasma, and in the presence of anti-TF antibody. In NPP the highest TG was induced by MDA.MB231, while the lowest by HEL and K562 cells. MDA.MB231 TG was not significantly affected by FXII and FVII depletion. In addition, the high TG of MDA.MB231 in FIXdef plasma and in presence of anti-TF antibody suggested an important contribution of FVII-independent procoagulants in their TG capacity. The NB4-induced TG was not FXII dependent, but was significantly reduced in FVII-def plasma, indicating a major role of TF for this cell line. HEL and K562 TG capacity was very low in both NPP and in FVII-def plasma, and became undetectable in FXII-def plasma; this indicated a main role of contact activation in the TG capacity of these cells. MCF7 H69, HMEC-1 and HEK-239 cells, all showed a slight decrease of TG in either FVII or FXII-def plasma, indicative of the role of both TF and contact activation. In conclusion, different tumour cells can induce TG through several patterns. The specific capacity of each cell type to activate TG could be used to select specific agents to block their procoagulant activity.

## P166 VEGF AND PROCOAGULANT FACTORS IN BEVACIZUMAB-BASED THERAPY RECURRENT MALIGNANT GLIOMA

Antenucci A.[(1)], Pace A.[(1)], Russillo M.[(2)], Koudriavtseva T.[(3)], Carapella C.M.[(4)], Sperduti I.[(5)], D'Alessandro G.[(1)], Tomao L.[(1)], Conti L.[(1)]
*[(1)] UOSD Patologia Clinica, Istituto Nazionale Tumori Regina Elena, Roma; [(2)] Oncologia Medica A, Istituto Nazionale Tumori Regina Elena, Roma; [(3)] UOSD Neurologia, Istituto Nazionale Tumori Regina Elena, Roma; [(4)] Neurochirurgia, Istituto Nazionale Tumori Regina Elena, Roma; [(5)]*

*Epidemiologia, Istituto Nazionale Tumori Regina Elena, Roma*

**Background** Malignant gliomas are the most common and aggressive primary brain tumours in adults. Significant therapeutic benefit has been observed for recurrent malignant glioma (MG) patients treated with bevacizumab (BV), a neutralizing monoclonal antibody against the vascular endothelial growth factor (VEGF). Anti-angiogenic therapies appear to be associated with an increased risk for thrombosis and, paradoxically, bleeding. The relationship between VEGF levels and response to BV has never been addressed so far.
**Methods** We conducted a prospective analysis of recurrent MG patients treated with BV alone or in combination with chemotherapy performing serial evaluations of serum and plasma VEGF (sVEGF/pVEGF) levels and procoagulant factors such as Tissue Factor (TF), Thrombin/Antithrombin Complex (TAT) and Prothrombin Activation Fragment 1+2 (F1+2) plasma levels (ELISA). Baseline and post-treatment samples were collected at each administration of BV.
**Results** Eighteen recurrent MGs (glioblastoma=7, anaplastic astrocytoma=7, oligodendroglioma=4) who received BV at 10 mg/kg intravenously every 14 days, of whom 13 in association with chemotherapy were included in the study. Median age was 39 years (27-65), median Karnofsky performance status was 80 (60-90). Out of the 12 evaluable, 4 partial responses (33%), 3 stable disease (25%) and 5 disease progressions (42%) were observed. Basal sVEGF lower than <224.25 pg/mL were observed in responding patients. Overall, serum and plasma VEGF, TF, TAT and F1+2 levels decreased during BV-based therapy.
**Conclusions** BV-based therapy showed activity in patients with heavily pretreated recurrent MGs. Low sVEGF levels at baseline might help predict response in recurrent MG patients treated with BV-based therapy. Further studies are needed to evaluate if pVEGF can be used as an additional marker in monitoring therapy.

## *EMOSTASI E GRAVIDANZA: DALL'ABORTO ALL'EMORRAGIA POST-PARTUM*

### P167 LOW MOLECULAR WEIGHT HEPARIN (LMWH) RESTORES DIRECT RELATIONSHIP BETWEEN HAEMOSTASIS AND ANGIOGENESIS IN PLACENTAE FROM THROMBOPHILIC WOMEN WITH PREVIOUS ADVERSE OUTCOMES

Chinni E.[(1)],Colaizzo D.[(1)], Tiscia G.L.[(1)], Vergura P.[(1)], Pisanelli D.[(1)], Sarno M.[(2)], D'Andrea G.[(2)], Margaglione M.[(2)], Grandone E.[(1)]
[(1)] *Emostasi e Trombosi, I.R.C.C.S. Casa Sollievo della Sofferenza, San Giovanni Rotondo;* [(2)] *Scienze Biomediche, Genetica M, OO.RR. Ospedali Riuniti di Foggia, Foggia*

Thrombophilia may play a role in the pathogenesis of preeclampsia (PE) and/or foetal growth restriction (FGR). Findings from observational studies also suggest that heparin reduces recurrence of such complications in women with thrombophilias.
In a previously study we found a direct relationship in placentae from uncomplicated pregnancies between expression of TF (tissue factor) and TFPI-2 and between TF and vascular endothelial growth factor (VEGF).
Furthermore, placentae from FGR foetuses lose these relationships.
The aim of this study was to evaluate these markers in placentae (n=13) from pregnancies treated with enoxaparin, because of previous obstetric complications in presence of known thrombophilias.
Placentae from 13 pregnancies ended in the delivery of 8 females and 5 males; median weight was 2,555 g range: 1,500-3,600g and gestational age median: 37.5$^{th}$ weeks, range 35$^{th}$-42$^{nd}$. From each placenta total RNA was obtained by means of Invitrogen Trizol, Reagent. Complementary DNA (cDNA) was prepared by means of RT-PCR. Quantity mRNA expression of TF, TFPI-2, VEGF, genes was evaluated by means of ABI 7700TM quantitative real time PCR system.
TF, TFPI-2, VEGF median and range were respectively 1.13 [0.26-2.02] 2-deltaCt; 31.23 [20.79-104.05] 2-deltaCt; 0.1 [0.04-0.37]. These values were similar to those previously observed in control pregnancies (TF 2.3 [1.9-2.9] 2-deltaCt; TFPI-2 48.40 [1.9-1351.2] 2-deltaCt; VEGF 0.1 [0.0-0.2] 2-deltaCt (Chinni et al Thrombosis Research, 2008). TF, TFPI-2 and VEGF were directly related to increasing weight of newborns.
A significant (Spearman rank test, r=0.79; p≤0,01) direct correlation between TF and its inhibitor TFPI-2 was found. Furthermore, a direct relationship between VEGF and TF (Spearman rank test, r=0.93; p≤0,01) was also observed.
Data from present work suggest that enoxaparin treatment during pregnancy in thrombophilic women with previous adverse obstetric outcomes restores the impairment of mechanisms involved in maintaining intervillous blood flow.

### P168 USE OF LOW MOLECULAR WEIGHT HEPARINS IN PREGNANCY: SAFETY AND EFFICACY

Sottilotta G.[(1)], Romeo E.[(1)], Oriana V.[(1)], Siboni S.M.[(2)], Luise F.[(1)], Latella C.[(1)], Trapani Lombardo V.
[(1)] *Centro Emofilia - Servizio Emostasi e Trombosi, Azienda Ospedaliera Bianchi-Melacrino-Morelli, Reggio Calabria;* [(2)] *Department of Medicine and Medical Specialties, "A. Bianchi Bonomi" Haemophilia and Thrombosis Centre, Milan*

**Introduction** There are several indication for low molecular weight heparin (LMWH) treatment during pregnancy: thromboprophylaxis and treatment of thromboembolism as well as the prevention of adverse pregnancy outcomes (recurrent abortions, foetal death, intrauterine growth restriction, etc.) related to congenital or acquired thrombophilia. Peer-reviewed international guidelines endorse the use of LMWH in pregnant women; however no LMWH has been completely licensed for pregnancy, and data regarding efficacy and safety come mostly from small case series. Aim of this study was to evaluate the pregnancy outcomes in patients treated with LMWH.
**Materials and Methods** We performed a review of pregnancy outcomes in patients treated with LMWH in our centre during the last 5 years. We examined 371 women (age 17-43, average 31.9) on LMWH therapy because recurrent abortions (96/371), thrombophilic states (146/371), pregnancy complications (25/371), thromboembolism (14/371), varicose veins (41/371), other causes (49/371). The start therapy median week was 25

(range 1-36).
**Results** 340/371 women (91.6%) had successful full-term delivery without any complications during pregnancy and postpartum. 31/371 women (8.4%) had adverse outcomes after LMWH therapy: 8 patients with thromboembolism, 7 with miscarriages, 5 with post partum haemorrhage (2 massive haemorrhages), 3 with gestosis, 2 with pre-term delivery, 2 with foetal hypoxia, 2 with foetal malformations, 1 with abruptio placentae, 1 with thrombocytopenia.
**Conclusions** The low prevalence of unsuccessful outcome observed (8.3%; 95% CI, 5.6%-11%), if compared to data reported from scientific literature about untreated women, seems to confirm the efficacy and safety of use of LMWH in pregnancy. The principal strength of our study is the high number of enrolled patients from a single centre. One limitation is the absence of a control group. The result of this study should be considered as preliminary.

## P169 THE PROTEIN C PATHWAY ON PLACENTA: MORE THAN JUST ANTICOAGULATION?

Faioni E.M.[(1)], Fontana G.[(1)], Razzari C.[(1)], Puozzo A.[(1)], Bulfamante G.[(2)], Avagliano L.[(2)], Cattaneo M.[(1)]
*[(1)] Dipartimento di Medicina, Chirurgia e Odontoiatria, Università degli Studi di Milano e Azienda Ospedaliera Anatomia Patologica, Milano; [(2)] Dipartimento di Medicina, Chirurgia e Odontoiatria, Università degli Studi di Milano e Azienda Ospedaliera Medicina III, Milano*

**Background** At the maternal-foetal interface an important requirement is to ensure adequate perfusion and prevent thrombi. Obstetric complications such as IUGR, pre-eclampsia, stillbirth and recurrent abortion are thought to be due to placental vascular under-perfusion and ischemia. However, no real biological evidence is available to sustain this concept and the association of these complications with thrombophilia is frail. Few studies have addressed the role of the anticoagulant pathway at the maternal-foetal interface. Furthermore, since the anticoagulant protein C pathway has anti-inflammatory as well as anticoagulant properties, but is also down-regulated by inflammation, it is interesting to establish if inflammation has a role in thrombotic□ obstetric complications.
**Aims of the study** To establish if the protein C pathway is present and functional in the placenta, and whether it is susceptible to inflammatory mediators. Since NK-mediated immunotolerance is important in pregnancy implantation and maintenance, a further aim is to analyze if the downregulation of the protein C pathway might interfere with NK tolerance.
**Methods** Placentae from normal pregnancies were studied at the three trimesters by immunohistochemistry to detect thrombomodulin (TM) and endothelial protein C receptor (EPCR). Three throphoblast cell lines (JEG-3, BEWO and JAR) were studied as well by flow-cytometry, Western blot, RT-PCR for TM and EPCR expression and for protein C activating capacity.
**Results and Discussion** Preliminary results indicate that EPCR and TM are strongly expressed on the villous syncytiothrophoblast of human placenta through the 3 trimesters, while in maternal decidua the extravillous trophoblast shows a positive immunoreaction for EPCR only. The throphoblast cell lines also express EPCR but not TM. Consequently, protein C activating capacity is absent. If these results will be confirmed with freshly obtained throphoblast cells, they would indicate that EPCR does not sustain anticoagulation at the maternal-foetal implantation site and its role in immunotolerance will be tested.

## P170 ENDOGENOUS THROMBIN POTENTIAL IN PATIENTS WITH THROMBOPHILIA AND HISTORY OF COMPLICATIONS DURING PREGNANCY

Consiglio P., De Michele S., Vitale G., Calabrò G.M., Paternò A., Salvia M., Amato S., Aiosa A., Palma B.
*Patologia Clinica A.R.N.A,S. O. Civico Palermo*

**Introduction** Thrombophilia in pregnant women leads to higher risk for thromboembolic complications, intrauterine growth restriction, miscarriage, foetal death and eclampsia. Investigation of thrombophilia in women with previous thrombosis or complications during pregnancy is a necessary condition to define the possible etiopathogenesis and the following antithrombotic prophylaxis in next pregnancies. ETP, suitable for assessing hypo and hypercoagulable states, can be another diagnostic test to define thrombophilia or the efficiency of the antithrombotic prophylaxis.
**Methods** We investigated 38 patients with thrombophilia who had complications during previous pregnancy. The thrombophilic markers found were: 7 x FV Leiden, 11 x 20210 GA, 16 x presence of ACA, 1 x protein S deficiency, 3 x FV Leiden + 20210 GA. The pregnancy complications were: 5 women with eclampsia, 28 with more miscarriages, 3 with IUGR, 2 with foetal death.
We investigated 36 healthy women as controls. Patients of the study received no antithrombotic therapy.
ETP was measured by a chromogenic test (ETP - Siemens) in platelet poor plasma. The results were compared with Enzyme immunoassay for the quantitative determination of human prothrombin fragment F 1+2 (Enzygnost F1+2 - Siemens).
**Results** Women with thrombophilia and previous complications during pregnancy showed higher ETP [median 117.75% (range 82.7%-157.2%)] than healthy controls [median 108.3% (range 80.9%-127.9%)] with $p<0.0010$.
**Conclusions** ETP is a useful test to define a thrombophilic condition, but its limit is the lack of standardization because of a large inter-individual and inter-laboratory variability that at the moment does not allow defining a normal value of reference.

## P171 SUPPLEMENTAZIONE CON ANTITROMBINA AD ALTE DOSI NELLA PREECLAMPSIA PRECOCE: STUDIO IN DOPPIO CIECO CONTROLLATO CON PLACEBO (ATIII-EPAS)

D'Angelo A.[(1)], Valsecchi L.[(2)], Sampietro F.[(1)]
*[(1)] Servizio di Coagulazione & Unità Ricerca Trombosi, Istituto Scientifico Universitario San Raffaele, Milano; [(2)] Dipartimento di Ostetricia e Ginecologia, Istituto Scientifico Universitario San Raffaele, Milano*

A fine 2004 è stato avviato uno studio in doppio cieco controllato con placebo per valutare efficacia e sicurezza della antitrombina (AT, Kedrion S.p.A, Italia, 3,000 UI/die per 7 giorni) in pazienti con gravidanza singola e preeclampsia

precoce (<30a settimana gestazionale). Per accertare una riduzione relativa del 30% del combinato di mortalità fetale/neonatale e di morbilità grave neonatale (ROP di grado III-IV, enterocolite necrotizzante, leucomalacia ventricolare, RDS severa, broncodisplasia, sepsi neonatale) era previsto l'arruolamento di 240 pazienti in 15 Centri Italiani. Lo studio è stato interrotto dallo sponsor nel 2007 con l'arruolamento di 40 pazienti -20 randomizzate ad antitrombina e 20 a placebo- senza differenze per età (32±5 anni), peso (61±13 kg), giorni di gestazione (191±12), e livelli di antitrombina (86±16%). Le pazienti randomizzate ad antitrombina, trattate con una media di 54±10 UI/kg/die, raggiungevano livelli plasmatici del 211±49% al 4 giorno e del 233±39% al 7 giorno. Il prolungamento medio della gravidanza risultava di 9 giorni in entrambi i bracci. L'endpoint primario si osservava, indipendentemente dal prolungamento della gravidanza, per 9 parti nel braccio antitrombina e 10 parti nel braccio placebo. Peso alla nascita, score Apgar e durata della ospedalizzazione in terapia intensiva neonatale erano anche simili nei due bracci. Il trattamento con antitrombina si associava ad un miglioramento di alcuni indici di laboratorio materni (LDH, uricemia); mentre la causa del parto era di origine materna per 4/5 pazienti trattate per 7 giorni con placebo, era di origine fetale per 6/6 pazienti che avevano completato il trattamento con antirombina (p=0.01).
Anche se suggestivi di un beneficio per le pazienti, questi risultati non permettono di trarre conclusioni definitive circa l'utilizzo di antitrombina nella preeclampsia precoce.

## *PROTOCOLLI DI STUDIO - REGISTRI DI PAZIENTI*

### P172 EVALUATION OF THE PREVALENCE OF SEVERE HYPERHOMOCYSTEINEMIA IN ADULT PATIENTS WITH THROMBOTIC EVENTS, WHO UNDERWENT SCREENING FOR INHERITED OR ACQUIRED THROMBOPHILIA

Lussana F.[(1)], Prisco D.[(1)], Cattaneo M.[(1)]
*[(1)] Dipartimento di Medicina, Chirurgia e Odontoiatria, Università degli Studi di Milano Medicina III, Azienda Ospedaliera San Paolo, Milano; [(2)] Centro Trombosi, Dipartimento di Area Critica Medico-Chirurgica, Università di Firenze, AOU Careggi, Firenze*

**Background** Homocysteine (Hcy) is a sulfhydryl amino acid derived from the metabolic conversion of methionine, which is dependent on B vitamins as cofactors and co-substrates. Severe hyperhomocysteinemia (homocystinuria), due to inherited severe metabolic defects of Hcy metabolism, is a rare disorder, which is usually diagnosed in pediatric life, characterized by mental retardation, ectopia lentis, musculoskeletal abnormalities and high risk of arterial and venous thrombosis. Treatment with high-dose B vitamins improves the clinical manifestations of the disease and decreases the risk of thrombosis. Despite its typical clinical features, the disease may not be recognized, and some patients may reach adulthood undiagnosed.
**Objectives** The primary objective of the proposed study is to gather information on the prevalence of severe, previously undiagnosed hyperhomocysteinemia among patients with thrombotic events (arterial and/or venous) who underwent a screening for thrombophilic states, including measurement of fasting plasma total Hcy (tHcy). Secondary objectives are to gather information on the clinical characteristics of these patients with severe hyperhomocysteinemia.
**Methods** Each participating centre will be invited to complete an online questionnaire on the number of patients for whom a diagnosis of severe hyperhomocysteinemia (fasting tHcy, >100 M) was made, among those who underwent thrombophilia screening for previous thrombotic episodes. For each patient with severe hyperhomocysteinemia, a second, more detailed questionnaire will be completed, detailing personal data, fasting plasma tHcy levels, type of molecular defect, clinical characteristics, co-morbidities, type of thrombotic event(s), presence of additional thrombophilic states, use of estrogens, type and duration of treatment. The gathered information will then be analyzed by descriptive statistics.
**Conclusions** The results of the proposed study will help to increase our knowledge on the natural history and treatment of severe hyperhomocysteinemia, and to better assess the clinical usefulness of tHcy measurement in the evaluation of adult patients with thrombotic events.

### P173 A MODEL FOR THE SURVEILLANCE OF IN-HOSPITAL VENOUS THROMBOEMBOLISM AND IMPLEMENTATION OF THROMBOPHYLAXIS

Faioni E.M.[(1)], Lussana F.[(1)], Bettamio V.[(2)], Moreno M.[(2)], Cattaneo M.[(1)]
*[(1)] Dipartimento di Medicina, Chirurgia e Odontoiatria, Università degli Studi di Milano e Azienda Ospedaliera Medicina III, Milano; [(2)] Azienda Ospedaliera San Paolo, Direzione Sanitaria, Milano*

**Background and Aims** Venous thromboembolism (VTE) is common in hospitalized patients. Though it is well established that prevention of VTE is cost-effective and safe, large surveys showed that as few as 25% of medical and 50% of surgical hospitalized patients are prescribed appropriate thromboprophylaxis. International guidelines promote the use of thromboprophylaxis and recommend active strategies of implementation in hospitals. Neither clear indications regarding the best approach to implementation of thromboprophylaxis in hospitals, nor largely validated algorithms for identifying at risk patients within surgical and medical wards are available.
We designed a strategic plan to promote the evaluation of thrombotic risk in each individual patient admitted to any ward of our hospital, and to make physicians aware of the importance of thromboprophylaxis.
**Methods** An electronic chart of risk factors for VTE was prepared, which also contains fields related to patient age, gender, weight and height, and a connected chart for reporting VTE outcome. Physicians are requested to fill these charts for every hospitalized patient. Two meetings with hospital staff were scheduled to explain the plan. In each ward two medical doctors and a nurse were designated as responsible for the implementation of the plan. In parallel, a schedule of meetings to write guidelines for in-hospital thromboprophylaxis in each area (medical, general surgical, critical area, special areas) was prepared. The thromboprophylaxis plan started in April 2010 on a trial run, and from the first of May 2010 is active in the hospital.

Results will be verified as follows: comparison between number of patients admitted to the wards and number of filled-in risk charts per month; number of VTE events, compared to expected incidence; actual preparation of in-hospital guidelines.
**Results and Discussion** Results from audits at 1, 3 and 6 months from start of VTE prophylaxis plan will be provided.

P174 **ATHEROSCLEROTIC AND THROMBOPHILIC RISK FACTORS IN PATIENTS WITH ISCHEMIC CENTRAL RETINAL VEIN OCCLUSION**

Giambene B.[(1)], Sodi A.[(1)], Marcucci R.[(2)], Sofi F.[(1)], Fedi S.[(2)], Abbate R.[(2)], Menchini U.[(1)], Prisco D.[(2)]
*[(1)] Eye Clinic, University of Florence, Florence; [(2)] Medical and surgical critical area, University of Florence, Florence*

**Purpose** To investigate atherosclerotic and thrombophilic risk factors in patients affected by acute ischemic and non ischemic central retinal vein occlusion (CRVO).
**Methods** One hundred and three patients with acute unilateral CRVO (forty-one ischemic, sixty-two non ischemic) were studied. The prevalence of traditional cardiovascular risk factors was assessed and the plasma levels of a variety of thrombophilic markers were measured. Multivariate logistic regression was performed to determine risk factors for ischemic CRVO.
**Results** Arterial hypertension, hypercholesterolemia, postmethionine hyperhomocysteinemia (HHcy), elevated factor VIII, and reduced folic acid and B6 plasma levels were more prevalent in patients with ischemic CRVO than in those with non ischemic CRVO ($p=0.030$, $p=0.025$, $p=0.011$, $p<0.001$, $p<0.001$, $p=0.044$, respectively). Independent risk factors for ischemic CRVO were arterial hypertension (OR 3.22, 95% CI 1.13-9.21, $p=0.037$), hypercholesterolemia (OR 3.03, 95% CI 1.06-8.65, $p=0.042$), reduced folic acid levels (OR 6.77, 95% CI 1.59-28.79, $p=0.011$) and elevated FVIII levels (OR 5.10, 95% CI 1.31-19.89, $p=0.014$). Postmethionine HHcy was associated with low folic acid levels ($r=-0.413$, $p=0.007$; OR 9.33, 95% CI 2.06-42.18, $p=.005$).
**Conclusion** The results of the present study suggest that some atherosclerotic and thrombophilic risk factors may increase the risk of having an ischemic form of CRVO.

# *ELENCO AUTORI*