

concentrations of thrombin lead to loss of contraction after 1 hour or longer incubation. Similar observations were made after addition of factor Xa ($p < 0.0001$, $n = 18$). Control experiments with flecainide showed a concentration-dependent reduction in contraction frequency ($p < 0.0001$, $n = 18$), figure A and B.

Conclusions: In a 3D-biohybrid system, cardiomyocyte contraction frequency is stimulated both by thrombin and factor Xa, but decreased by flecainide. These data demonstrate a functional link between thrombus-associated molecules and cardiomyocyte contraction, suggesting a potential pro-arrhythmic effect of thrombus formation in the setting of myocardial infarction.

PP6.1-2

Cardiovascular risk factors and the risk of venous thromboembolism

Macarone Palmieri N¹, Tufano A¹, Guida A¹, De Gregorio A¹, Russolillo A¹, Di Minno M¹, Di Capua M¹, Cerbone A¹

¹Regional Reference Centre For Emocoagulopathies, Naples, Italy

Introduction: The association between established cardiovascular risk factors, and the risk of venous thromboembolism (VTE) is not yet understood.

Methods: We screened for hyperlipidaemia, hypertension, smoking, obesity-overweight 565 consecutive patients (256 men and 309 women; mean age 43.59 ± 14.49 yrs) referred to Our centre for a first episode of VTE. 358 DVT of lower extremities, 118 associated with PE, 85 isolated PE, 4 with PE associated with SVT. DVT was documented by ultrasonography and PE was diagnosed using ventilation/perfusion scans and/or pulmonary angiography. As many as 577 age- and sex-matched apparently healthy subjects (237 men and 340 women; mean age 42.29 ± 12.31 yrs), from the same ethnic background, served as controls. Hypertension was found in 146/563 (25.9%) of VTE patients and in 115/573 (20.1%) of controls ($p = 0.020$; OR: 1.39; 95%CI 1.05–1.84; Chi-squared test). Hypercholesterolemia (total cholesterol levels ≥ 190 mg/dl in repeated evaluations) and low HDL-C levels (< 35 mg/dl) were more common in VTE patients than in controls (306/490, 62.4% vs. 227/446, 50.9%; $p < 0.001$; OR: 1.60, 95%CI 1.23–2.08, Chi-squared test) HDL-C levels (47/427, 11.1% versus 18/279, 6.5% $p < 0.045$ OR: 1.8 95% CI 1.02–3.17). The association between obesity-overweight and DTE was statistically significant compared with controls (BMI ≥ 25) (365/519, 70.3% vs. 305/556 54.9%; $p < 0.001$, OR: 1.95; 95% CI 1.51–2.5; Chi-squared test). The prevalence of smoking was 291/563, (51.7%) in cases vs. 240/572 (42%), in controls; $p < 0.001$, OR: 1.48; 95% CI 1.17–1.87.

Conclusion: The association between some established cardiovascular risk factor and VTE implies that adequate treatment/prophylaxis of them should be seriously considered

PP6.1-3

Alpha-2 adrenergic receptor contributes to platelet reactivity in stable coronary artery disease patients on dual antiplatelet therapy

Béres B¹, Tóth-Zsámboki E^{1,2}, Vargová K¹, Préda I^{1,2}, Kiss R^{1,2}

¹Hungarian Academy Of Sciences - Semmelweis University Research Group For Inflammation Biology And Immunogenomics, Budapest, Hungary ²State Health Center - Department of Cardiology

Combined antiplatelet therapy reduces recurrent atherothrombotic events in stable coronary disease patients, however, ex vivo measured high residual platelet reactivity still raises concerns as a condition related to treatment failure. Alpha-2 adrenoceptor enhances platelet reactivity and might contribute to this phenomenon.

Methods: 121 stable angina patients on standard dual antiplatelet therapy (75mg clopidogrel and 100mg acetylsalicylic acid) were recruited. Born aggregometry was performed with adenosine diphosphate (ADP), collagen and epinephrine. To verify platelet adrenergic activity, potentiation by low-dose epinephrine and inhibition by selective alpha-2 receptor blocker atipamezole were determined. To assess the P2Y₁₂-specific residual activity, cangrelor was used. Plasma norepinephrine, soluble CD40-ligand, high-sensitivity-C-reactive protein (hsCRP) – and in 24 subjects platelet P-selectin positivity were measured.

Results: Epinephrine - at very low concentration (10–9g/ml) - significantly potentiates (1.25µM ADP:26.5% vs 43%; 5µM ADP:53% vs 64.5%; collagen:17% vs 42%, $p < 0.001$) while atipamezole inhibits ADP- and collagen-induced platelet aggregations (1.25µM ADP:26.5% vs 23%; 5µM ADP:53% vs 47%; collagen:17% vs 11%, $p < 0.001$). Patients with high adrenergic activity have significantly increased baseline ADP- and collagen-induced platelet aggregation. Based on cangrelor's efficacy, these patients have significantly more residual P2Y₁₂ activity as well. HsCRP and soluble CD40-ligand levels were similar.

Conclusions: Stable coronary heart disease patients with prominent in vitro adrenoceptor activity have significantly increased platelet aggregability and more functional P2Y₁₂ receptor, indicating poor inhibitory response to thienopyridines. Therefore, platelet adrenergic receptor represents a considerable, dynamic factor of high residual platelet reactivity and might contribute to cardiovascular events indicating failure of antiplatelet therapy.

PP6 Thrombotic Disorders and Antithrombotic Therapy

PP6.1 Epidemiology and Diagnosis: Cardiovascular Disease

PP6.1-1

Effect of thrombin and Factor Xa on cardiomyocytes in a three-dimensional cell culture model

Alesci R¹, Sueselbeck T², Liebe V², Kaden J², Bartholomae P³, Thielecke H³, Dempfle C²

¹Hämophiliezentrum Frankfurt, Germany ²Universitätsklinikum Mannheim, Germany ³Fraunhofer Institute for Biomedical Engineering, St. Ingbert, Germany

Background and Objectives: The occurrence of ventricular arrhythmia during acute thrombotic coronary occlusion or reperfusion may be related to direct action of thrombin on cardiomyocytes since concentrations of thrombin are high in thrombus formation. The aim of the present study was to investigate the potential proarrhythmic effects of thrombin and factor Xa on cardiomyocytes in a three-dimensional (3D) cell culture model.

Methods: Cardiomyocyte microspheres (spheroids) were prepared from chicken embryo hearts. The contraction frequency of cells was monitored in a three-dimensional biohybrid measurement system after addition of different concentrations of thrombin or factor Xa.

Results: Thrombin causes a concentration-dependent and time-dependent increase in contraction frequency of cardiomyocyte 3D spheroids ($p < 0.0001$, $n = 18$). High



PP6.1-4

Thrombophilia in young patients with acute myocardial infarction

Macarone Palmieri N¹, Guida A¹, Tufano A¹, Di Capua M¹, De Gregorio A¹, Quintavalle G¹, Cimino E¹, Carbone A¹

¹Regional Reference Centre For Emocoagulopathies, Naples, Italy

Thrombophilia is a prominent risk factor for venous thromboembolism, its role in arterial events is less defined. We screened for MTHFR C677T, Factor V Leiden, Factor II G20210A, antithrombin, protein C and S deficiencies, lupus anticoagulant, anti-cardiolipin antibodies, hyperhomocysteinemia and conventional risk factors (hyperlipidaemia, hypertension, smoking, impaired fasting glucose (IFG), diabetes mellitus, and overweight), 137 consecutive patients (99 m and 38 F; mean age 44.27 ± 10.5 yrs) with a first episode of acute coronary syndrome in young age (≤50 years), and 203 age- and sex-matched controls (131 m and 72 F; mean age 42.68 ± 6.09 yrs). MTHFR homozygosity was found in 22.1% of patients and in 16.4% of controls, Factor V Leiden and Factor II G20210A were found respectively in 7.4% cases vs. 8.5% controls and 8.1% vs. 9.5%; these differences were not significant. There was no difference between patients and controls as to the protein C, S and antithrombin deficiency. We found hypertension in 44.1% of patients and in 19.5% controls (p<0.0001; OR:3.26; 95% CI 2.00–5.3), cigarette smoking (80.9% vs. 41.0%; p<0.0001; OR:6.00, CI 3.65–10.16) and diabetes (9.7% vs. 0.8%; p=0.002; OR:13.8, CI 1.72–111.10). There was no statistical difference in prevalence of hyperlipidemia (46.4% vs. 40.5%), IFG (19.2% vs. 11.6%), overweight (55.6% vs. 53.1%) and hyperhomocysteinemia (38.8% vs. 39.1%). Cardiovascular risk factors, except for hyperlipidemia, increase the risk of coronary artery disease in our population, whereas prothrombin G20210A mutation, FV Leiden, MTHFR C677T mutation, protein C, S and antithrombin deficiencies and hyperhomocysteinemia did not increase the risk.

PP6.1-5

A case of venous thrombosis of the popliteal vein through compression by exostosis in a seventeen-year old male patient

Hecking C¹, Linnemann B¹, Lindhoff-Last E¹

¹Universitätsklinik Für Angiologie, Frankfurt am Main, Germany

We report the case of a seventeen-year old male patient who developed first symptoms of exostoses at his right femur and humerus. His mother and brother also presented with exostoses, whereas his father had a history of recurrent venous thromboses and a heterozygous Factor V-Leiden mutation. The patient presented with pain and swelling in his right calf after two 4-hour trainrides and sitting in a lecture with angled legs for several hours. Deep venous thrombosis of the right popliteal vein, the fibular veins and the posterior tibial veins was diagnosed by contrast-mediated venography and colour-coded duplex ultrasound. (Fig 1 and 2) The ultrasound showed compression of the veins by exostoses of the proximal tibia and fibula in sitting position. The exostoses were examined by X-ray. The patient was tested positive for heterozygous Factor V-Leiden mutation. The patient was anticoagulated with body weight-adjusted LMWH (nadroparin) followed by phenprocoumon (INR 2–3) for six months. The exostoses of the femur and tibia were excised one month before ending the anticoagulation. The ultrasonic examination after excision did not show compression of the veins any longer, but advancing recanalization of the posterior tibial veins and the fibular veins, with complete recanalization of the popliteal vein.

PP6.1-6

Baseline and stress-related levels of coagulation and fibrinolysis factors in patients with anxiety disorders: A case-control study

Harbrecht U¹, Meier C², Liedtke R², Oldenburg J¹, Geiser F²

¹Institute for Experimental Haematology and Transfusions Medicine, University of Bonn, Germany

²Department of Psychosomatic Medicine and Psychotherapy

Objective: Enhancement of procoagulant activity is one of various reaction patterns to acute and chronic mental stress. Increased platelet function, hypercoagulability and hypofibrinolysis may contribute to premature atherosclerosis. The aim of this study was to assess baseline coagulation and fibrinolysis factors and haemostatic stress reactivity in patients with clinically relevant anxiety disorder.

Design and Methods: In a case-control study comprising 31 anxiety patients and 31 healthy controls matched by age and gender the following parameters of haemostasis were investigated at rest and immediately after a ten minute standardized challenge to acute stress (Stroop-test): fibrinogen, FVII, VIII, vWF:Ag, vWF:RCO, vWF:CB, TAT, F1+2, PAP, DD, a2-antiplasmin, t-PA, PAI-1, INR, aPTT, PFA-100.

Results: At baseline, anxiety patients in comparison to healthy controls exhibited shorter in vitro bleeding times, activation of the coagulation system with significantly higher levels of FVII (p=0.05), F1+2 (p=0.05) and PAI-1 (p=0.01). Stress condition elicited significant increases in coagulant activity in both groups (anxiety

patients: vWF, PAP; controls: FVII, vWF, PAP, DD), being more pronounced in controls. Mean heart rate rose in patients (p=0.02) and controls (p=0.004) indicating that stress reaction did occur.

Conclusions: Severe anxiety disorder is associated with a general activation of the haemostatic system, although differences in single parameters are moderate and within the physiological range. Enhanced procoagulant activity may contribute to the higher risk for cardiovascular disorders in anxiety patients.

PP6.1-7

Thrombophilia in ArAbs. in Kuwait

Jadaon M¹, Dashti A¹, Lewis H¹

¹Faculty of Allied Health Sciences - Kuwait University

Objectives: Venous thromboembolic diseases (VTE) occur due to different genetic and acquired abnormalities in the coagulation factors and their regulatory proteins. The prevalences of such abnormalities were found to be different in different ethnic groups. VTE in Kuwait was reported to be as common as in Western countries. This study report the prevalence of different genetic and acquired abnormalities associated with VTE in ArAbs. living in Kuwait.

Design and Methods: 640 VTE cases and 222 healthy controls were tested, all of whom were ArAbs. living in Kuwait. Coagulometric testing was performed for presence of lupus anticoagulants (LA) and activated protein C resistance (APCR), and to determine levels of protein C (PC), proteins (PS) and antithrombin (AT). PCR, RFLP and DNA sequencing were used to detect Factor V Leiden (FVL), prothrombin G20210A mutation and HR2 haplotype.

Results and Conclusions: Abnormalities were found in 275 patients (43%) and 31 controls (14%). In details, abnormalities in patients and controls, respectively, were as follows: LA 4.7% & 1.4%; APCR 16.1% & 1.8%, PC deficiency 12.8% & 4.5%; PS deficiency 18.4% & 7.7%; AT 5.3% & 1.8%; FVL heterozygous 14.1% & 1.8%; FVL homozygous 2% & 0%, prothrombin G20210A heterozygous 6.4% & 1.3%; prothrombin G20210A homozygous 0.5% & 0%. HR2 haplotype heterozygous 15% & 7%; HR2 haplotype homozygous 1.6% & 0%. 84 patients and 7 controls had more than one abnormality. Combination of more than one abnormality had a higher risk of developing VTE than the risk of a single abnormality (6.3-fold vs. 4.2-fold).

PP6.1-8

Evaluation of primary hemostasis in patients undergoing cardiac surgery

Ludwig A¹, Waldow T², Plötze K³, Kuhlisch E³, Gehrich S⁴, Knöfler R¹

¹Dept. of Pediatric Hematology and Oncology, University Hospital Dresden, Germany ²Herzzentrum Dresden, Department of Cardiac Surgery, Germany ³Institute of Medical Statistics, University Hospital Dresden, Germany ⁴Institute of Clinical Chemistry, University Hospital Dresden, Germany

Objective: Aim of study was to investigate the influence of different cardiosurgical procedures on primary hemostasis.

Design and Methods: Prospectively 79 elective patients (age: 71±10 years) without antiplatelet medication or with acetylsalicylic acid monotherapy were enrolled. Three groups were under investigation: coronary artery bypass grafting (CABG; n=29), single valve replacement (SVR; n=25), and double valve replacement or combination of CABG and valve replacement (COMPLEX; n=6+19=25), respectively. Tests were performed pre- and postoperatively within 2h after surgery: hemogram, aggregometry in whole blood (WB) induced by ADP, collagen and ristocetin, PFA-100[®] collagen/ADP-closure time (CT), von Willebrand factor antigen (VWF:Ag), ristocetin cofactor (VWF:RCo) and collagen binding activity (VWF:CB).

Results: Hemoglobin concentration and platelet count were significantly reduced after surgery. Postoperatively leukocyte counts showed a significant increase with the highest values for SVR and COMPLEX compared to CABG. The values of aggregometry parameters were significantly decreased postoperatively with no significant differences among the groups. PFA-100[®] CT were preoperatively prolonged for SVR (208±79 s) and COMPLEX (186±82 s) but not for CABG (96±61 s). Postoperatively, a significant reduction of CT values was observed reaching the normal range in all groups. The VWF:Ag and VWF:RCo were higher after surgery, reaching statistically significant niveau for SVR and COMPLEX.

Conclusions: Cardiosurgical procedures led to a decreased platelet aggregation in WB which can be explained at least in part by the procedure-associated diminished platelet counts. The postoperative normalization of PFA-100[®] CT are most likely due to the increase of VWF:Ag levels as a compensatory mechanism for primary hemostasis.

PP6.1-9

Pituitary function in young ischemic stroke: preliminary study

Macaroni Palmieri N¹, Tufano A¹, Guida A¹, Somma C¹, De Gregorio A¹, Cimino E¹, Quintavalle G¹, Cerbone A¹¹Regional Reference Centre For Emocoagulopathies, Naples, Italy

Both animal and human studies suggest that the GH-IGF axis is involved in the pathogenesis of ischemic stroke. The aim of this study was to assess the presence of endocrine alterations in young patients experiencing ischemic stroke. At this purpose, in 13 patients with a history of ischemic stroke, pituitary function was tested 6–12 months after the thrombotic event. In all patients (5 Males, 8 Females; aged 15–50 yrs; BMI 26.9±3.6 kg/m²), basal endocrine parameters and the GH response to GHRH + arginine test (using BMI-dependent cut offs) were evaluated. Hypopituitarism was found in 38.5% of the patients. The most common pituitary deficits were, in decreasing order: GH deficit in 23.1%, LH/FSH deficit in 15.4%. In contrast, deficit of ACTH, deficit of TSH, and diabetes insipidus were not recorded in any patients. In conclusion, hypopituitarism was found in young patients 6–12 months after an episode of ischemic stroke. Thus, endocrine evaluation and neuroendocrine follow-up of patients experiencing ischemic stroke should be performed on a regular basis, in order to monitoring pituitary function and, eventually, providing appropriate replacement treatment. Whether this finding can influence the clinical outcome of the ischemic disease remain to be clarified.

PP6.1-10

Coagulation factor V g allele, HR2 haplotype, 6533T>C and the risk of myocardial infarction

Kostka H¹, Kuhlisch E², Gehrisch S¹¹Institut Für Klinische Chemie Und Laboratoriumsmedizin, Universitätsklinikum Dresden, Germany²Institut für Medizinische Informatik und Biometrie, Medizinische Fakultät

In a previous study we found associations of the non Leiden g allele [2391A>G; 2663A>G; 2684A>G; 2863A>G] coding for amino acids Ser739, Lys830Arg, His837Arg and Lys897Glu with high FV levels and with a protective effect on deep venous thrombosis (DVT). The HR2 haplotype and 6533T>C (Met2120Thr) have been reported to be associated with reduced FV levels. Because high FV levels have been found to be associated with an increased risk of myocardial infarction (MI), we examined how the presence of the g allele, the HR2 haplotype and 6533T>C affected the risk of MI in men older than 50 years. The above mentioned polymorphisms were examined in 90 men with MI and in 63 healthy men without MI and without DVT. Among the men with MI 30 (33%) were heterozygous and 5 (5.6%) homozygous carriers of the g allele. In the group of the healthy men were 23 (36%) heterozygous and two (3.2%) homozygous carriers of the g allele. 11 (12%) men with MI and 8 (13%) men of the controls were heterozygous carriers of the HR2 haplotype. Three (4.8%) heterozygous, one (1.6%) homozygous carriers of the 6533T>C polymorphism were found in men with MI and three (3.3%) heterozygous carriers in controls. The frequencies of the g allele, the HR2 haplotype and 6533T>C did not differ in the two groups.

Conclusion: The risk of myocardial infarction for men older than 50 years is not associated with the g allele, the HR2 haplotype or the polymorphism 6533T>C in the FV gene.

PP6.1-11

Association between plasma lipoprotein(a) concentration and restenosis after stent implantation in the superficial femoral artery

Gary T¹, Stojakovic I², Froehlich H¹, Scharnagl H², Hafner F¹, Pilger E¹, Brodmann M¹¹Klinische Abteilung Für Angiologie; Medizinische Universität Graz, Austria ²Klinisches Institut für Medizinische Labordiagnostik; Medizinische Universität Graz, Austria

Background: Elevated levels of Lipoprotein(a) [Lp(a)] are associated with atherosclerotic and thrombotic vascular disease. In recent literature plasma Lp(a) is described as an independent predictor of stent restenosis after percutaneous coronary intervention. The aim of our study was to evaluate the association between plasma Lp(a) concentration and in-stent restenosis in peripheral artery occlusive disease (PAOD). We decided to include only patients with stenting of the superficial femoral artery because restenosis after stentimplantation in this area is a clinical problem and the reason therefore unsolved so far.

Methods and Materials: 66 patients (34 male/ 32 female) with a mean age of 63.7 years for male and 76.4 for female patients with stenting of the superficial femoral artery were included in our study. Plasma Lp(a) concentration was measured 3 months after stentimplantation. In-stentrestenosis was assessed with duplex scan of the femoral superficial artery 3, 6 and 12 months after stent implantation. A stenosis was considered as relevant when stenosis grade was > 50%.

Results: 17 of the 66 patients developed restenosis > 50% after stent implantation in the superficial femoral artery, 3 of these 17 patients developed a stent-occlusion.

In these patients the plasma concentration of Lp(a) (median 21 mg/dl) was not statistically significant higher (p 0.533) compared with the patients who developed no restenosis (median 18 mg/dl).

Conclusion: We conclude that patients with higher plasma Lp(a) concentration are not on a higher risk for stent-restenosis in the superficial femoral artery than patients with lower Lp(a) levels.

PP6.1-12

The association of circulating Factor Seven Activating Protease with clinical outcome in patients with atrial fibrillation

Parahuleva M¹, Kanse S², Zheleva K¹, Guenduez D¹, Soydan N¹, Tillmanns H¹, Erdogan A¹¹Department of Internal Medicine, Division of Cardiology and Angiology, University Hospital of Giessen and Marburg, Germany ²Institut für Biochemie, Justus-Liebig Universität, Giessen, Germany

Objectives: The atrial fibrillation (AF) is accompanied by a hypercoagulable state that may contribute to the development of atrial thromboembolism. FSAP regulates hemostasis and may influence the progression of atherothrombotic cardiovascular disease. It is not known if FSAP is related in any way to the clinical outcome in patients with different forms of arrhythmias. The present study was performed to examine the relation between plasma FSAP and hypercoagulable state in patients with AF.

Design and Methods: 80 patients with AF comprised the study group. FSAP concentrations was assessed in these patients and was compared to control healthy subjects with sinus rhythm (SR).

Results: The median FSAP concentration in control subjects (1053 mPEU/ml, range 855–1195 mPEU/ml) were significantly different from those in patients with AF (1731 mPEU/ml, range 1385–2274 mPEU/ml; p < 0.01). The FSAP level was positively correlated with AF duration and the left atrial diameter (P < 0.05), but when compare with baseline values, there were not significant changes in the plasma FSAP requiring cardioversion. The following medications did not influence FSAP concentration and activity: warfarin, β-blockers, or an ACE inhibitor/angiotensin II receptor blocker (P values < 0.05). Furthermore, the influence of omega-3 fatty acid treatment on plasma FSAP in vivo was tested, showing that omega-3 fatty acid was without effect.

Conclusions: Enhanced FSAP concentration might be a novel risk factor for stroke mediating hypercoagulable state in patients with AF. Plasma FSAP was an independent prognostic marker, suggesting its potential role in risk stratification and clinical management of AF.

PP6.1-13

Clinical outcome of patients with acute pulmonary embolism (PE) after one year

Hoffmanns P¹, Ames M¹, Diehm C¹, Lawall H¹¹SRH Klinik Karlsbad-Langensteinbach

Introduction: This retrospective study analyzed the clinical outcome of hospitalized patients with acute PE without hemodynamic instability after one year.

Methods: The data are collected retrospectively from medical reports.

Results: 84 patients with PE were analyzed (male 52,6%; female 47,6%) and follow-up was done after 1 year. In 65% of the medical patients the admitting diagnosis was VTE. 35% of the medical patients were hospitalized due to different medical disorders. In 69% the PE were diagnosed by ct, in 31% by szintigraphy, in 52% an additional echocardiography was done. All patients were in a stable cardiopulmonary situation (Grosser I / II) without hemodynamic instability. All patients were initially treated conservative due to national guidelines with heparins (UFH 38%; LWMH 68%). 53% of the patients got oral anticoagulation for an average time of 9,03 months, 27% were treated with low molecular weight heparins for 11,4 months. 20% of the patients were not treated effectively with an anticoagulant therapy within the first year after the acute PE, mainly surgical patients with complications and neurological patients. 16,7% of the patients died within the first year, 3,6% had a relapse. In 19% of the patients an echocardiography was done after 1 year.

Conclusions: Surprisingly 20% of patients were not treated effectively with anticoagulant therapy within the first year after acute PE. 19% of the patients get investigated after one year to determine right heart strain. It has to be shown why so many patients in ambulant section were not treated effectively.

PP6.1-14

Antithrombotic prophylaxis and therapy. The Hungarian guideline and praxis

Pfliegler G¹¹Medical And Health Science Center, Univ. Debrecen, Hungary

G. Pfliegler on behalf of the Hungarian Society of Thrombosis and Haemostasis 1 Div. Rare Diseases, 2nd Department of Medicine, Institute of Medicine, Medical and Health Science Center, University of Debrecen, Hungary The 4th National Antithrombotic Guideline has been elaborated by experts of the Hungarian Soci-



ety of Thrombosis and Haemostasis in collaboration with non-haemostasis specialists to build in the views of the „consumers.“ The current Hungarian everyday practice, methods to improve implementation of the guideline will be overviewed, finally attention will be draught to the fact that despite highest quality internationally accepted standards, several unanswered questions remained, e.g.: Case 1: anticoagulation. Female 37 y, 170 kg bw, pregnant. Despite combined thrombophilia and previous multiple triggers (e.g. oral anticoncept) first thrombosis occurred only during pregnancy. Despite therapeutic anticoagulation, early recanalisation leg ulcer developed. Case 2: INr. Male, 87 y, combined mechanical heart valve. Despite INR between 1.0 and 8.0 in fifteen years (!) unexpectedly and „illogically“ no valve-thrombosis at time of autopsy. Case 3: thrombophilia. Male, 20 y, deep vein thrombosis. Thrombophilic screening: severe PS deficiency (PS-activity: repeatedly between 10–20 per cent, PS-antigen: from unmeasurable to 40 per cent). Father, 51 y: PS-activity: 35–40 per cent, PS-antigen: 40 per cent, but no thromboembolism in history. A genetic analysis failed to detect any small deletion, insertion or point mutation.

PP6.1-15

Fibrinogen influences the progression of peripheral atherosclerosis in type 2 diabetic patients

Bosevski M¹, Georgievska-Ismail L¹, Tosev S¹, Borozanov V¹
¹University Cardiology Clinic, Skopje, Macedonia

The purpose of the study was to determinate the influence of fibrinogen (F) on the progression of peripheral atherosclerosis in type 2 diabetic pts.

Design and methods: 62 pts with type 2 diabetes and diagnosed coronary artery disease were enrolled in a cohort prospective study. We measured in them, at all, progression of peripheral atherosclerosis, defined as change of ankle-brachial index (ABI) after 36 months. Multiple linear regression analysis was built to define continuous variables with predictive value for F and ABI.

Results: Study population was on age 60.28 ± 27 years (231 men and 109 women) and mean diabetes duration of 8.58 ± 6.17 years. Mean plasma fibrinogen level was 4.12 ± 0.85 g/L. Multivariate analysis showed F value has been determinate with non HDL - cholesterol (\hat{a} = 1.093, p = 0.027). Linear regression analysis defined F as predictor for minimal value of ABI, found at the end of investigation (\hat{a} = 0.469, p = 0.007).

Conclusion: Our data indicate that plasma determination of fibrinogen have clinical utility in defining the process of progression of peripheral atherosclerosis in type 2 diabetic population.

PP6.1-16

Risk stratification of recurrent venous thromboembolism

Zotz R¹, Segendorf H², Claus K², Funck S², Karrenberg M², van Dornick M², Vinke C², Gerhardt A³

¹Praxis Für Hämostaseologie und Transfusionsmedizin, Düsseldorf, Germany ²Institut für Hämostaseologie und Transfusionsmedizin, Universitätsklinikum Düsseldorf, Germany ³Blutgerinnung Ulm, Germany

Background: A better risk stratification for recurrent venous thromboembolism (VTE) in patients with a first episode of idiopathic venous thromboembolism (VTE) is urgently needed.

Methods: Retrospective study covering more than 20 years after a first venous thromboembolic event in a group of 1,440 patients with VTE.

Results: In the subgroup of patients (n=515) with a first spontaneous VTE, the yearly incidence of a recurrent spontaneous VTE was 8% for the time period 0–2 years and 4–6% in the following 8 years, after a first VTE, triggered by a transient risk factor (oral contraceptives, surgery or immobilization, pregnancy), the yearly incidence of a recurrent spontaneous VTE was 2% (first 2 years) and 1.3–3% (following 8 years). The hazard ratio for recurrent spontaneous VTE in patients with a first spontaneous VTE for specific predictors were as follows: prothrombin mutation heterozygous 1.2 (95% CI 0.9–1.7), FVL heterozygous 1.3 (95% CI 0.92–1.8), male sex 1.9 (95% CI 1.4–2.7), D-Dimer 2.3 (0.9–6.4), protein C (<60% activity) 2.6 (95% CI 1.2–5.7), FVL homozygous 3.0 (1.3–7.7), AT (<60% activity) 3.0 (95% CI 0.96–9.6).

In Conclusion, in patients with a first spontaneous VTE the yearly recurrence rate of 5% is more than doubled in the presence of relevant thrombophilic risk factors supporting the need of long-term oral anticoagulant therapy after a first idiopathic VTE. In contrast to current ACCP recommendations, thrombophilic risk factors are of clinical relevance.

PP6.2 Epidemiology and Diagnosis: Venous Thromboembolism

PP6.2-1

Validation of a predictive model for indentifying an increased risk for thromboembolism in children with acute lymphoblastic leukemia

Nowak-Göttl U¹, Flege S¹, Heller C², Schobess R³, Bidlingmaier C⁴, Mauz-Köhrholz C⁵, Frühwald M¹

¹Pädiatrische Hämatologie-Onkologie, Münster, Germany ²Pädiatrische Hämatologie-Onkologie, Frankfurt, Germany ³Universitätskinderklinik Halle, Saale, Germany ⁴Universitätskinderklinik München, Germany ⁵Universitätskinderklinik Düsseldorf, Germany

The aim of the study was to develop a simple model for predicting acute lymphoblastic leukemia (ALL)-chemotherapy-associated venous thromboembolism (VTE) using baseline clinical and laboratory variables. For development of the risk model the predictive variables were scored as follows: treatment with E. coli asparaginase (ASP: 5000–10000/m²) in combination with prednisone or dexamethasone, presence of central venous lines, thrombophilic genetic abnormalities, e.g. positive family history for VTE or identification of a single thrombophilic trait (1 point each), or carrier status of combined thrombophilic traits (2 points). A definition of VTE risk by score was low (1–2) and high (> 3). The risk score was than retrospectively evaluated in a derivation cohort (n=552) and prospectively validated in an independent cohort of 136 newly recruited patients enrolled into the German database. Patients ascertained in the derivation and validation cohort were comparable ($p=0.97$). The cumulative VTE rates at 3.5 months in the validation cohorts were 3.6% (95% CI 1%-9%) in the low-risk group (4 of 112), and 47% (95% CI 23%-72%) in the high-risk category (8 of 17). In multivariate analysis [Cox regression] the high risk group was significantly associated with VTE when compared to the low risk group even after adjusting for age at ALL-onset, duration of ASP administration, steroid administered.

PP6.2-2

Fibrinogen alpha and gamma genes and Factor VLeiden in children with thromboembolism: Results from two family-based association studies

Nowak-Göttl U¹, Weiler H², Thedieck S¹, Seehafer T³, Stoll M³

¹Pädiatrische Hämatologie-Onkologie, Münster, Germany ²Blood transfusion Center Milwaukee, USA, ³Leibniz Institut für Arterioskleroseforschung, Univ. Münster, Germany

Background: A previous large case-control study showed that genetic variation in the fibrinogen gamma gene (FGG) increased the risk for venous thrombosis (VT) in adults.

Methods: We investigated the association of haplotypes comprising the fibrinogen alpha (FGA) and FGG genes, carriership of the Factor VLeiden-mutation and risk for VT in two family-based study samples. (VT: n=244) and stroke (TS: n=268). Association was assessed using the Transmission Disequilibrium Test (TDT) corrected for multiple testing.

Results: Association analysis revealed that the FGA-H1 haplotype, and the FGG-H2 and -H3 haplotypes, were significantly associated with VT (FGA-H1, $P=0.05$; FGG: H2, $P=0.032$; H3, $P=0.0216$). In an independent study sample, FGA-H1 ($P=0.0085$) and FGG-H2 ($P=0.05$) were significantly associated with TS. When stratifying for FVLeiden carriership, the association between FGA and FGG and VT was more pronounced in FVLeiden-negative families. Homozygous carriership of the FGG-H2 risk haplotype resulted in the lowest fibrinogen α content (α levels: 22.7±13.7 vs. 26.8±12.0, $P=0.013$; α : 7.63±3.05 vs. 9.46±3.17, $P=2.3 \times 10^{-5}$), with increasing concentrations of fibrinogen α in heterozygote H2 carriers. Compound heterozygote carriers of one FGG-H2 risk and one FGG-H3 protective haplotype, showed significant increase in fibrinogen α ($P=0.000032$), while fibrinogen levels remained unchanged. In contrast, homozygote carriers of the protective FGG-H3 haplotype showed the highest concentration of fibrinogen α content (α : 9.21±3.09, $P=0.0031$) with decreased total fibrinogen.

Conclusion: Our data suggest that the genetic architecture of VT is complex and involves subtle influences through susceptibility and protective haplotypes in FGG with a genetic interaction with the FVLeiden-mutation.

PP6.2-3

Blood count score enables to predict thromboembolic events in cancer patients – results from the Vienna Cancer and Thrombosis Study (CATS)

Chiriac A¹, Vormittag R¹, Ay C¹, Schwarzwinger I¹, Steger G¹, Jäger U¹, Zielinski C¹, Pabinger I¹

¹Medizinische Universität Wien, Austria

Introduction: Venous thromboembolic events (VTE) are a frequent complication in cancer patients. In order to identify reliable markers of risk prediction, we assessed a Blood Count Score (BCS) as predictor for cancer-associated VTE.

Methods: The Cancer and Thrombosis Study (CATS) is an ongoing prospective observational study in patients with newly diagnosed cancer or disease progression. Occurrence of VTE and information on the patients' anti-cancer-treatment during

follow-up are recorded. Observation ends with occurrence of VTE, death or after 2 years. At enrolment a blood sample was taken. A VTE risk score was calculated based on the blood cell count. A hemoglobin level below 100 g/L, platelet count above $350 \times 10^9/L$ and leucocyte count above $11 \times 10^9/L$ increased the score by 1, respectively.

Results: Data on 635 patients with solid tumours were available. Patients were followed for a median observation time of 366 days, during which 44 objectively confirmed VTEs occurred. 477 patients had a score of 0, 120 of 1, 32 of 2 and 6 patients had a score of 3. Compared to patients with a score of 0 those with a score of 1 had a hazard ratio of 1.6 [0.8 – 3.4, $p=0.19$] and those with a score of 2/3 had a hazard ratio of 3.8 [1.6 – 9.2, $p=0.003$], respectively, adjusted for age and sex.

Conclusions: The BCS offers a simple method to determine which cancer patients are at highest risk of suffering a VTE and might be used for risk stratification in future interventional trials.

PP6.2-4

Inferior vena cava thrombosis and its relationship to chronic myeloproliferative disorders, the JAK2V617F mutation and prv-1 mRNA expression

Linnemann B¹, Kraft C¹, Roskos M², Zgouras D¹, Lindhoff-Last E¹

¹J.W.Goethe University Hospital, Division Of Vascular Medicine, Department Of Internal Medicine, Frankfurt/Main, Germany, ²Center of Laboratory Medicine, Jena, Germany

Objectives: Splanchnic vein thrombosis (SVT) is a typical manifestation of polycythaemia vera (PV) or essential thrombocytosis (ET). The recently discovered JAK2V617F somatic mutation and an increased expression of granulocyte PRV-1 mRNA are closely related to chronic myeloproliferative disorders (CMD). We investigated if thrombosis involving the inferior vena cava (IVC) is related to the JAK2V617F mutation, an increased expression of PRV-1 mRNA or the presence of cmD.

Design and Methods: Blood samples were obtained from 40 IVC thrombosis patients seen in our University Hospital's outpatient department. Fifty-three patients with isolated lower extremity DVT (LE-DVT) and 15 SVT patients served as controls. The presence of JAK2V617F or PRV-1 mRNA was assessed by real-time polymerase chain reaction (RT-PCR).

Results: The JAK2V617F allele was not detected in any of the IVC thrombosis patients, and no patient presented with an increased prv-1 mRNA expression. However, four patients (10%) had borderline levels of prv-1 mRNA (i.e., 0.7–1.5%). These patients did not suffer from known cmD. Even after a median observation period of 25 months (range 20–27) we did not observe any increase of white or red blood cell or platelet count in these four patients. In contrast, the JAK2V617F allele was detected in 3 patients with SVT and known cmD (PV n=2, ET n=1) as well as in one LE-DVT patient.

Conclusion: According to our data, there is no evidence that thrombosis involving the inferior vena cava is related to the JAK2V617F mutation, an elevated prv-1 mRNA expression or the presence of myeloproliferative disorders.

PP6.2-5

Cytomegalovirus infection is associated with venous thromboembolism of immunocompetent adults – a case-control study

Schimanski S¹, Linnemann B², Luxembourg B², Rochon J³, Seifried E⁴, Jilg W¹, Lindhoff-Last E², Schambeck C⁵

¹Institute of Medical Microbiology and Hygiene, Division of Virology and Infectious Immunology, University of Regensburg, Regensburg, Germany, ²Department of Internal Medicine, Division of Vascular Medicine, Johann Wolfgang Goethe University Hospital, Frankfurt/Main, Germany, ³Center for Clinical Studies, University of Regensburg, Regensburg, Germany, ⁴Institute of Transfusion Medicine and Immunohaematology, German Red Cross Baden-Württemberg-Hessen/Johann Wolfgang Goethe-University Frankfurt, Frankfurt/Main, Germany, ⁵Hämostasikum, München, Germany

Objectives: Cytomegalovirus (CMV) is known to contribute to the development of venous thromboembolism (VTE) in immunocompromised patients while literature data on the role in immunocompetent individuals is limited to case descriptions. We initiated this study to investigate the role of cmV infection regarding the occurrence of VTE in a large cohort of immunocompetent patients.

Design and Methods: In a case-control study cmV-IgG and cmV-IgM antibody titres were determined in blood samples from 187 VTE patients and 187 age- and sex-matched blood donors without a history of VTE. cmV-IgG avidity was measured in cmV-IgM positive samples.

Results: cmV-IgG antibodies were found more frequently in VTE patients compared to controls (59.9% vs. 43.3%; OR 1.95 [95%-CI 1.30–2.95]; $p=0.002$). cmV-IgM antibodies could be detected more often in patients with spontaneous VTE compared to controls (7.4% vs. 1.1%; OR 7.36 [95%-CI 1.31–41.3]; $p=0.024$). Mean cmV antibody titres were significantly higher in the case group (109.3 vs. 1.8 AU/ml for IgG [$p<0.001$]; 0.22 vs. 0.17 index for IgM [$p=0.008$]). cmV-IgG avidity was high in 8/9 cmV-IgM positive samples from the patient group (one sample was determined as "greyzone").

Conclusions: Our data indicate that cmV might be a relevant risk factor for the development of VTE in immunocompetent individuals. The overall low rate of IgM antibodies in the patient group and the results of avidity testing favour recurrent and not primary cmV infection to be relevant for the development of venous thrombosis in this cohort.

PP6.2-6

The role of plasminogen activator inhibitor-1 Activity and Factor XII activity in venous thromboembolism

Bukreeva L¹, Kiesewetter H¹, Salama A¹, Hoppe B¹, Shilova N²

¹Institute Of Transfusion Medicine, Campus Virchow-Klinikum, Charite Berlin, Germany

²Siberian State Medical University, Tomsk, Russia

Objectives: Venous thromboembolism (VTE), including deep venous thrombosis (DVT) and pulmonary embolism (PE), occurs secondary to a number of hereditary and acquired disorders of hemostasis. Widespread screening of patients with venous thromboembolism (VTE) for thrombophilic risk factors has become common clinical practice. Because of the increasing number of risk factors, assessing the risk of recurrence in an individual patient is intricate; therefore, a laboratory method that measures multifactorial thrombophilia is required.

Design and Methods: In 127 patients with idiopathic, recurrent deep venous thrombosis (>2 incidents) the activity of antithrombin, protein C, S, APTT, D-Dimer, prothrombin time and INR were investigated. Fibrinogen, factor VIII, factor XI, factor XII, PAI-1 activity were determined. Patients with idiopathic DVT, after elimination of most important thromboembolism risk factors, were qualified for the study. Results were compared with a group of not recurrent DVT (485 patients).

Results: Analysis of fibrinolysis system demonstrated significant higher PAI-1 activity ($p=0.021$) than patients with not recurrent DVT. The patients demonstrated factor XII activity decrease ($p=0.003$) than patients with not recurrent DVT.

Conclusions: Reduced factor XII activity and higher PAI-1 activity were found in patients with recurrent DVT. These factors may be associated with the risk of recurrent venous thromboembolism (VTE).

PP6.2-7

Homocysteine remains a risk factor in patients with peripheral arterial disease

Hoellerl F¹, Hoebaus C¹, Plank C², Koppensteiner R¹, Scherthaner G¹

¹Medical University Of Vienna, Internal Medicine II, Angiology, Vienna, Austria ²Medical University Of Vienna, Radiology, Vienna, Austria

Objectives: Homocysteine (HC) $>12\mu\text{mol/L}/>15\mu\text{mol/L}$ in women/men is a cardiovascular risk factor (RF). Epidemiology described an odds ratio (OR) of 7 for hyperhomocysteinemia (HHC) as RF for peripheral arterial disease (PAD). Since recent studies, which lowered HC with a B-vitamin-combination, found an augmentation of coronary events, it is questionable whether in the era of new therapeutic strategies (ACE-I/ARBs/statins) HHC in PAD remains a RF deserving to be treated.

Design and Methods: 2002–2003 521 consecutive patients of Angiology were screened for HHC. After 5½ years of observation death, cardiovascular death and events were noted.

Results: 252 of 521 patients had elevated HC (women: $15.3\pm 8.4\mu\text{mol/L}$; men: $15.1\pm 5.6\mu\text{mol/L}$); 39% of men but 63% of women had HHC. During the observation period 107 patients (20.5%) died; 20.3% of men and 20.9% of women. In women, mortality was elevated by HHC from 18.2% to 22.5% ($p=ns$), in men from 12.0% to 33.3% ($p<0.001$). Per elevation of HC by $5\mu\text{mol/L}$, the risk for death in women was augmented by 12%, in contrast in men by 44%.

Conclusion: Under modern vascular-protective therapies in PAD patients, HHC remains a substantial RF in men, whereas HC is more frequent in women due to lower cut-offs. It has to be questioned whether the lower cut-offs in women which are derived from the research of recurrent thrombosis are justified in the arterial setting and how one can treat HHC in men (which have an OR of 2.8 for death) without doing harm.

PP6.2-8

Right-to-left shunt, atrial septal aneurysm and thrombophilia in patients with cryptogenic stroke or TIA versus those with venous thromboembolism

Macarone Palmieri N¹, Tufano A¹, Guida A¹, De Gregorio A¹, Di Capua M¹, Di Minno M¹, Cerbone A¹, Coppola A¹

¹Regional Reference Centre For Emocoagulopathies, Naples, Italy

Whether intracardiac right-to-left shunt (RLS) is an independent risk factor for cerebrovascular accidents is dispute. In patients with RLS, venous thromboembolism (VTE) may represent a predisposition to stroke/transient ischemic attack (TIA). Whether thrombophilia is associated with RLS is unclear. We compared prevalences of intra- and extracardiac RLS and of atrial septal aneurysm (ASA) between 29 selected nondiabetic patients with cryptogenic stroke ($n=17$)



or TIA (n=12) and 19 selected patients with VTE, but without history of stroke/TIA, autoimmune systemic disease and migraine. We performed contrast (agitated saline) transthoracic and transesophageal echocardiography, ultrasound evaluation of carotid arteries, thrombophilia (lupus anticoagulant, V Leiden factor, protein S, protein C and antithrombin deficiency, hyperhomocysteine, G20210A Factor II homozygosis/heterozygosis, use of estrogens). RLS and ASA were also evaluated in 30 healthy volunteers. Stroke/TIA patients were younger than VTE patients ($p < 0.05$), whereas prevalence of male gender, proportion of hypertension, hypercholesterolemia and smoking did not differ (all $p > 0.1$). Thrombophilia was similar in both groups (43% in stroke/TIA, 47% in VTE, $p > 0.1$). Intracardiac RLS (28%) and ASA (21%) were detected in stroke/TIA patients but not in VTE patients (both $p < 0.05$); however, prevalences of RLS and ASA did not differ between stroke/TIA and controls (20% intracardiac RLS, 7% ASA, respectively, both $p = \text{NS}$). Within patients, thrombophilia was not associated with intracardiac RLS, but tended to be associated with ASA (83% vs. 40%, $p = 0.08$). Intracardiac RLS may have a role in the multi-factorial pathogenesis of stroke/TIA of embolic origin. ASA seems an independent risk factor for stroke/TIA with possible interaction with thrombophilia.

PP6.2-9

Antithrombin Cambridge II (A384S): Frequency in Austrian patients

Höller B¹, Pauer-Metzker R¹, Dossenbach-Glaninger A¹, Hopmeier P¹
¹Krankenanstalt Rudolfstiftung, Zentrallabor, Vienna, Austria

Well over 120 mutations causing antithrombin deficiency have been identified. One mutation - Antithrombin Cambridge II (A384S) - is associated with a reduced heparin cofactor activity, and the variant is regarded a mild risk factor for thrombotic disease. The mutation was found with a relatively high frequency in the British population (1.45%)¹ and in Spanish patients (1.7%)² with deep venous thrombosis (DVT) or pulmonary embolism (PE). In French patients, a lower frequency was found (0.4%)³. All reported patients were heterozygotes. Aim of our study was to establish the frequency of Antithrombin Cambridge II in Austrian patients with DVT or PE. Patients: 1010 consecutive hospitalized patients with at least 1 established episode of DVT or PE. Test Method: Real time PCR.

Results: Antithrombin Cambridge II was found in only 1 patient (0.1%), a female who had developed DVT. She also tested positive for FV Leiden in the heterozygous state.

Conclusion: Antithrombin Cambridge II is rare in Austrian patients with DVT. No patient with PE and Antithrombin Cambridge II was detected. The variant seems to occur in Austria at a lower frequency than in several Western European countries. References: 1 Tait at al Br J Haematology 1994 2. Corral at al Blood May 2007 3. Picard at al Blood October 2007

PP6.2-10

Thrombotic events in children with malignancy

Serban M¹, Mihailov M, Arghirescu S, Stana L, Doros G, Badeti R, Zoica B
¹Children's Hospital „Louis Turcanu“, Timisoara, Romania

Introduction: Haemostatic disturbances and cancer are intimately interconnected in a bidirectional manner. Despite of protective antithrombotic factors and mechanisms, thrombotic events are steadily increasing in paediatric cancer patients. Objective of the study was to evaluate frequency, type and site of thrombosis in cancer patients ≤ 18 years vs. > 18 years, underlying disease and its impact on evolution.

Patients and methods: It is a descriptive and analytic retrospective study, conducted on a group of 603 consecutive patients, 507 ≤ 18 years and 96 > 18 years; 205 had leukemia, 333 solid tumors and 65 hematopoietic stem cell transplantation.

Results: Based on clinical and exploratory data (color Doppler ultrasound, conventional and MRI angiography), thrombosis was identified in 40 cases (6.63% of patients), with a significantly lower proportion in patients ≤ 18 years (4.53% vs. 16.66%); most prone to develop thrombotic events have been those with leukemia (9.75%) compared to those with solid tumors (5.4%) and transplanted patients (3.07%). Clinical presentation was dominated by superficial (47.5%), followed by deep venous thrombosis (40%) - upper (27.5%) and lower (12.5%) limb; in 10% of cases, visceral (mesenteric, renal and duodenal) thrombosis was identified; arterial ischemic thrombosis was recorded in only one patient. In 75% of cases we correlated the complication with central and long-lasting peripheral venous catheter, or medication (Asparaginase, Corticotherapy, etc). Thrombotic events were cause of death in 3 cases.

Conclusions: The identification of thrombosis in paediatric malignancy is steadily increasing. It urges us to improve our management in diagnosis, prophylaxis and treatment.

PP6.2-11

Frequency of established risk factors for deep-venous-thrombosis in a collective of Austrian patients

Pauer-Metzker R¹, Höller B¹, Hopmeier P¹, Dossenbach-Glaninger A¹, Doleschel W¹

¹Department of Laboratory Medicine / Krankenanstalt Rudolfstiftung, Vienna, Austria

Aim of our study was to determine to what extent established risk factors for deep vein thrombosis were associated with the occurrence of venous thrombotic disease. 209 consecutive hospitalized patients with spontaneous deep-venous-thrombosis (proved by ultrasound or phlebographically) were included in the study.

Results: Risk-factor / No. of patients / %

Mutation in Prothrombingene (het&hom) 20.210G->A / 19 / 9,1%

Factor-V-Leiden-mutation (het&hom) 1.691G->A / 52 / 24,9%

Antithrombin-deficiency (activity) (<70%) / 2 / 1,0%

Protein C-deficiency (activity) (<65%) / 6 / 2,9%

Protein S-deficiency (free antigen) (<60%) / 2 / 1,0%

Lupus-anticoagulans / 13 / 6,2%

Increased homocysteine (>20umol/l) / 15 / 7,2%

Increased F VIII-activity (>200%) / 12 / 5,7%

Increased F XI-activity (>170%) / 4 / 1,9%

Patients with identified risk factor* / 125 / 59,8%

Patients with no identified risk factor / 84 / 40,2%

*19 of the patients were positive for 2 or more risk factors.

Conclusion: In accordance with published papers, our results show that spontaneous deep vein thrombosis is associated with at least one established risk factor in nearly 60% of cases. However, in about 40% of patients the occurrence of disease cannot be explained by our screening panel.

PP6.2-12

FXII T46T genotype is not associated with a higher risk of cerebral venous thrombosis

Dick A¹, Kutsch M¹, Giebl A¹, Kauke T¹, Lison S¹, Spannagl M¹

¹Klinikum der Universität, Klinik für Anaesthesiologie, Abteilung für Transfusionsmedizin, München, Germany

Objectives: The importance of the Factor XII C46T gene polymorphism as a risk factor for venous thromboembolism is still under discussion. Recently an association between the TT genotype of FXII C46T gene polymorphism and the risk of cerebral venous thrombosis CVT was published by Reuner et al. (Neurology, 2008). Therefore we were interested in the prevalence of the TT genotype in our patients with CVT.

Design and Methods: 84 age and sex matched blood donors (71 females, 13 males) serving as healthy controls and 71 patients (60 females, 11 males) with CVT were included in our study. We focused on the FXII T46T, the FVL and Prothrombin 20210 genotypes, lupus anticoagulants and coagulation inhibitor deficiencies. Furthermore we documented the intake of oral contraceptives OCs, pregnancy and puerperium during manifestation of CVT.

Results: 3/71 patients revealed FXII T46T genotype (1 with heterozygous FVL) and 2/84 controls. 15/71 (21%) of patients with CVT showed FVL, 11/71 (15%) Prothrombin 20210 mutation, 3/71 lupus anticoagulants and 3/71 proteins deficiency. The well known risk factors intake of OCs (32/60) and puerperium (6/60) were often found in our 60 females with CVT. In healthy controls the common prevalence of FVL and prothrombin 20210 mutation was detected. About 30% of the female blood donors used OCs.

Conclusion: According to our data the prevalence of the FXII T46T genotype among patients with CVT is not increased while established thromboembolic risk factors were more often detected compared to healthy controls.

PP6.2-13

Thrombin generation in the course of bone marrow transplantation

Kentouche K¹, Voigt A¹, Fuchs D¹, Glaser D¹, Beck J¹

¹Klinik Für Kinder- und Jugendmedizin, Friedrich Schiller Universität Jena, Germany

Transplantation of haematopoietic stem cells (HSCT) is often complicated by haemostatic complications. Patients are prone to severe bleeding during thrombocytopenia and alteration of the mucous membranes for radiation, cytostatic therapy and infections. In contrast patients may exhibit thrombotic complications like veno occlusive disease (VOD) or transplantation associated thrombotic microangiopathy. We were interested in whether the thrombin generation assay may serve as diagnostic tool to predict haemorrhagic or thrombotic complications in the setting of HSCT. 14 consecutive pediatric patients to receive an allogeneic HSCT (MDS: 5; ALL: 4; AML: 1; Various: 4) were analysed before HSCT and weekly until discharge. VOD was diagnosed in two patients, one developed haemorrhagic cystitis (HC), one had gastrointestinal bleeding.

Results: Thrombin generation was measured using a commercial assay (Technoclone TGA) expressing the results in peak - thrombin (PT) and area under the curve (AUC). The assay comprises two start reactants (RCL - RCH). Before BMT PT was within the normal range (PT- RCL: 157,1 nm - normal 125 - 225, PT-RCH: 215,9nm - normal 175 - 450), whereas AUC was elevated (AUC-RCL: 3863,6 - normal: 2200 - 3100; AUC-RCH: 4194,4 - normal: 2200 - 3600). All parameters decrease until week 3 after HSCT to increase then further on above the pre-HSCT values. VOD patients had lower PT and AUC values. 5 patients received defibrotide-treatment. Although not significant PT and AUC were lower in these patients. **Conclusion:** TGA may be helpful to assess the risk of coagulation abnormalities in the course of HSCT.

PP6.2-14

D-dimer in the diagnosis of proximal and distal venous thrombosis of the lower limbs

Luxembourg B¹, Schwonberg J¹, Hecking C¹, Schindewolf M¹, Zgouras D¹, Lehmeier S¹, Lindhoff-Last E¹

¹Schwerpunkt Angiologie und Hämostaseologie, J.W. Goethe-Universität Frankfurt, Germany

Introduction: The diagnostic value of D-dimer (DD) in the exclusion of deep venous thrombosis (DVT) is well established, but is less well known in distal (infrapopliteal) and superficial venous thrombosis (VT). We evaluated the performance of different DD assays in the diagnosis of symptomatic proximal and distal VT of the legs.

Methods: 243 outpatients with symptoms suspicious of VT were enrolled in the study. All patients received compression ultrasonography (CUS) of the whole symptomatic leg(s). Five different DD assays were performed: Vidas-DD (bioMerieux, n=242), Liatest-DD (Stago, n=243), HemosIL-DD (IL, n=215), HemosIL-DDHS (IL, n=212), Innovance DD (Siemens, n=206). All patients with a negative CUS were followed up for 3 months for thromboembolic complications.

Results: 38 proximal DVT, 17 distal DVT, 14 muscle vein thromboses (MVT), and 20 superficial thrombophlebitides (ST) were diagnosed by CUS. None of the 147 patients with normal CUS developed thromboembolic complications in the following 3 months. Six patients who were lost from follow-up and one patient who developed VT of the contralateral, former asymptomatic leg, were excluded for this analysis. The sensitivity for a proximal DVT, distal DVT, MVT and ST was: Vidas-DD 100 %, 88 %, 79 %, 75 %, Liatest-DD 97 %, 88 %, 79 %, 70 %, HemosIL-DD 97 %, 82 %, 70 %, 72 %, HemosIL-DDHS 97 %, 88 %, 100 %, 72 %, Innovance-DD 97 %, 88 % (MVT and ST not analysed). The overall specificity for VT was 49 % (Vidas-DD), 55 % (Liatest-DD), 52 % (HemosIL-DD), 54 % (HemosIL-DDHS), 53 % (Innovance-DD).

Conclusion: The sensitivity of different DD assays varies between 97-100 % for proximal DVT, 82-88 % for distal DVT, 70-100 % for muscle VT, and 70-75 % for superficial thrombophlebitis.

PP6.2-15

Patients with venous thrombosis and pulmonary embolism have different risk factors

Krebs H¹, Meixner T¹, Dick A¹, Spannagl M¹

¹University Hospital Munich, Department of Transfusion and Hemostasis, Munich, Germany

Objectives: Venous thromboembolism (VTE) including venous thrombosis and pulmonary embolism is a common disease with potentially severe complications. Former studies have shown that there might be a difference in the prevalence of hereditary risk factors between patients with predominant venous thrombosis and predominant pulmonary embolism. Our investigation aimed to identify differences between these two groups regarding acquired and hereditary risk factors for VTE.

Design and Methods: We analyzed retrospectively 543 consecutive patients (women: n = 336 / age 38.9 +/- 14.8; men: n = 207 / age 48.6 +/- 15.1) of our outpatient clinic having experienced at least one VTE. Acquired risk factors and highly prevalent hereditary risk factors (factor-V-Leiden and prothrombin G20210A mutation) of thrombophilia were analyzed statistically.

Results: The prevalence of acquired risk factors was not different between patients with venous thrombosis (with or without additional pulmonary embolism, group one, n = 454) and patients with isolated pulmonary embolism (group two, n = 68). However the groups differed significantly (p < 0.036, Fisher's exact test) regarding the prevalence of factor-V-Leiden and prothrombin mutation (group one 33.7 %, group two 20.6 %).

Conclusions: We could harden hints that hereditary risk factors for VTE are found less often in patients with predominant pulmonary embolism. Therefore venous thrombosis and pulmonary embolism might be two different entities though sharing several hereditary and acquired risk factors.

PP6.3 Thrombophilia and Familial Thrombosis

PP6.3-1

Usefulness of Innovin dilute prothrombin time for the detection of lupus anticoagulant

Loreth R¹, Schreyer B¹, Klose G¹, Albert F¹

¹Westfal-Klinikum GmbH Medizinische Klinik III, Kaiserslautern, Germany

Objectives: Lupus anticoagulants (LA) are antibodies which inhibit in vitro phospholipid-dependent tests of coagulation. No single screening test can detect all LA-positive patients, so the SCC Subcommittee for the Standardization of LA recommends at least two independent tests for LA-screening. Commonly used screening tests are based on the Kaolin Clotting Time (KCT), a LA-sensitive aPTT or on the dilute Russell's Viper Venom Time (dRVVT). Dilute prothrombin time (dPT) has also reported as a sensitive test for LA-screening. Therefore we evaluated the usefulness of a homemade dPT in comparison to different commercial available tests.

Methods: All tests were performed on the BCS analyser (Siemens Healthcare Diagnostics, Germany). In a first step quality and performance of the homemade dPT using recombinant thromboplastin Innovin (Siemens Healthcare Diagnostics, Germany) in a 1/200 dilution was evaluated. Following we estimated in 22 patients, previously tested positive for LA, dPT, KCT (Kaoclot, Life Diagnostics, USA), dRVVT (LA1, LA2, Siemens Healthcare Diagnostics, Germany) and MixConLA (Instrumentation Laboratories, Germany).

Results: Intra-assay coefficient of variation (CV) for dPT was 1 % and inter-assay CV 5,6 %. Normal values assigned in 50 healthy individuals ranges from 35 to 51 sec. 51 sec was chosen as the cut-off value. We obtained negative results in 2 patients with dPT, in 1 patient with MixConLA, in 8 patients with KCT and in 3 patients with dRVVT.

Conclusion: Innovin dPT is a low-cost but high sensitive screening test for LA and improves the sensitivity of LA screening in combination with other commercial assays such as LA-sensitive aPTT.

PP6.3-2

Pulmonary embolism in a patient with severe Factor V deficiency

Groß J¹, Stephan B¹, Schenk J¹, Eichler H¹

¹Institut für Klinische Hämostaseologie und Transfusionsmedizin, Homburg, Germany

Objectives: Severe factor V (FV) deficiency is known to be associated with a clinical relevant bleeding tendency. This case report describes a patient with FV deficiency suffering from pulmonary embolism and showing so far unknown mutations of the FV gene.

Methods: The clinical course was analyzed retrospectively.

Results: Case report: The 40 year old male patient was historically tested with a significant prolongation of both prothrombin time and partial thromboplastin time around 20 years ago; interestingly, he did not report any bleeding tendency. Further investigation revealed FV activity of 5 %, while an inhibitor could be excluded; FVIII activity was measured within the normal range. In his 31st year of life ulcerative colitis was diagnosed. The patient now developed a thromboembolic event of the pelvis and leg, and pulmonary embolism could be confirmed by computed tomography. Some days before, steroid therapy of the colitis was performed. Further investigation of the coagulation system revealed normal values for protein C/S and antithrombin. Neither antiphospholipide antibodies nor polymorphisms of FV (G1691A) and FII (G20210A) could be detected. Molecular genetic investigation revealed a first described deletion (Tyr1554_Tyr1555 del/ins Tyr) and missense-mutation (Met1339Lys) within the FV gene. Anticoagulation with phenprocoumon for at least 12 months was initiated in this patient.

Conclusions: Patients with FV deficiency cannot only present with a bleeding tendency, but might also suffer from serious thromboembolic events which possibly are favoured by the underlying disease and concomitant therapy.

PP6.3-3

A novel mutation causative for type I antithrombin-deficiency combined with other thrombophilic defects in a family with severe thromboembolic disease

Schmeink U¹, Luxembourg B², Lindhoff-Last E², Oldenburg J³, Pavlova A³

¹Gefäßzentrum Aachen, Germany ²Schwerpunkt Angiologie, Zentrum der Inneren Medizin,

Medizinische Klinik I, Klinikum der Johann Wolfgang Goethe-Universität Frankfurt am Main, Germany

³Institut für experimentelle Hämatologie und Transfusionsmedizin, Universitätsklinikum Bonn, Germany

Objectives: Patients with inherited antithrombin deficiency (ATD) are rare. Little is known about clinical relevance of ATD combined with other thrombophilic defects. Since 1996 we are observing the occurrence of venous thromboembolism (VTE) in four generations of a family in which ATD segregates with factor V Leiden-mutation (FVL) or protein C-deficiency. 12 family underwent labora-



tory thrombophilia screening and were interviewed and examined for VTE. VTE occurred in 8 persons and lead to death of great-grandfather. 10/12 persons show thrombophilic defects. Patients who suffered from VTE all show at least ATD. VFL was detected in 3 individuals (2 heterozygous, 1 homozygous). One patient showed protein C-deficiency combined with ATD. The antithrombin gene was sequenced in 6 persons and showed a deletion of 53 base pairs in exon 2 of the antithrombin gene (c.266_318 del. p.Arg57fsX22) associated with decreased plasma antithrombin activity and antigen levels. All individuals were heterozygous for the mutation. Interestingly, the age at first VTE differs widely in family members with combined thrombophilia (14 years for the individual with ATD and homozygous FVL, and 34, 59, 61 years).

Conclusions: A small deletion in exon 2 of the antithrombin gene was shown to be associated with type I antithrombin deficiency in a family with VTE. The high penetrance of VTE in this kindred demonstrates the high risk of type I antithrombin deficiency combined with other coagulation defects.

PP6.3-4

Antiphospholipid antibodies in young Indian patients with stroke

Mishra M¹, Rohatgi S², Gupta M³

¹Command Hospital - Southern Command, Pune, India ²Armed Forces Medical College, ³Bombay Hospital, India

Stroke is defined as the sudden occlusion or rupture cerebral arteries or veins resulting in focal cerebral damage and neurological deficits. It may be caused by antiphospholipid antibodies (APL) especially in young persons without other risk factors. In this study, the prevalence of two clinically significant APL - anticardiolipin antibody (ACL) and lupus anticoagulants (LA) in young patients presenting with sudden neurological deficit was studied and compared with age- and -sex matched controls. Fifty healthy volunteers and 51 young patients (less than 45 yrs) diagnosed as ischemic stroke were recruited for the study. Overall, the risk factor profile was: smoking (43%), positive family history (28.5%), hyperlipidemia (8%), and diabetes mellitus (3.6%). APL (LA and ACL) were present in 29.4% of the samples and in 4% of controls. The 'p' value for both ACL and LA was 0.03 and 0.02 respectively, but the maximum level of ACL was 25 GPL units only. One-fourth of the patients were smokers and one-sixth had a family history of thrombosis ('p' < 0.05 for both). Our study showed an association between ischemic stroke in young patients with both: the presence of APL risk factors. We advocate screening for APL in all young patients with stroke.

PP6.3-5

Markers of thrombin generation and parameters of overall haemostatic potential assay in individuals with antiphospholipid antibodies

Milijic P¹, Antovic A², Elezovic I¹, Colovic M¹

¹Institute of Haematology, Clinical Center of Serbia, Belgrade, ²Karolinska Institute, Karolinska Hospital, Stockholm, Sweden

Objectives: Blood hypercoagulability may be one of the underlying mechanisms of thrombotic tendency or pregnancy loss in antiphospholipid syndrome, but it is not clear whether assays which reflect thrombin or fibrin generation may distinguish individuals who are prone to develop these complications

Design and Methods: In this retrospective study we measured concentration of prothrombin fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT), and D-dimer in 53 individuals with primary occurrence of antiphospholipid antibodies. In the same patients we also determined parameters of overall haemostatic potential (OHP) assay (first described by Blombäck et al. 2001, which reflect the balance between fibrin generation and lysis in microtiter plate wells. Thirty six patients were asymptomatic (pregnancy loss in 12, and thrombosis in 24), while 17 individuals were symptomatic.

Results: Mean concentrations of F1+2, TAT and D-dimer were higher in group of symptomatic individuals than in asymptomatic group, but the differences were not significant, (p > 0.05, for all three markers). Results of overall coagulation potential, overall haemostatic potential and overall fibrinolytic potential were also similar in both groups, (p > 0.05, for all three parameters). Sensitivity and specificity of all investigated parameters for occurrence of thrombosis or pregnancy loss in individuals with antiphospholipid antibodies were quite low.

Conclusions: Although our study is limited by size and retrospective nature, it could be concluded that neither markers of thrombin generation nor parameters of OHP assay are reliable tools in recognition of individuals with antiphospholipid antibodies who are at increased risk for development of thrombotic complications or spontaneous pregnancy loss.

PP6.3-6

Incidence of thrombophilic disorders in women of childbearing age and a history of thromboembolism or recurrent pregnant loss vs. a healthy control group

Schinzl H¹, Rolfes E¹, Walter L¹, Interthal C², Jachmann N³, Peetz D³

¹Universitätsklinik Mainz II. Med. Klinik, Germany ²Universitätsfrauenklinik Mainz, Germany

³Zentrallabor der Universitätsklinik Mainz, Germany

Background: Thromboembolism (VTE) is the major reason for motherly morbidity and mortality during gravidity and puerperium. The risk of VTEs during pregnancy is five to six times higher than for non-pregnant women. Inherited and acquired thrombophilic disorders further raise the risk. Some of these disorders are additionally responsible for recurrent abortions. Is screening these women for thrombophilia useful?

Patients and Methods: 133 pregnant patients with history of thromboembolism and/or recurrent fetal losses were included in this study (group 1). They were screened for factor V Leiden mutation, Prothrombin mutation (G20210A), deficiency of Protein C, S, Antithrombin and Plasminogen, lupus anticoagulants and anti-cardiolipin antibodies. Reference was 135 women of childbearing age (group 2).

Results: Group 1: The most common histories were thromboembolic events, recurrent fetal losses followed by combinations of thromboembolism and abortions. The control group was composed of women between 18 and 48 years, without a history of events. 47% of all pregnant patients had one thrombophilic disorder, 7% two or more. The reference collective had 6% single prothrombotic defects, in 1% two combined disorders were found. The most frequent disorder was the Faktor V Leiden mutation (63% in group 1 vs. 46% in the control group) followed by prothrombin mutation 21% vs. 27%.

Conclusion: In more than 50% of women with a history of thromboembolism or recurrent abortions an underlying thrombophilic disorder was detected vs. 7% in the control group. Therefore screening for thrombophilia is recommended in these patients.

PP6.3-7

Antibodies to lepirudin in subcutaneous long-term treatment of a patient with recurrent venous thromboembolism due to Behcet's disease – a case report

Linnemann B¹, Schwonberg J¹, Zgouras D¹, Greinacher A², Lindhoff-Last E¹

¹Division of Vascular Medicine, Department of Internal Medicine, J.W. Goethe University Hospital, Frankfurt/Main, Germany, ²Institute for Immunology and Transfusion Medicine, Ernst-Moritz-Arndt

University, Greifswald, Germany

The direct thrombin inhibitor lepirudin is mainly applied in heparin-induced thrombocytopenia. We report here the case of a 37-year-old Kurdish woman in whom Behcet's disease was diagnosed in 1998 due to Budd Chiari syndrome (BCS) complicated by pulmonary embolism. Recurrent VTE occurred despite anticoagulant therapy with phenprocoumon and various immunosuppressive therapy regimens. In 2001, when BCS recurred ultimately i.v. lepirudin was administered. When the patient improved and remained clinically stable lepirudin was applied subcutaneously. During long-term treatment with twice-daily 50 mg no further VTE was observed over the following years. In May 2005 anticoagulant therapy was switched to phenprocoumon. BCS recurred when INR values were suboptimal in February 2007, and lepirudin treatment was immediately restarted. After admission the patient received 50 mg b.i.d. lepirudin s.c. with plasma levels in the therapeutic range. Over the following months, lepirudin levels repeatedly exceeded the upper limit of this range and the dosage was stepwise reduced. Finally, 20 mg b.i.d. were sufficient to obtain therapeutic levels. Renal function was normal, but lepirudin antibodies were present in high titer, as assessed by ELISA. We suppose that these antibodies reduce renal filtration of lepirudin thus leading to increased plasma levels.

Conclusion: This case is an example for the efficacy of long-term therapeutic-dose anticoagulation with s.c. lepirudin in patients with recurrent venous thromboembolism despite therapeutic-dose anticoagulation with LMWH or vitamin K antagonists. However, regular measurement of lepirudin plasma levels is needed. If stepwise dose lowering is required over time, the presence of lepirudin antibodies should be considered.

PP6.4 Hormones, Pregnancy, Women's Issue

PP6.4-1

Association of elevated soluble P-selectin levels and fetal loss in women with a history of venous thromboembolism

 Ay C¹, Kaider A², Koder S¹, Husslein P³, Pabinger I¹
¹Division of Haematology and Haemostaseology, Department of Internal Medicine I, Medical University of Vienna, Austria ²Section of Clinical Biometrics, Core Unit for Medical Statistics and Informatics, Medical University of Vienna, Austria ³Department of Obstetrics and Gynecology, Medical University of Vienna, Austria

Objectives: Pregnancy induces hypercoagulability with an increased risk of venous thromboembolism (VTE) and pregnancy complications. Recently, the cell adhesion molecule P-selectin has been identified to be strongly associated with VTE.

Design and Methods: We investigated the significance of elevated soluble P-selectin (sP-selectin) for fetal loss in women with a history of VTE and evaluated retrospectively data on pregnancy-associated complications in 304 women (mean age [+/-SD]: 45.6 [+/-11.5] yrs) with a history of VTE. sP-selectin plasma levels were measured with ELISA.

Results: At the time of sP-selectin measurement none of the women was pregnant and did not have an acute VTE. The prevalence of miscarriage (=intrauterine fetal death before the 24th gestation week or when fetus weighed <500g) was 21.8% and of stillbirth (=intrauterine fetal death at or after the 24th gestation week) was 4.3%. Median [IQR] sP-selectin level of the total study population was 38.0 [21.7–44.4] ng/mL. The cut-off point for elevated sP-selectin was set at the 75th percentile (44.4 ng/mL). The prevalence of stillbirth was significantly higher in subjects with elevated sP-selectin levels compared to those with lower levels (10.5% vs. 2.6%, p=0.008), whereas no statistically significant difference in prevalence of miscarriage was observed between women with and without elevated sP-selectin (17.1% vs. 22.9%, p=0.303). The odds ratio [95% CI] of elevated sP-selectin for stillbirth was 4.2 [1.5–12.7] and for miscarriage 0.7 [0.4–1.3].

Conclusions: Elevated sP-selectin plasma levels were associated with a 4-fold risk for stillbirth in women with a history of VTE and may have a possible role in the aetiology of late pregnancy complications.

PP6.4-2

Menorrhagia is common in patients on oral anticoagulation with vitamin-k-antagonist phenprocoumon

 Rott H¹, Kappert G¹, Halimeh S¹
¹MVZ Labor Duisburg, Germany

Objectives: To assess the menstrual blood flow of 46 women on long term oral anticoagulation (OAC) with phenprocoumon in comparison to normal values from the literature.

Design and Methods: Menstrual blood loss was assessed by a pictorial blood loss assessment chart (PBAC) over three menstrual cycles in each woman. 34 women were on OAC due to thromboembolism and 12 women for cardiac diseases (10 after prosthetic heart valve replacement, 2 with atrial fibrillation). All women were on INR-self-management with a coagu-chek device. 39 patients measured INR at least every 2 weeks, the others had longer control intervals (up to 6 weeks). Medium age of the patients was 37,5 years. The medium duration of OAC was 6,3 years.

Results: 33 from 46 patients (72%) had menorrhagia defined as a PBAC score > 100. Medium PBAC-Score of women on OAC was 191, ranging from 15 to 1064. Medium duration of menstrual bleeding was 6,5 days. Medium cycle length was 27,8 days. 4 of the women were taking oral contraceptives, two had an intrauterine device.

Conclusions: Menorrhagia is common in patients on OAC (72%). Patients on long-term OAC had an almost doubling of blood loss compared with normal controls. There is a noteworthy undersupply of contraception methods to control bleeding and prevent pregnancy in women on OAC.

PP6.4-3

Interaction between homocysteine and lipoprotein(a) increases the risk of coronary sclerosis/myocardial infarction in Hungarian women

 Katona É¹, Balogh E², Bereczky Z¹, Czuriga I², Édes I², Muszbek L¹
¹University of Debrecen, Medical and Health Science Center, Clinical Research Center, Debrecen, Hungary ²University of Debrecen, Medical and Health Science Center, Department of Cardiology, Debrecen, Hungary

Objectives: The aim of the study was to evaluate the effect of high homocysteine (HCY) and lipoprotein (a) (Lp(a)) levels on the risk of coronary sclerosis (CS) and myocardial infarction (MI) and to investigate their interaction in both genders.

Design and methods: 1010 consecutive patients undergoing coronary angiography were categorized according to the presence or absence of significant CS (CS+, CS-)

and according to the history of MI (MI+, MI-). Serum Lp(a), plasma HCY, B12 vitamin, folate, MTHFR C677T polymorphism and a number of other laboratory and clinical parameters used for adjustment were determined.

Results: As compared to clinical controls (CS-MI-) adjusted HCY and Lp(a) levels were significantly elevated and B12 vitamin levels were decreased only in the CS+MI+ group. Folic acid concentration didn't differ significantly. There was no association between MTHFR genotype and CS or MI. Elevated HCY (>12.5 micromol/L) increased the risk of CS (OR: 2.04 (1.27–3.28)) and MI (OR: 2.60 (1.43–4.71)) only in women. Similar results were obtained with elevated Lp(a) (>300 mg/L); in this case ORs were 1.64 (1.03–2.61) for CS and 1.89 (1.06–3.38) for MI. Parallel elevation of HCY and Lp(a) increased the ORs to 3.75 (1.80–7.83) and 5.05 (2.08–12.31), respectively. In women under the age of 55 ORs 6.43 (1.67–24.73) and 16.40 (3.46–77.67) were obtained.

Conclusions: The elevation of HCY or Lp(a) level confers a moderate risk of CS and MI in women. The elevation of both parameters additively increases the risks, and in women below 55 the risk becomes especially high.

PP6.4-4

Fondaparinux during pregnancy

 Siegemund A¹, Oppermann J, Scholz U¹
¹Laboratory Practice Dr. G. Reising-Ackermann and colleagues, Centre for Coagulation Disorders and Hemophilia

Objectives: In the literature only few reports exist about fondaparinux in pregnancy and there are no controlled clinical trials. On the other side we know from in vitro studies that there occurs a minimal transplacental passage of fondaparinux. In our center five pregnant women with thrombophilia, allergic reactions against LMWH, or history of heparin-induced thrombocytopenia, respectively, received fondaparinux over several weeks in pregnancy and in the postpartal period.

Subjects and Methods: Five women with pregnancy and thrombophilia were compared with five pregnant women with nearly the same history of thrombophilia and the same time schedule for taking plasma samples. The dosage was 2.5 and 5 mg/day. Markers of activation (D dimer, F1+2) and thrombin generation (TG) were determined in both groups. TG was measured at the BCS (ETP, Siemens Healthcare) and Calibrated Automated thrombogram (CAT, Thrombinoscope).

Results: Fondaparinux was well tolerated in all women. Coagulation activation were nearly identical in both groups. There were also no significant differences between TG. Both methods demonstrate a moderate coagulation during pregnancy and a significant decrease in the postpartal period.

Conclusions: The data demonstrate that the selective Xa inhibitor fondaparinux can be used in patients with allergic and immunologic reactions against LMWH with the same effectiveness and safety like the established therapy with LMWH. There are no significant differences regarding thrombin generation in both groups. All pregnancies were successful without any complications for mother and child and fondaparinux is an alternative therapy in a this group of patients.

PP6.4-5

Global coagulation markers in women with and without anticoagulation during pregnancy

 Hron G¹, Philipp K², Kaider A³, Kyrle P¹, Eichinger S¹
¹Klinik Für Innere Medizin I, Medizinische Universität Wien, Wien, Österreich, ²Sozialmedizinisches Zentrum Ost, Wien, Österreich, ³Besondere Einrichtung für Medizinische Statistik und Informatik, Medizinische Universität Wien, Wien, Österreich

Background: Pregnancy is associated with a hypercoagulable state. We investigated if the hemostatic system alterations can be monitored by global coagulation markers.

Methods: In a prospective case-control study, we followed 61 women with low molecular weight heparin (LMWH) thromboprophylaxis throughout pregnancy. 113 healthy, pregnant women without LMWH served as controls. ProCGlobal[®] and the endogenous thrombin potential (ETP) were measured by commercially available assays (both Dade Behring Siemens, Germany) in the 1st, 2nd, and 3rd trimester.

Results: ProCGlobal values decreased significantly from 1st to 2nd and from 2nd to 3rd trimester and patients had lower ProCGlobal values than controls (p<0.001 in all comparisons). ProCGlobal values (mean [IQR]) were 0.94 [0.84–1.02], 0.82 [0.74–0.90], and 0.73 [0.66–0.79] in controls, and were 0.76 [0.89–0.82], 0.70 [0.67–0.74], and 0.64 [0.61–0.68] in patients without hereditary defects in the protein C pathway and 0.51 [0.46–0.53], 0.46 [0.42–0.49], and 0.43 [0.40–0.46] in patients with respective defects. ETP values remained unchanged during the 1st and 2nd trimester (p=0.18), but decreased significantly from 2nd to 3rd trimester (p<0.001). ETP values (mean [IQR]) were slightly higher in patients than in controls in 1st, 2nd, and 3rd trimester, respectively (111.9% [102.4–127.7] vs. 108.2% [100.0–120.8], p=0.08; 114.2% [105.6–121.7] vs. 110.9% [104.9–116.7], p=0.08; and 109.8% [100.4–118.5] vs. 105.2% [99.5–111.7], p=0.04).



Conclusion: The hypercoagulable state during pregnancy was reflected by decreased ProCGlobal values in women with and without anticoagulation. In contrast, ETP which indicates the plasma's potency to generate thrombin in response to a thrombotic stimulus remained unaffected by hemostatic system activation during pregnancy.

PP6.4-6

Monitoring of substitution therapy in two pregnant women with severe antithrombin deficiency

Siegemund A¹, Siegemund T¹, Scholz U¹, Thamm-Mücke B¹

¹Laboratory Practice Dr. G. Reising-Ackermann and colleagues, Centre for Coagulation Disorders and Hemophilia

Objectives: Patients with antithrombin deficiency have a high risk for thromboembolic complications especially during pregnancy. Low molecular heparins (LMWH) or antithrombin (AT) substitution are possible therapeutic options. The individual therapy should be assessed on the basis of molecular defects. Both patients had a type I deficiency, one had a spontaneous abortion in the first pregnancy (16th week) under prophylaxis with LMWH. With AT therapy, both pregnancies were successful. Therapy monitoring was done using thrombin generation (TG) methods.

Subjects and Methods: Both patients are descended from a family with AT deficiency (5354delCTT) and multiple thromboembolic events. TG was measured at the BCS (ETP, Siemens Healthcare) and with Calibrated Automated Thrombogram (CAT, Thrombinoscope). In addition to the in vivo data, we made in vitro experiments to estimate the effect of different anticoagulants as possible treatment regime in these patients.

Results: LMWH prophylaxis is not effective in both patients, whereas AT substitution reduces the excessive TG. TG measurements also show which kind of anticoagulant are therapeutic alternatives in patients with AT deficiency. The CAT displayed problems regarding substrate consumption.

Conclusions: The described TG methods are suitable to describe the state of hypercoagulability and are suitable to monitor patients with high risk of thrombosis during pregnancy. TG methods may be helpful to select custom-made anticoagulant therapy.

PP6.4-7

D-Dimer as guiding parameter for LMWH prophylaxis: Therapeutic outcome of patients with thrombophilia and thrombotic history treated with LMWH during pregnancy

Siegmund B¹, Richter H¹, Pollmann H¹

¹Institut für Thrombophilie und Hämostaseologie, Münster, Deutschland

Objective: In this study D-Dimer was used to indicate imbalance of haemostasis towards activation of blood coagulation. Dependent on D-Dimer level LMWH (dalteparin, enoxaparin, certoparin) prophylaxis was administered during variable time periods in pregnant women with thrombophilia and an own or family thrombotic history.

Design and Methods: Medical history of thrombotic events and markers of thrombophilia were recorded. D-Dimer was measured every 3–4 weeks. LMWH prophylaxis was started patient orientated depending on the development of its D-Dimer level and ended 6 weeks post partum. Data are given as mean +/- SD.

Results: 128 pregnant women (age at delivery 32.1 +/- 4.9) with a thrombotic past medical history and positive for thrombophilia were included. D-Dimer levels were measured on average 8.4 (range 1 - 18) times per gravidity and post partum. 81 % of patients received LMWH prophylaxis during pregnancy, 19 % only post partum. LMWH prophylaxis was started patient dependent on average at a D-Dimer level of 0.93 +/- 0.51 mg/l and reached under LMWH a level of 1.29 +/- 0.91 mg/l. A control group of 7 pregnant women (6 measurements each) without thrombophilia and heparin treatment 0.51 +/- 0.25 mg/l D-Dimer was measured. Birth weight was 3.337 +/- 601 g. 6.7 % of the newborns had a birth weight lower than normal. 3 abortions (2.3 % of pregnancies) and 5.3 % preterm deliveries (<37 weeks of pregnancy) were observed despite LMWH treatment.

Conclusions: D-Dimer is a suitable parameter to manage LMWH prophylaxis in patients with pregnancy at risk.

PP6.4-8

Thromboprophylaxis during pregnancy (estimated risk based thromboprophylaxis on pregnancy)

Kovács L^{1,2,3}, Horváth B^{1,2,3}, Riba M^{1,3}, Farkas G³, Bódis J²

¹Markusovszky Teaching Hospital, Szombathely, Hungary ²University of Pécs, Faculty of Health Sciences, Hungary ³Thrombosis Club Szombathely, Hungary

Objective: Pregnancy and the puerperium in themselves are periods of increased risk for venous thromboembolism. This risk is clearly increased by the presence of familiar and/or acquired thrombophilic risk factors. Study design The study pres-

ents the experience gained over the past ten years in the prophylaxis of thrombosis with pregnant patients with increased hazard of thromboembolism.

Results: The study period involved 350 pregnant patients with increased hazard of thromboembolism. The majority (86 %) belonged to a low risk group, while the remaining 14 % fell into groups of average, high, or extreme high risk levels. Of the patients 29 had been treated for familiar thrombophilia, in a few cases the combined type, 22 for thromboembolism, one had artificial mitral valve, and 16 had been treated for antiphospholipid antibody syndrome and repeated miscarriage. In the last group as a result of LMWH and salicyl therapy 86 % of the pregnancies was successful compared to the earlier 20 % with no therapy. The authors emphasise the significance of rigorous anamnesis, angiology examination and a risk analysis considering the haemostaseologic characteristics, on the basis of which they chose the method and the extent of the prophylactic treatment.

Conclusions: The LMWH prophylaxis must be chosen individually in accordance with the body weight, the gestation age and the personal risk level. Identifying the four groups (low, average, high, and extreme high risk) when estimating the risk arising from the thrombophilic factors seems a practical measure.

PP6.4-9

Fibrinolytic therapy in tricuspidal valve prosthesis thrombosis after vaginal delivery

Dämgen-von Brevern G¹, Luxembourg B¹, Fichtlscherer S², Kohl J³, Reitter A³, Lindhoff-Last E¹, Louwen F³, Zeiher A²

¹Department of Internal Medicine, Division of Vascular Medicine, University Hospital Frankfurt/Main; Germany, ²Department of Internal Medicine, Division of Cardiology, University Hospital Frankfurt/Main; Germany, ³Department of Obstetrics & Gynaecology, University Hospital Frankfurt/Main; Germany

Women with prosthetic valve disease are at high risk for thromboembolism as well as bleeding complications during pregnancy. A 33 year old woman developed retroplacental hematoma in the second trimester of pregnancy under therapy with acetylsalicylic acid (ASS) and low molecular weight heparin because of mechanical tricuspidal valve prosthesis. ASS was stopped immediately. Despite of moderate thrombocytopenia no progression of the hematoma occurred under low molecular weight heparin therapy monitored by anti-Xa-level controls. Clinical observation showed a satisfactory development of the foetus. The baby was delivered at 37 weeks of gestation by forceps extraction. Because of retention of placental tissue increased vaginal blood loss occurred and therefore uterine abrasion and also a short delay (6 hours) of the anticoagulation was necessary. Three days post partum tricuspidal valve thrombosis with reduction of the prosthetic valve function was found by echocardiography and radioscopy. Low dose fibrinolytic treatment with recombinant tissue plasminogen activator (r t-PA 2 mg /hour) via central venous catheter and anticoagulation with unfractionated heparin in therapeutic dose was effective in thrombolysis and repair of valvular function. Vaginal bleeding required substitution of erythrocyte- and thrombocyte concentrates, fresh frozen plasma, oxytocin, methylergometrinhydrogenmaleat and sulproston.

Conclusion: Dose modified fibrinolytic therapy can be a successful treatment in heart valve thrombosis and is also available after complicated vaginal delivery.

PP6.4-10

Risk factors for deep vein thrombosis in women 18–50 years of age: a retrospective analysis

Binder B¹, Lackner H², Salmhofer W¹, Hofmann-Wellenhof R¹

¹Universitätsklinik Für Dermatologie und Venerologie, Medizinische Universität Graz, Austria

²Technische Universität, Graz, Austria

Background: Deep vein thrombosis (DVT) occurs in one of every 1000 individuals per year. Various inherited and acquired risk factors are known. We investigated the importance of oral contraceptives (OC) as risk factor in women of child bearing age.

Objectives: To evaluate the risk factors for DVT in our female patient collective.

Methods: We analyzed the records of 99 women between 18 and 50 years with DVT. We documented age, identifiable risk factor of DVT, localisation of the thrombus, use of OC and thrombophilia.

Results: 52 were 40–50 years old. 46 patients had an unprovoked DVT; the most common risk factor was immobilisation in 41. Thrombophilia was found in 18 cases. 29 patients used OC, no influence of OC on any of the other risk factors was found.

Conclusions: In our patient group the most important risk factors were higher age and immobilisation. DVT is typically idiopathic, maybe caused by subtle non-detectable thrombophilic disorders. The use of OC was distributed in DVT patients just as in the general Austrian community and seems not to be a severe additional risk factor. To examine the whole lower extremity during ultrasonography is important not to overlook a DVT of the lower leg.

PP6.4-11

Deep venous floating thrombus during pregnancy

Vorobyeveva N^{1,2}, Anisimov M¹Northern State Medical University, Archangelsk, Russia, ²Northern Filial of The Gematologicheskoy Science Centre of RAMS Archangelsk, Russia

Objective: The increased thrombogenic potential of blood has recently been identified as the cause of deep venous floating thrombus during the thrombophilia. Endovascular prophylaxis PTE and treatment of deep venous floating thrombus were considered with patient DVT. This problem is now addressed by using implantation of temporary cava filter during the pregnancy.

Method: The investigation included 89 women of the pregnancy aged 19–36 with of deep venous floating thrombus. The blood of all patients was analyzed for 6 genes of hemostatic system: factor I (fibrinogen), factor V (Leiden), prothrombin 20210A, methylenetetrahydrofolate reductase (MTHFR), PAI-1 (4G-5G), platelet receptor of fibrinogen.

Results: Was revealed linear interrelation between of deep venous floating thrombus and frequency of genetic polymorphism gene PAI-1 ($p=0.02$). Fibrinogen G/F 455 genetic polymorphism in homozygous position (A/A) and PAI-1 genetic polymorphism genotype are the most significant of deep venous floating thrombus ($p<0.007$, $p=0.036$, accordingly). The results showed, patients with PAI-1 genetic polymorphism in homozygous position - 4G/4G have been patients with of deep venous floating thrombus ($p=0.02$) and with revealed higher frequency of PTE ($p=0.05$). The results were analyzed with SPSS for Windows 13.0. Data were represented as $M\pm S.D.$ One-way ANOVA was used. Significant of differences was determined by nonparametric tests - Mann-Whitney, Fridman.

Conclusions: This methods of used for the purpose highly efficiently prevented massive pulmonary embolism during the pregnancy. Was revealed positively correlation between deep venous floating thrombus and frequency of genetic polymorphism gene PAI-1 ($r=0.34$, $p=0.02$). We could find a relationship between frequency of genetic polymorphism gene PAI-1 and gene fibrinogen G/F 455 and deep venous floating thrombus ($r=0.36$, $p=0.036$).

PP6.4-12

Successful management of portal vein thrombosis during pregnancy

Struve S¹, Griesshammer M²¹Universitätsklinikum Ulm, Germany ²Johannes Wessling-Klinikum Minden, Germany

We report about a 29-year old woman who was referred to our centre in early pregnancy. Five months ago immune thrombocytopenic purpura with a platelet count $< 10.000/\mu\text{l}$ had been diagnosed. Since primary treatment with high-dose dexamethasone had not led to a sustained response splenectomy had been performed followed by abdominal pain and fever two weeks later. CT scan had revealed portal and splenic vein thrombosis, whereupon treatment with vitamin K antagonists had been started. Anticoagulation was switched to enoxaparin 1 mg per kilogram twice daily immediately, i.e. at six weeks' gestation. Follow up by ultrasonography at sixteen weeks' gestation showed cavernous transformation with hepatocentral flow; hence enoxaparin was reduced to 1 mg per kilogram once daily, achieving anti-factor Xa levels around 0,4 IU/ml. After an uneventful pregnancy the patient gave birth to a healthy infant by spontaneous vaginal delivery at 37 weeks' gestation, afterwards anticoagulation was continued as before. The patient did not show any clinical problems during pregnancy or puerperium. Platelet counts were slightly elevated during pregnancy and decreased to the upper normal range in puerperium. Further ultrasounds showed persistent cavernous transformation. Other predisposing factors for portal vein thrombosis, especially inherited thrombophilia, could be excluded, therefore splenectomy with reactive thrombocytosis seemed to be the sole cause. Regarding the benign clinical course we decided to stop anticoagulation six months after delivery. There are only few reports concerning portal vein thrombosis associated with pregnancy. In our case management with intermediate doses of low-molecular-weight heparin proved to be safe and effective.

PP6.4-13

Hormon replacement therapy, angiosarcomatosis and disseminated intravascular coagulation

Kovács E¹, Urbán K¹, Péter M², Sáy P³, Pfliegler G¹¹Div Rare Dis, Inst Medicine, MHSC, Univ Debrecen, Hungary, ²Dept Radiol, MHSC, Univ Debrecen, Hungary, ³Inst Surgery, MHSC, Univ Debrecen, Hungary

A 50-year-old woman presented herself with fever, haemoptoe in a county hospital. D-dimer test was positive and lung scintigraphy suggested microembolisation, therefore low molecular weight heparin was immediately started. Her condition deteriorated and abdominal sonography revealed liver cavernous haemangiomas. She was transmitted to our department, laboratory reevaluation proved disseminated intravascular coagulation, pulmonary embolism could be ruled out. A sudden decrease in haemoglobin and abdominal pain was caused by rupture of one of the

haemangiomas. Arterial embolisation, rFVIIa was given but after two days while waiting for surgery, rupture repeatedly occurred and an urgent liver resection had to be carried out. Her condition due to postoperative complications (sepsis, pleuropneumonia), only slowly improved, and subsequent control examinations discovered rapidly growing haemangiomas in the lung. Because of generalized appearance surgical removal and pig-tail coil could not be administered. Histological reevaluation of liver haemangioma confirmed expected haemangiosarcomatosis for what no effective therapy is currently available, and sporadic suggestions (prednisone, alpha-interferon, doxycyclin, antiangiogenic) in her case remained ineffective or was not enough time left for therapeutic response and she died. Haemangiosarcomatosis is a rare disease as a causative factor thorium dioxide, vinylchloride toxicity and oestrogen therapy had been suggested. In our patient 6 months prior her symptoms norethisterone therapy was introduced by the previously healthy woman, therefore a possible relationship cannot be ruled out.

PP6.4-14

The significance of endothelial markers of activation in the prediction of subsequent development of preeclampsia

Procházka M¹, Slavik L², Procházková J², Pilka R¹, Úlehlová J², Michurová A³¹Medical Faculty Of Palacky University, Dept of Obstet. and Gynaecology., Olomouc, Czech Republic²Medical Faculty of Palacký University, Dept of Hematonocology, Olomouc, Czech Republic ³Institute for the Care of Mother and Child, Prague, Czech Republic

Introduction: The hypertension and preeclampsia in pregnancy are multisystemic diseases characterized by generalized systemic vasoconstriction. The ischaemia of the fetoplacental unit cause the release of specific factors into maternal vessels and subsequent activation of the endothelium and vasoconstriction. There is a rush development a new markers of the endothelial activation have been found. Eg. t-PA, PAI-1, vWF, EPCR, thrombomodulin and endothelial microparticles with procoagulant activity. The aim of the study: To detect of above mentioned markers of endothelial activation in healthy pregnant women compared to those with pregnancy complicated by hypertension, diabetes mellitus and preeclampsia. The work hypothesis: We suppose that plasma specimens of the women with preeclampsia and diabetes mellitus will contain a higher levels of endothelial activation markers compared to healthy pregnant.

Methods: All included patient have to assign an informed consent. The blood sampling were taken by the routine way at the time of the first blood pregnancy sampling the end of the first trimester. The second specimen will be taken between 24.- 28. weeks of gestation. The following tests were performed: t-PA, PAI-1 - ELISA, vWF:Ag - EIA (immunologic detection by immunoturbidimetry), ePCR, mmp-2,9 - ELISA (fluorogenic detection), endothelial microparticles - Flow cytometry.

Results: We have found significant increase in the levels of of almost all endothelial activation markers within physiological pregnancy. Statistically significant difference was found in t-PA, PAI-1 vWf, ePCR and endothelial microparticles between normal and preeclamptic pregnancy. Supported by the Grant IGA of Min. of Health Czech Republic IGA NH 6986-3/2002 and NR 9282-3(2007)

PP6.5 Thrombotic Disorders in Children

PP6.5-1

Recurrent pulmonary embolism in a 14-year-old boy with antiphospholipid syndrome

Girsch M¹, Rauch R¹, Wiegand G¹, Hofbeck M¹¹Universitätsklinik Für Kinder und Jugendliche, Tübingen, Germany

Introduction: Anti-phospholipid syndrome (APS) is a potentially life-threatening autoimmune disease, characterized by arterial and venous thrombosis and presence of anti-phospholipid antibodies (aPL).

Case Report: A 14 year old boy (BW 58 kg, BL 172 cm) with cough, fever and chest pain was referred to hospital. Pneumonia was diagnosed and treated with antibiotics. After 3 days of immobilisation he developed thrombosis of the left leg and severe chest pain recurred. CT scan yielded pulmonary embolism (PE). Complete screening for hypercoagulability showed high titres of immunoglobulin G anticardiolipin (59 U/ml; normal < 10), β -2-glycoprotein 1-IgG (69 U/ml, normal < 10) and elevated antiphospholipid (aPL) antibodies. The patient was treated with unfractionated heparin and corticosteroids. However, he sustained another PE attack (confirmed on CT scan) and corticosteroids were increased. Now anticoagulation was switched to phenprocoumon (INR 3.0–3.5) and the boy discharged from hospital. Three weeks later a left sided relapse of PE led to admission to our hospital. Anticoagulation therapy was switched to LMWH (anti-Xa-level 0.6–1 IE/ml) and steroid treatment was tapered down and stopped. No further episodes of thromboses were noted since 3 month. Discussion: Pediatric primary APS is very rare and



onset before 15 years is only 2.8%. In adults APS is well characterized, but there are only few studies of children.

Conclusion: Due to the rarity of APS in children and youngsters there is little evidence on the optimal substance, intensity and duration of anticoagulant and anti-inflammatory treatment.

PP6.5-2

Longterm treatment of a severely protein C deficient infant by protein C concentrate substitution

Hertfelder H¹, Horneff S¹, Rey M², Fleischhack G³, Bartmann P⁴, Oldenburg J¹, Heep A⁴

¹Institute Of. Exp. Haematology and Transfusion Medicine University Hospital Bonn, Bonn Germany,

²Department Of Paediatrics, Karolinen-Hospital, Arnsberg, Germany, ³Department Of Paediatric Haematology&Oncology University Hospital Bonn, Bonn Germany, ⁴Department Of Neonatology University Hospital Bonn, Bonn Germany

Objectives: Severe protein C (PC) deficiency is a rare autosomal disease predisposing to severe thrombohemorrhagic complications. Few casuistic experiences about the outcome on long-term thromboprophylaxis by coumarins and/or PC substitution are available.

Patient and Methods: The treatment of a female infant with severe compound heterozygous protein C deficiency (protein C < 1% at birth) over 32 months is reported. The child was born with cerebral thrombohemorrhagic infarctions. Prophylaxis has been performed with coumarins (warfarin, phenprocoumon) and PC concentrate substitution (PCC, Ceprotin®) via a permanent femoral Broviac® catheter. At the age of 20 months the Broviac catheter was replaced via left jugular vein. Therefoere, bridging by intensified PCC substitution and low-molecular weight heparin was necessary. Monitoring includes INR, protein C activity and antigen, and D-Dimer levels.

Results: 500 IU PCC was given every 96 to 120 h over 20 months. During the first 7 months of life, warfarin was applied but INR was very unstable (INR range 1.5 to 4.7). Since then with 1.2 to 1.4 mg/d phenprocoumon anticoagulation stable INR 2.5 to 3.5 is achieved. For replacement of the Broviac catheter, PCC was applied subcutaneously resulting in prolonged PC halflife of 20 to 30 h. Over the new Broviac 500 IE PCC are continuously applied over 72 to 96 h respectively. PC levels between 6 and 9% are observed. D-Dimer levels are below 0.2 µg/ml. No bleeding or thrombotic complications occurred until now.

Conclusions: Infants with severe PC deficiency can be sufficiently treated with phenprocoumon and continuous PCC substitution to prevented from thrombotic relapses.

PP6.5-3

Primary antiphospholipid antibody syndrome and systemic lupus erythematosus during childhood in two sisters with C4 deficiency

Acham-Roschitz B¹, Mache C¹, Ring E¹, Muntean W¹

¹Univ. Klinik f. Kinder/Jugendheilkunde Graz, Austria

Objectives: Complement deficiencies within the classical pathway predispose to autoimmune disease. C4 deficiency is associated in 75% with systemic lupus erythematosus (SLE). Patients with hypocomplementemia show a higher prevalence to primary antiphospholipid antibody syndrome (APS). This subgroup has a higher risk for SLE if positive antinuclear antibodies and a positive Coombs test are found. We report on two siblings with C4 deficiency and different clinical courses. Patients: Patient 1 experienced left femoral venous thrombosis due to high antiphospholipid antibodies (APA) at the age of nine years. Oral anticoagulation was initiated. Ten years after onset she has not yet progressed to SLE. Her sister (patient 2) presented with epistaxis due to thrombocytopenia at the age of 11 years and developed full-blown SLE including lupus nephritis. She responded well to induction therapy and is on maintenance therapy in remission for two years.

Results: Patient 1 showed APA/anticardiolipin antibodies positive. Sapporo criteria for APS were fulfilled. Two years later she developed antinuclear antibodies, dsDNA and positive Coombs test. Patient 2 presented with antinuclear- and dsDNA antibodies, APAs/anticardiolipin antibodies and positive Coombs test. C4a and C4b were decreased in both siblings. Genetic analysis is pending.

Conclusions: Complement activation is the rule in childhood SLE, resulting in hypocomplementemia and deposition of complement at sites of tissue damage. On the other hand complement deficiencies predispose for autoimmune disease. Our siblings with C4 deficiency had different clinical presentations at nearly the same age.

PP6.5-4

Management of subcutaneous protein C substitution in a child with severe protein C deficiency

Oliveri M¹, Bidlingmaier C¹, Engelsberger I², Kurnik K¹

¹Abt. für Pädiatrische Hämostaseologie, Dr. von Haunersches Kinderspital, LMU, München, Germany

²Neonatalogie, Kinderklinik Schwabing, TU München, Germany

Introduction: Homozygous protein C deficiency is a rare and severe congenital disorder associated with purpura fulminans, multiple arterial and venous thrombosis and retinal haemorrhage. Therapy consists in intravenous or subcutaneous replacement of protein C concentrate and long term anticoagulation with vitamin k antagonists.

Case report: We report a preterm newborn (35+2 gestational week) of consanguine parents born by caesarean because of growth retardation and pathological cardiococogram. The ophthalmologic examination and cranial MRI showed a bilateral retinal haemorrhage and a large infarction area with secondary haemorrhage. Screening for thrombophilia showed a severe protein C deficiency. Genetic analysis approved the diagnosis by showing a Gly292(GGC)>Ser(AGC) mutation in the protein C gen. We started treatment with intravenous substitution of 50U/kg purified protein C concentrate every 6 hours. (chromogene protein C levels prior substitution 20%-30%). In course dose was increased to 67U/kg every 8 hours. After the acute phase of disease we switched to subcutaneous replacement therapy with 62U/kg every 8 hours, increased to 121U/kg every 12 hours and lastly to 230U/kg/day (chromogene 49%-67%, activity 14%). Because of low protein C activity levels we increased dose to 333U/kg (chromogene 87%-114%, activity 36%-48%).

Conclusion: Several thrombosis, purpura fulminans and retinal haemorrhage are pathognomonic for severe protein C deficiency. Subcutaneous protein C substitution is a good method to bridge over acute phase until starting long term oral anticoagulation. Because of the difference of protein C levels in the chromogene test and in the activity test we presume a protein C deficiency type IIb.

PP6.5-5

Malignant stroke in an adolescent with a homozygous MTHFR 677CT mutation and intake of oral contraceptives: Report of a case

Pfurtscheller K¹, Senning B¹, Bernhard H¹, Novak M¹, Zobel G¹, Plecko B¹, Muntean W¹

¹Department of Pediatrics, Medical University of Graz, Austria

Stroke is a rare disorder in childhood, but ranges among the top ten causes of death in children. Over the last ten years the incidence seems to have increased from two to six cases per 100.000 children per year. The reason for this increase might partly be explained by the enhanced detection rate of minor stroke events or transitory ischemic attacks due to improved clinical awareness as well as new neuroimaging- and laboratory techniques. Another explanation for the increase may be the improved survival of children with previously lethal diseases predisposing to stroke e.g. cardiac malformations. In 40% the cause of stroke in infancy and childhood remains unclear and half of the cases were found to have multiple risk factors. Stroke in this age group is often associated with non atherosclerotic vasculopathy, congenital or acquired heart disease, and central nervous system infections. Hereditary prothrombotic risk factors are suspected to contribute to the risk of ischemic stroke, but in most cases an additive acquired factor seems to be necessary to trigger a stroke. We report the case of an 18 year-old girl with a malignant, fatal stroke of her non-dominant right hemisphere. The risk factors found were a homozygote MTHFR-677CZ mutation, elevated plasma homocysteine and the history of recently started oral contraceptives. Since prevention seems easily feasible, general screening for MTHFR mutations might be worthwhile in women before starting oral contraceptives.

PP6.6 Preclinical Studies

PP6.6-1

Autologous bone marrow-derived stem cell therapy: A promising and prospective approach in the treatment of patients with severe Buerger's disease

Boda Z¹, Rázsó K¹, Szarvas M¹, Ilonczai P¹, Tóth J², Kappelmayer J³, Farkas K⁴, Rajnavölgyi E⁵

¹University Of Debrecen, 2nd Department of Medicine, Debrecen, Hungary ²University of Debrecen,

Department of Radiology, Debrecen, Hungary ³University of Debrecen, Department of Clinical

Biochemistry and Molecular Pathology, Debrecen, Hungary ⁴St. Imre County Hospital, Budapest,

Hungary ⁵University of Debrecen, Department of Immunology, Debrecen, Hungary

Objectives: No effective blood-flow enhancement therapies are available for patients with severe peripheral arterial disease (SPAD). Amputation remains the only option for relief of rest pain or gangrene. Autologous bone marrow-derived stem cell therapy (ABMSCT) is an emerging modality to induce angiogenesis.

Methods and Results: Seven limbs of six patients with Buerger's disease were treated by ABMSCT using isolated CD34+ cells with characterized phenotype and administered by intramuscular injections. The follow-up before and 1-, 3-, 6-, 9- and 12 months after ABMSCT was based on clinical and laboratory measurements. Therapeutic benefit was demonstrated by complete regression of rest pain in all the 6 patients, and by the significant improvement of pain-free walking distance. The average of ABI improved significantly on the treated (before: 0.41, after twelve months: 0.83), but did not change on the contra-lateral limb. Non-healing ischemic ulcers disappeared in five, and became smaller and thinner in two lower limbs, the ulcer remained unchanged in one case, only. In all of the seven cases we observed improvement on the treated limb only, the contra-lateral symptoms and ulcers remained unchanged or worsened. Severe adverse events, complications were not observed.

Conclusions: ABMSCT with isolated CD34+ cells is safe, effective, localized and results in local and sustained clinical benefit for patients with severe forms of Buerger's disease. We show for the first time that low number of isolated bone marrow-derived CD34+ stem cells involving CD34+CD133+, CD34+CD133-, CD34+CD45- and CD34+CD45+ cells with different vessel forming ability confers vasculogenesis to patients with Buerger's disease.

PP6.6-2

Increased antithrombotic and bleeding effects of contaminated heparins; hematological implications

Litinas E¹, Adiguzal C¹, Jeske W¹, Hoppensteadt D¹, Messmore H¹, Walenga J¹, Fareed J¹
¹Loyola University Medical Center, Maywood, United States

Introduction: Earlier this year batches of unfractionated heparin (UFH) were recalled due to the presence of oversulfated chondroitin sulfate (OSCS). While structural and molecular characteristics of the contaminant are reported, no data on its impact on antithrombotic and bleeding effects is available. This investigation characterizes the effects of OSCS on the safety and efficacy of contaminated heparin (CH) in animal models.

Materials and Methods: CH (Baxter; lot 117050) and contaminant free UFH (CFH) (Abraxis; lot 405651) were studied at doses of 2 and 5 mg/kg IV using a rat model of jugular vein clamping induced thrombosis and a rat tail transection model. Ex vivo studies were carried out to measure anticoagulant and anti-Xa effects.

Results: CH contained 30% OSCS; CFH did not contain OSCS. In the bleeding studies, no differences between CH (37.0±5 min.) and CFH (35.0±5.7 min.) were observed at a dose of 2 mg/kg. At 5 mg/kg, CFH (67.8±1.8 min.) produced stronger bleeding effects than CFH (57.1±1.9 min.). In the jugular vein clamping model, CH produced stronger antithrombotic effects (6.2±1.2 vs. 5.2±0.3 clampings) at 2 mg/kg and at 5 mg/kg (7.6±0.8 clampings vs. 6.2±0). The ex vivo analysis showed a stronger anticoagulant effect with the CH. Anti-Xa activity and anti-IIa activities were higher in the CH groups. Physiologic distress was not observed at either dosage.

Conclusions: It appears that the presence of the OSCS contaminant augmented the anticoagulant and bleeding effects of CH and may have contributed to the increased clinical bleeding observed in patients treated with these agents.

PP6.6-3

A fluorescent microplate assay for quantification of heparins and other sulfated polysaccharides

Lühn S¹, Alban S¹
¹Pharmazeutisches Institut, Kiel, Germany

The problem of quantification of heparins is as old as their application. On the one hand there are many biological tests, which only indirectly detect effects of AT-binding material (ABM). On the other hand direct quantitative methods are available but all of these methods are extremely time-consuming and expensive. The aim of this study was to develop a direct, rapid, accurate and sensitive assay, which is suitable for routine analysis. We report on a microplate assay using Polymer6 (0.075 mg/mL), a heparin complexing, fluorescence labeled synthetic polymer developed by Schrader et al (2007). The influence of different heparins and other structurally defined glucan sulfates (GS) on its fluorescence characteristics was determined (λ (em) 330nm, λ (ex) 510nm). The fluorescence intensity of Polymer6 showed to be amplified by heparins and GS. This increase is strongly dependent on their concentration. The best sensitivity, accuracy and linearity were observed in a range from 0.625 to 5.0 μ g/mL heparins and GS, respectively. No differences in the fluorescence between various heparins were observed. Studies on structure-activity relationships using GS revealed that the fluorescence intensity increased with increasing degree of sulfation (DS) up to a DS of ~1.4, higher DS have no further impact. In contrast, influences by molecular mass and type of the polysaccharide backbone played only a minor role. In conclusion, this assay provides the opportunity to detect not only the ABM but to exactly quantify heparins and other GS. In contrast to pharmacodynamic assays only one standard curve for all heparins is required.

PP6.6-4

Increased levels of Anaphylatoxin (C5a) and bradykinin in end-stage renal disease patients. Potential relevance to heparin mediated hemodynamic responses

Adiguzal C¹, Bansal V², Cunanan J¹, Litinas E¹, Hoppensteadt D¹, Fareed J¹
¹Dept. of Pathology Loyola University Medical Center, Maywood, United States ²Dept. Of Nephrology Loyola University Medical Center, Maywood, United States

Introduction: In addition to upregulation of inflammatory mediators, patients maintained on hemodialysis because of end stage renal disease (ESRD) are subjected to periodic exposure to heparin and contact activation due to procedural settings. Recently the presence of a heparin contaminant, oversulfated chondroitin sulfate was linked with the adverse reactions and deaths in these patients.

Materials and Methods: We measured C5a anaphylatoxin and bradykinin levels in ESRD patients prior to and after maintenance hemodialysis using sandwich ELISA methods. A control group comprised of 40 normal healthy individuals was included to establish the normal level of these mediators.

Results: Both C5a and bradykinin levels were elevated in pre-dialysis samples from ESRD patients (C5a: 14.2±4.6 vs. 3.2±0.6 ng/ml, bradykinin: 9.3±2.4 vs. 4±1.8 ng/ml). Dialysis itself produced an increase in both the C5a and bradykinin levels. The postdialysis samples were further increased, suggesting that dialysis and heparinization itself result in the up-regulation of these mediators. Supplementation of heparin, contaminated heparin or isolated contaminant to the plasma also resulted in the generation of C5a and bradykinin. The plasma samples included in these studies were obtained from patients who were not treated with contaminated heparin.

Conclusions: These results suggest that both C5a and bradykinin are up-regulated in ESRD patients and this level can be further augmented by dialysis and heparinization. Therefore, additional factors may have contributed to the complex adverse reaction profiles and deaths in patients administered with contaminated heparin.

PP6.6-5

Pharmacoequivalence of enoxaparin and contaminated enoxaparin

Kuziej J¹, Jeske W¹, Hoppensteadt D¹, Litinas E¹, McGeehan E¹, Adiguzal C¹, Fareed J¹, Kennedy R¹
¹Loyola University Medical Center, Maywood, United States

Introduction: The use of heparin contaminated with oversulfated chondroitin sulfate (OSCS) was associated with severe adverse reactions. Some batches of enoxaparin also contained low levels of OSCS (<5%). To address the bioequivalence of enoxaparin and its contaminated version, studies were undertaken in established animal models of bleeding and thrombosis.

Materials and Methods: Contaminant-free enoxaparin (CFE) and one commercially available contaminated enoxaparin (CCE) batch were administered at a dosage of 2.5 mg/kg SC. Blood pressure and heart rate were measured 90 minutes post-administration, followed by jugular vein clamping model 120 minutes post-administration. Upon reaching the thrombotic endpoint, blood was collected for ex-vivo monitoring of anti-coagulant and anti-protease effects.

Results: No differences in blood pressure or heart rate were observed between the two groups. Relative to saline treated rats (3.5±0.5 clampings), both CCE and CFE treated animals required a higher number of clampings to induce thrombosis (4.8±0.7 and 5.0±0.6, respectively; p=0.001 vs. saline). A slight elevation in whole blood aPTT was observed in both enoxaparin treated groups (CFE: 36.8±18.6 sec; CCE: 30.5±10.9 sec vs. saline: 26.7±3.9 sec). Plasmatic anti-Xa activity was significantly higher with CFE (84.4±1.5% inhibition) compared to CCE (80.5±2.9% inhibition; p=0.026) while anti-IIa activity was comparable in the two groups (37.1±22.0 and 30.6±17.9% inhibition).

Discussion: Since OSCS is highly charged, it is likely not absorbed following subcutaneous administration and at levels <5%, does not impact the anti-thrombotic effects enoxaparin. The impact of repeated administration of contaminated enoxaparins and long-term pharmacodynamic and immunogenic effects need to be explored further.

PP6.6-6

Molecular and functional heterogeneity in contaminants isolated from recalled Heparin. Impact on anticoagulation and potential adverse reactions

Fareed J¹, Hoppensteadt D¹, Jeske W², Adiguzal C¹, Iqbal O¹, Walenga J²
¹Dept. Of Pathology Loyola University Medical Center, Maywood, United States ²Cardiovascular Institute Loyola University Medical Center, Maywood, United States

Introduction: The primary contaminant in recalled batches of unfractionated heparin (UFH) is reported to be oversulfated chondroitin sulfate (OSCS). It has been assumed that different heparin batches contained similar forms of OSCS.

Materials and Methods: Non-heparin contaminants were isolated from four batches of contaminated UFH and two batches of LMWH by digestion of heparin followed by alcohol precipitation and ion-exchange chromatography. Anticoagulant activities were measured using whole blood and plasmatic assays. Thrombin



generation inhibition and protamine/PF4 neutralization studies were carried out in human plasma. Each contaminant's interaction with AT and HCII was characterized.

Results: The contaminated UFHs did not exhibit major differences in molecular weight profile (14.8–15.6 kDa), USP potency or anticoagulant actions. There were differences in their anti-Xa:anti-IIa ratios (0.93–1.24) and in the amount of heparinase resistant material (14–30%). Two heparins also contained significant amounts of dermatan sulfate. Each isolated contaminant exhibited distinct neutralization profiles with PF4 and protamine. The LMWHs were comparable in molecular weight and biologic actions, but differed in heparinase-1 digestion profile. The molecular weight of the contaminant isolated from LMWH was lower (12.8 vs. 14.1–16.8 kDa). The contaminants also exhibited differences in thrombin generation inhibition. The contaminants isolated from heparin and LMWH had potencies of 28–46 USP U/mg and 38–46 USP U/mg, respectively.

Conclusions: Contaminants isolated from recalled batches of heparin are heterogeneous. Moreover, the contaminants obtained from LMWHs may exhibit additional structural and biologic differences. The variations observed in the adverse reactions with recalled heparins may be due to compositional variations in the contaminants.

PP6.6-7

Influence of naturally occurring humic acids and synthetic humic acid like polymers on the coagulation Factors IIa, VIIa and Xa

Klöcking H¹, Mahr N¹, Klöcking R², Heise K³, Herdering W⁴

¹Institute of Pharmacology and Toxicology/Working Group Erfurt, University Jena, Germany ²Research Institute for Peat and Natural Products, Zittau/Goerlitz University of Applied Sciences, Germany

³Institute of Radiochemistry, Research Centre Rossendorf, ⁴Institute of Anorganic and Analytical Chemistry, University Hamburg, Germany

Objectives: Previous studies have shown that naturally occurring humic acids (HA) and synthetic HA-like polymers prolong the clotting time of blood *in vitro* and *in vivo*. In the present study, we investigated the influence of HA and synthetic HA-like polymers on the coagulation factors IIa, VIIa and Xa *in vitro*.

Material and Methods: Test substances comprised sodium humate (NaH) from a rain moor peat, the commercially available Aldrich HA, the synthetic HA-like caffeic acid oxidation product (KOP), oxidation products from hydroquinone with L-glutamic acid (HS 130) and L-lysine (HS 136), respectively, as well as two melanoidins. FXa activity and thrombin activity were determined using the chromogenic substrate S-2765 and S-2238. FVIIa activity was determined by monitoring the clotting time in FVIIa-depleted human plasma.

Results: All the test substances inhibit FXa activity dose-dependently. The strongest effect was observed with HS 136 (IC₅₀ 36.5 µg/ml), followed by KOP (45.1 µg/ml), NaH (67.0 µg/ml), Melanoidin M42 (70.2 µg/ml), Aldrich HA (83.7 µg/ml), HS 130 (88.4 µg/ml) and Melanoidin M1 (274 µg/ml). Anti-IIa effectiveness was found for KOP (IC₅₀ 11.6 µg/ml), HS 130 (16.7 µg/ml), and NaH (60.8 µg/ml). Moreover, the test substances were able to prolong the extrinsically activated coagulation by inhibition of FVIIa. The strongest effect was observed with KOP (IC₅₀ 27.6 µg/ml), followed by HS 130, HS 136, Aldrich HS, melanoidin M1 and NaH.

Conclusions: From these results we conclude that besides thrombin also FVIIa and FXa may be considered targets of anticoagulatory effective substances of the humic acid type.

PP6.6-8

Direct thrombin inhibitors' activity measurement in plasma

Peyrafitte M¹, Vissac A¹, Amiral J¹

¹Hyphen Biomed Research, Neuville sur Oise, France

Direct Thrombin Inhibitors (DTIs: Lepirudin, Bivalirudin, Argatroban, etc.) have increasing applications in severe clinical situations associated with a high risk context, and can have promising applications as prothylactic drugs (oral absorption). When used for curative applications, laboratory methods are required for drug efficacy adjustment and for avoiding overdosage. These methods must present the most limited impact to the progressive activity of plasma Anti-Thrombin (AT), which must have no interference in the assay. A clotting method and a chromogenic assay were developed for quantitating DTIs. For the clotting assay, a substrate normal plasma pool (or a procoagulant mixture containing purified fibrinogen) is mixed with diluted test plasma (1:8 to 1:20), and coagulation is initiated with human thrombin (in the alpha form) in presence of calcium, and clotting time (CT) is recorded. A linear relationship is obtained between DTI concentration and CT (from about 30 seconds to 90 seconds). This assay has no matrix effect and can be used for any DTI. In the chromogenic kinetics assay, tested specimen (1:10) is incubated with thrombin substrate, and human thrombin is then added. Colour development measured at 405 nm is an inverse relationship of DTIs' concentration. This assay has excellent performances with Hirudin and its analogues, but is

not sensitive for Argatroban concentrations in the usual therapeutic range (this DTI has a potent anti-coagulant effect but its inhibitory potency on the thrombin chromogenic activity is low and 'protected' by AT). These methods have a dynamic range for Hirudin from 0.1 to 2.0 µg/ml, and this range can be extended from 0.25 to 5.0 µg/ml in some applications such as ECC. Specific calibrations with the DTI used are required. The clotting method offers a working range from 0.1 to 2.0 µg/ml of Argatroban. Both methods offer safe, reliable and rapid tools for measuring DTIs' activity in plasma. They introduce appropriate tools for analytical and pre-clinical studies for emerging DTIs.

PP6.7 Antithrombotics: Clinical Trials

PP6.7-1

Pharmacokinetics of dalteparin in therapeutic dosage in patients with renal insufficiency

Schmid P¹, Brodmann D², Odermatt Y¹, Fischer A², Wuillemin W^{1,3}

¹Division of Haematology and Central Haematology Laboratory, Luzerner Kantonsspital, Luzern, Switzerland, ²Division of Nephrology, Luzerner Kantonsspital, Luzern, Switzerland, ³University of Berne, Berne, Switzerland

Objectives: Low-molecular-weight heparins (LMWH) are effective, safe and convenient for therapeutic anticoagulation. Their use is limited in patients with renal insufficiency (RI) due to bioaccumulation. The aim of this study was to evaluate pharmacokinetics of dalteparin in therapeutic dosage in patients with various levels of renal function.

Design and Methods: Prospective observational cohort study. Inpatients therapeutically anticoagulated with dalteparin were included according to stage of RI: group A = no RI, group B = mild/moderate RI (30–59 ml/min/1.73m²), group C = severe RI (GFR < 30 ml/min/1.73m²). Peak plasma anti-Xa activity (anti-Xa) was measured and adjusted to applied dalteparin dose and body weight after first dose, on day 2, and every 2nd day afterwards. Bioaccumulation factor R was calculated as quotient of last and first adjusted anti-Xa. Data is shown as median (interquartile range, IQR).

Results: Thirty-two patients (23 men) receiving dalteparin for ≥ 2 days were analyzed. Median follow up was 6 days (IQR 4–10, range 2–22). Median dose was 90 (73–106) units/kg/12h s.c. without difference among the groups (p=0.68). Calculated R was 1.46 (1.15–1.82, n=18) in group A, 1.36 (1.20–2.16, n=9) in group B, and 2.28 (1.53–2.93, n=5) in group C. Although there was a trend for increased R in patients with severe RI, group C was not significantly different from group A (p=0.17) or group B (p=0.51).

Conclusions: There is a trend for increased bioaccumulation of therapeutically dosed dalteparin in patients with severe RI. A larger study is necessary to investigate the significance of these findings.

PP6.7-2

Effects of pantoprazole and esomeprazole on platelet inhibition by clopidogrel

Siller-Matula J¹, Spiel A¹, Lang I¹, Kreiner G¹, Christ G¹, Jilma B¹

¹Medizinische Universität Wien, Österreich

Objectives: Clopidogrel is activated by CYP2C19 which also metabolizes proton pump inhibitors (PPI). As proton pump inhibitors are metabolized to varying degrees by CYP2C19, we hypothesized that the reported negative omeprazole-clopidogrel drug interaction may not be a class effect.

Design and Methods: Responsiveness to clopidogrel was assessed by the vasodilator stimulated phosphoprotein (VASP) phosphorylation assay and aggregometry (Multiplate Analyzer) in 300 patients with coronary artery disease (CAD) undergoing percutaneous coronary intervention (PCI).

Results: The mean platelet reactivity index (PRI, assessed by VASP assay) was nearly the same in patients with (n=226; PRI=51%) or without PPI treatment (n=74; PRI=49%; p=0.724). Likewise, the ADP-induced platelet aggregation did not differ significantly between patients with or without PPI treatment (45U vs. 41U; p=0.619). Similarly, there was no difference in the PRI or the ADP-induced platelet aggregation between patients with pantoprazole (n=152; PRI=50%; aggregation=47U), esomeprazole (n=74; PRI=54%; aggregation=42U) or without PPI (n=74; PRI=49%; aggregation=41U; p=0.382).

Conclusion: In contrast to the reported negative omeprazole-clopidogrel drug interaction, the intake of pantoprazole or esomeprazole is not associated with impaired response to clopidogrel.

PP6.7-3

VKORC1-, CYP2C9- and CYP3A5-polymorphisms in patients with complicated phenprocoumon therapy

 Werner D¹, Werner U², Würfel A¹, Lestin H³, Grosch A³, Eschenhagen T², Rau T²
¹Department of Cardiology / Angiology, Protestant Hospital Ludwigslust, Germany ²Institute of Experimental and Clinical Pharmacology, University Hamburg, Germany ³Institute for Laboratory Medicine, HELIOS-Hospital Schwerin, Germany

Introduction: Different studies demonstrated the impact of genetic polymorphisms of vitamin-K-epoxide-reductase (VKORC1) and the hepatic cytochromes CYP2C9 and CYP3A5 on pharmacodynamics and pharmacokinetics of vitamin K antagonists. However, data regarding a pharmacogenetic tailored anticoagulation are conflicting. In the present study, we asked for the distribution of these polymorphisms in patients with difficult anticoagulation

Patients and Methods: We investigated allele- and genotype-frequencies of polymorphisms in VKORC1 (Haplotypes A, B; tag-SNP: G6853C), CYP2C9 (reduced function alleles: *2, *3) and CYP3A5 (active allele *1) in 60 consecutive patients with clinically complicated anticoagulation. Patients were included due to: A) low phenprocoumon-dosing (≤ 1.5 mg/d; n=46); B) overanticoagulation for ≥ 1 week after treatment-start (n=6) or C) lack of normalization of the INR for ≥ 1 week after phenprocoumon-pausing (n=8). Results were compared to frequencies obtained in 120 healthy controls.

Results: Allele frequencies of interest between patients and controls were distributed as following: CYP2C9*2: 17.5 vs. 10.8 % ($p < 0.05$); CYP2C9*3: 11.7 vs. 5.8 % ($p < 0.001$), and CYP3A5*1: 16.8 vs. 8.3 % (ns.) The rate of two non-wildtype alleles of CYP2C9 was 4-fold higher in patients. The frequency of the less active VKORC1-C allele was 70.0 % vs. 41.7 % ($p < 0.0001$) in patients compared to controls. Two less active VKORC1-haplotypes A were 2.7-fold more frequent in patients.

Conclusions: Patients had increased frequencies of genotypes associated with reduced VKORC1-activity and CYP2C9-genotypes with non-wildtype alleles. Thus genotyping of VKORC1 and CYP2C9 may serve as predictor of recurrence of over-anticoagulation or bleedings in patients with problematic anticoagulation.

PP6.7-4

An oral vitamin K protocol to reverse over-anticoagulation in patients presenting with an INR above 10.0

 Denas G¹, Padayattil Jose S¹, Cucchini U¹, Pengo V¹, Illiceto S¹
¹University Of Padova School Of Medicine, Padova, Italy

The use of low dose oral vitamin K to correct over-anticoagulation is nowadays widely accepted, but apparent differences between preparations and the INR-related dosages vary. We conducted a retrospective cohort study to assess the efficacy and safety of a 3 mg oral vitamin K protocol in correcting INR values > 10 .

Methods and materials: The protocol, designed and used at our Anticoagulation Clinic (Padua), consisted in the dispensation of 3 drops of oral vitamin K (Konaktion[®], 1 drop=1mg) together with the omission of the day's dose of warfarin to all asymptomatic patients with INR > 10 . INR values were checked the following day and the warfarin dosage adjusted accordingly. Patients were followed-up for 30 days and bleeding and thromboembolic events were recorded; the eventual vitamin K-induced warfarin-resistance (the mean of the two INRs after resuming warfarin below 1.5) was also assessed.

Results: Of the 225 events considered from 1997 to 2007, 105 (104 patients) met the selection criteria. The median INR (interquartile range) at presentation was 11.3 (10.6-13.1) and it fell to 2.9 (2.2-3.7) within 20 hours of vitamin K administration ($p < 0.0001$); 86 % of the INRs were below 4.5. Sixteen percent of the INRs were overcorrected (INR < 2.0), while 2 patients developed warfarin-resistance. During follow-up, 2 minor bleeding episodes and no thromboembolic events were reported.

Conclusion: The results of this study are satisfactory and our protocol proves to be safe, effective, and easily applied in the primary care setting.

PP6.7-5

Venous thromboembolism risk and prophylaxis in the Acute Hospital Care Setting: German and global results

 Zoltz R¹, Cohen A², Tapson V³, Bergmann J⁴, Goldhaber S⁵, Kakkar A⁶, Anderson F⁷
¹Praxis Für Hämostaseologie Und Transfusionsmedizin, Düsseldorf, Germany ²King's College Hospital, London, UK, ³Duke University Medical Center, Durham, NC, USA, ⁴Hôpital Lariboisière, University Paris 7, Paris, France, ⁵Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA, ⁶Barts and The London School of Medicine Thrombosis Research Institute, London, UK, ⁷Center for Outcomes Research, University of Massachusetts Medical School, Worcester, MA, USA

Introduction: ENDORSE (Epidemiologic International Day for the Evaluation of Patients at Risk for Venous Thromboembolism in the Acute Hospital Care Setting), is a multinational, observational, cross-sectional survey, designed to assess

the prevalence of VTE risk in the acute hospital care setting, and to determine the proportion of at-risk patients who receive effective prophylaxis.

Methods: Patients were enrolled from 358 randomly selected hospitals in 32 countries, encompassing 6 continents. Enrolled patient charts were reviewed including, medical history, current medical conditions, type of surgery, initiation and type of VTE prophylaxis. The American College of Chest Physicians (ACCP) evidence-based consensus guidelines were employed to evaluate VTE risk and prophylaxis use.

Results: In Germany [global results in brackets] of 2,370 [68,183] patients, 1,210 (51 %) [30,827 (45 %)] and 1,160 (49 %) [37,356 (55 %)] were categorized as surgical or medical, respectively. Based on ACCP criteria, a mean of 41 % [52 %] of enrolled hospital in-patients were judged to be at risk for VTE, including 69 % [64 %] of surgical and 41 % [42 %] of medical patients. Of the surgical and medical patients, 92 % [59 %] and 70 % [40 %] received recommended VTE prophylaxis, respectively.

Conclusions: ENDORSE demonstrates the high prevalence of patients at risk for VTE and the low rate of prophylaxis use worldwide. In Germany the rate of prophylaxis use appears to be higher than the global average rate. However, our data reinforce the rationale for urgently implementing hospital-wide strategies for systematically assessing patient VTE risk and for providing appropriate prophylaxis.

PP6.7-6

Capillary bleeding under oral anticoagulation

 Leithäuser B¹, Mrowietz C², Pindur G³, Sternitzky R⁴, Jung F⁵
¹Angiologische Praxis, Hannover, Germany ²Institut für Herz- und Kreislaufforschung,

³Universitätsklinik des Saarlandes, Germany ⁴Praxisklinik Herz und Gefäße, ⁵Berlin Brandenburg Zentrum für Regenerative Therapien, Germany

Background: Oral anticoagulants are routinely used for prevention of thromboembolism in cardiac, arterial or venous diseases. Hemorrhages are serious treatment complications, frequently occurring under long-term or high-dose regimens. From animal experiments it is known that coumarin-type anticoagulants may cause increased capillary permeability, red blood cell extravasation and punctate bleeding. Controlled human trials are lacking.

Methods: 31 patients under oral anticoagulation were examined by video capillary microscopy. 48 patients with comparable diseases and treatment but without oral anticoagulation served as controls. Nailfold capillaries of four fingers of each hand were examined and analyzed off-line according to the following criteria: 1. Numbers of capillaries investigated, 2. numbers of capillary bleedings, and 3. bleeding incidence (bleedings per 100 capillaries).

Results: In 23 out of 31 patients (74.2 %) capillary bleedings were observed. The bleeding incidence ranged from 0.33 to 4.29 per 100 capillaries. In contrast, only 4 out of 52 controls were detected with capillary bleedings (2.1 %, $p < 0.001$). The bleeding incidence was 0.34 - 2.41. In patients on anticoagulation there was no correlation between the number of capillary bleedings and the INR or Quick values. During a two year follow-up of patients on oral anticoagulation no significant difference was found in terms of clinically obvious bleedings in patients with or without capillary bleedings.

Conclusion: This study shows that punctate bleedings can be demonstrated in patients on oral anticoagulation. Bleedings occur independent of the INR-value. Thus, other factors than the vitamin-k-dependent coagulation effect seem to be causal for the damage of microvessels.

PP6.7-7

Incidence and causes of heparin-induced skin lesions

 Schindewolf M¹, Schwane S², Wolter M², Kroll H³, Kaufmann R², Boehncke W², Ludwig R⁴, Lindhoff-Last E¹
¹Universitätsklinikum Frankfurt am Main - Zentrum der Inneren Medizin, Medizinische Klinik III, Angiologie/Hämostaseologie, Frankfurt am Main, Germany ²Universitätsklinikum Frankfurt am Main - Zentrum der Dermatologie und Venerologie, Frankfurt am Main, Germany ³DRK-Blutspendedienst NSTOB, Dessau - Institut für Transfusionsmedizin, Dessau, Germany ⁴Universitätsklinikum Schleswig-Holstein - Campus Lübeck - Klinik für Dermatologie, Allergologie und Venerologie, Lübeck, Germany

Objectives: Despite heparin-induced skin lesions are increasingly being reported, little is known about the incidence and causes. Life-threatening, immune-mediated, heparin-induced thrombocytopenia (HIT) and delayed-type hypersensitivity reactions (DTH) are among the two most frequently reported causes for heparin-induced skin lesions. Hence, there has been a great medical need to determine incidence and causes of heparin-induced skin lesions.

Design and Methods: We prospectively examined medical patients treated subcutaneously with heparins for presence of heparin-induced skin lesions. If a skin lesion was observed, the underlying cause was determined by histology and/or allergologic skin testing and diagnostics for HIT. Overall, 320 patients were recruited.

Results: We observed 24 patients with heparin-induced skin lesions, amounting to an incidence of 7.5 %. DTH reactions were identified as the underlying cause in



all patients. Comparison of study subjects with DTH reactions to patients without heparin-induced skin lesions, identified clearly obesity, pregnancy, long duration of heparin therapy and female gender as patient-related risk factors for the development of heparin-induced skin lesions.

Conclusions: In conclusion, heparin-induced skin lesions have to be considered common adverse events and are in the vast majority of patients due to a type IV allergic response. In contrast, other causes of heparin-induced skin lesions such as HIT or type I hypersensitivity responses are rare. Yet, diagnosis of heparin-induced cutaneous DTH does not rule out systemic HIT. Physicians need to be aware of the frequency and differential diagnosis of heparin-induced skin lesions.

PP6.7-8

Anticoagulant effects of low-molecular-weight heparin certoparin in combination with phenprocoumon in patients with recent atrial fibrillation undergoing cardioversion

Harenberg J¹, Wierdack J¹, Giese C¹, Weiss C², Abletshauser C³, Tebbe U⁴

¹Clinical Pharmacology University Medicine Mannheim, Germany ²Institute for Biometrics and Statistics University Medicine Mannheim, Germany ³Novartis Pharma GmbH Nuremberg, Germany ⁴University Hospital Lippe, Germany

Objectives: The effect of a combined treatment with low-molecular-weight heparin (LMWH) certoparin and phenprocoumon was analysed in patients with recent onset of atrial fibrillation (AF) on coagulation parameters.

Design and Methods: Patients with recent AF (n=200) received 8.000 IU LMWH certoparin bid upon entry into the study. Phenprocoumon was started after electrical cardioversion. LMWH was stopped after 2 days within therapeutic INR range of phenprocoumon. Blood samples were taken before LMWH (visit 1, V1), before phenprocoumon (V4) and after reaching the desired INR range and before stopping LMWH (V5). The study was accepted by the local ethics committees and patients gave written informed consent.

Results: Activated partial thromboplastin time, chromogenic factor Xa assay, heparin, prothrombinase-induced clotting time and tissue factor pathway inhibitor differed between V1 and V4 as well as between V1 and V5 (all p<0.001) but not between V4 and V5. Prothrombin time was prolonged at V5 versus V1 and V4 (both p<0.001). D-Dimer and prothrombin fragment F1+2 significantly decreased from V1 to V4 and from V4 to V5 (all p<0.001). Endogenous thrombin potential assay (ETP) was inhibited stronger at V4 compared to V1, and at V5 compared to V4 (both p<0.001).

Discussion: D-Dimer, F1+2 and ETP are the parameters reflecting the combined anticoagulant effect of certoparin and phenprocoumon while overlapping anticoagulation with these compounds in patients with recent AF for electrical cardioversion. The clinical effects on thromboembolic or bleeding incidences remain to be determined.

PP6.7-9

Influence of genetic polymorphisms of VKORC1 and CYP2C9 in patients on phenprocoumon steady-state dose requirements

Harenberg J¹, Wu W¹, Weiss C², Kirchheiner J³, Stehle S³, Fuhr U⁴, Gleiter C⁵

¹Clinical Pharmacology, University Medicine Mannheim, Germany ²Department of Medical Statistics, University Medicine Mannheim, Germany ³Institute of Pharmacology of Natural Products and Clinical Pharmacology, University Ulm, Germany ⁴Department of Pharmacology, University Cologne, Germany ⁵Department of Clinical Pharmacology, University Tuebingen, Germany

Objectives: The variants of the (Vitamin-K epoxid reductase subcomplex 1) VKORC1 and of cytochrome P450 2C9 (CYP2C9) genes influence the steady state dose of the vitamin-K antagonist (VKA) phenprocoumon during oral anticoagulant therapy.

Design and Methods: We have analysed the influence of the VKORC1 1173CC and 1173CT variants in combination with the CYP2C9 variants on the steady state dose of phenprocoumon (n= 114 patients). The weekly phenprocoumon dosage was calculated after a 3 to 6 months of stable anticoagulation by the international normalized ratio (INR) which had to be in a range from 2 to 3. VKORC1 1173CC, CT, TT and CYP2C9*1/*1, *1/*2, *1/*3, *2/*2, *2/*3 and *3/*3 genotypes were determined using direct DNA sequencing and realtime PCR.

Results: Patients carrying VKORC1 1173CT and CYP2C9*1/*1 genotypes required a 33% lower steady state dose of phenprocoumon compared to VKORC1 1173CC and CYP2C9*1/*1 genotypes (p=0.0001). Patients with VKORC1 1173CC in combination with CYP2C9*1/*2, *1/*3 needed 10% and 34% lower steady state dose of phenprocoumon compared to the combination with CYP2C9*1/*1, respectively (p=0.0001). Patients carrying VKORC1 1173CT needed lower doses of phenprocoumon in combination with CYP2C9*1/*1 (-32%), *1/*2 (-29%), or *1/*3 (-33%), and VKORC1 1173TT in combination with CYP2C9*1/*3 (-44%) compared to VKORC1 1173CC combined with CYP2C9*1/*1 (all p<0.001). CYP2C9 variants did not influence the dose of carriers of VKORC1 1173CT gene.

Discussion: Both genotypes influenced the phenprocoumon maintenance dosage. Genotyping for CYP2C9 and VKORC1 therefore might be a suitable tool for dose individualization in patients at oral anticoagulant drug therapy.

PP6.7-10

Tinzaparin compared to unfractionated heparin for initial treatment of DVT in very elderly patients with renal insufficiency – the IRIS trial

Bauersachs R¹, Leizorovicz A² on behalf of the IRIS Investigators

¹Dept. Vascular Medicine, Klinikum Darmstadt, Germany, ²Unité de Pharmacologie Clinique, Université Claude Bernard Lyon I, France

Objectives: Tinzaparin pharmacokinetics suggests little accumulation in impaired renal function. Safety and efficacy of Tinzaparin in therapeutic dose was tested against aPTT adjusted UFH in elderly patients.

Design and Methods: IRIS (Innohep® in renal insufficiency): International, multicentre, open, centrally randomised, parallel group study. Patients ≥75yrs with creatinine-clearance (CrCl) of ≤60ml/min, or ≥70yrs with CrCl of ≤30ml/min and DVT were randomised to Tinzaparin 175 IU/kg,od,sq or aPTT titrated heparin, bid,sq for at least 5 days and until therapeutic INr. Both groups received VKA to d90. Primary endpoint: Clinically relevant bleeds (CRB) by d90, secondary endpoints recurrent VTE and death. After a planned interim analysis, DMC advised premature stopping of the study.

Results: Full analysis set of 537 patients followed to day 90: Mortality rates were 6.3% vs. 11.2% for UFH and Tinzaparin (p=0.049). Mortality difference was not due to recurrent VTE or bleeding. The Kaplan-Meier curves diverged on average 20 days after UFH/Tinzaparin had been discontinued. Most of the difference in mortality was in those >90yrs. Below 90yrs mortality was 5.9% vs. 7.8%. Bleedings: CRB was 11.9% in both groups by d90 and was also equal (7.1%) until d12. Recurrent VTE was 1.1% for heparin vs. 2.6% for Tinzaparin (p=0.203). Several risk factors were imbalanced at randomisation, multivariate analysis showed no difference between the two treatments.

Conclusions: IRIS showed no hint of excess bleeding of Tinzaparin in elderly subjects with renal insufficiency. Whether there is a true difference in terms of VTE or mortality in the elderly remains to be confirmed.

PP6.7-11

Thromboprophylaxis with Certoparin in acutely ill, non-surgical patients – certain study

Schellong S¹, Haas S², Greinacher A³, Sieder C⁴, Abletshauser C⁵, Riess H⁶

¹II. Med. Klinik, Krankenhaus Dresden-Friedrichstadt, Germany, ²Institut für experimentelle Onkologie, Klinikum rechts der Isar der Technischen Universität München, Germany, ³Institut für Immunologie und Transfusionsmedizin, Universitätsklinikum Greifswald, Germany, ⁴Medical Department, Novartis Pharma Nuernberg, Germany, ⁵Medical Department, Novartis Pharma Nuernberg, Germany, ⁶Medizinische Klinik, Campus Virchow - Klinikum der Charité – Universitätsmedizin Berlin, Germany

In general medical patients, only a limited number of studies did evaluate the efficacy and safety of either low-dose UFH or LMWH. This pilot study was designed to generate outcome data for thromboprophylaxis in acutely-ill, non-surgical patients with the LMWH Certoparin (Mono-Embolex®NM) when compared to low dose UFH.

Design/Methods: Acutely ill, hospitalized, non-surgical patients aged >40 years were randomized to open-label Certoparin (3000U aXa od) or to UFH (7500IU bid) for a treatment period of 10±2 days. The primary efficacy endpoint included objectively confirmed, asymptomatic or symptomatic proximal or distal DVT, symptomatic PE, and death related to VTE. Bilateral complete compression ultrasound was performed at end of treatment. The non-inferiority-margin for Certoparin versus UFH was set at 4%. A blinded central adjudication committee did evaluate all endpoints. Standard criteria for endpoints, adverse events, serious adverse events, and major bleeds were applied.

Results: 337 patients were randomized, 244 had a valid outcome assessment. Mean age was 71 years. AEs leading to discontinuation occurred in 5.2% (UFH) versus 2.5% (Certoparin) of the patients. The incidence of thromboembolic events was 14.9% (UFH) versus 8.9% (Certoparin). Hence, non-inferiority could be demonstrated (-5.9%; 95CI: -14.0,+2.2), and a strong trend in favor for Certoparin emerged. SAEs occurred in 6.3% (UFH) versus 3.7% (Certoparin), major bleeds in 2 (UFH) versus 0 (Certoparin) patients, respectively.

Conclusions: In acutely ill, non-surgical patients, thromboprophylaxis with Certoparin 3000U anti Xa od seems to have a favorable efficacy/safety-profile. A large double-blind study based on this data is warranted.

PP6.7-12

Teststrip-based genotyping to assist in the prediction of anticoagulant dose requirement

Puehringer H¹, Klose G², Schreyer B², Krugluger W³, Loreth R², Oberkanins C¹
¹ViennaLab Diagnostics GmbH, Vienna, Austria, ²Clinical Haemostaseology, Westpfalz-Klinikum GmbH, Kaiserslautern, Germany, ³Institute of Laboratory Medicine, Donauespital, Vienna, Austria

Background: Coumarin derivatives (warfarin, phenprocoumon) are the most widespread oral anticoagulant drugs for the prevention and treatment of thromboembolic disorders. However, these vitamin K antagonists have a narrow therapeutic range and a wide interindividual variability in dose requirement. Despite adjustment for clinical variables, adverse events are frequently encountered during the initial phase of therapy. Genetic polymorphisms in the drug-targeted vitamin K epoxide reductase complex 1 (VKORC1) and in the drug metabolizing enzyme CYP2C9 have been reported to account for the majority of variations in the therapeutic response to warfarin.

Aims and Methods: A genetic test (StripAssay) for the simultaneous detection of two VKORC1 polymorphisms (-1639G>A, 3730G>A) and the functionally defective CYP2C9 variants *2 (430C>T) and *3 (1075A>C) was developed. The protocol is based on multiplex PCR and reverse-hybridization of biotinylated amplification products to allele-specific probes on membrane teststrips. The new StripAssay is currently being used in an ongoing clinical study to classify patients into high, intermediate and low dose responders to coumarin anticoagulants.

Results: Preliminary data based on more than 130 patients treated with phenprocoumon (Marcumar) indicated a considerably lower stable dosage required for therapeutic anticoagulation in carriers of a combined VKORC1 -1639A and CYP2C9 *2 or *3 genotype compared to carriers of a single variation or wildtype alleles. The VKORC1 3730G>A polymorphism seemed to have no additional predictive power for phenprocoumon dose variability.

Summary and Conclusions: The new diagnostic assay and the results obtained during our study will assist clinicians to achieve a safer and more individualized anticoagulant therapy.

PP6.7-13

Prevention of venous thrombosis in cancer patients: a prospective, randomized, double-blind study comparing two different dosages of low-molecular weight heparin (LMWH)

Traby L¹, Kaider A², Quehenberger P³, Kyrle P¹, Eichinger-Hasenauer S¹
¹Univ. Klinik für Innere Medizin I, Klinische Abteilung Für Hämatologie und Hämostaseologie, Wien, Österreich ²Besondere Einrichtung für Medizinische Statistik und Informatik, ³Klinisches Institut für Medizinische und Chemische Labordiagnostik

Objectives: In surgical cancer patients thromboprophylaxis with ~5000 aXa U LMWH is standard, but non-surgical cancer patients may benefit from a higher dose.

Methods and Design: In a prospective, double-blind trial non-surgical cancer patients with thrombotic risk factors were randomised to enoxaparin 40mg or 80mg od sc. D-Dimer (Asserachrom® D-Di, Roche, Germany), and thrombin generation (peak thrombin, Technothrombin TGA, Austria) were measured in venous blood before enoxaparin on day 1–4 and 2, 4, and 6 hours after enoxaparin on day 1.

Results: 22 patients were randomized to enoxaparin 40mg and 27 to 80mg. D-dimer levels [median (range)] before enoxaparin (=baseline) were elevated [40mg:957.0 ng/ml (254.1–4419.6); 80mg:1054.9 ng/ml (197.0–14761.0)], and decreased after 6 hours in both groups (p=0.001). D-Dimer baseline levels in the 80mg group were lower on day 4 [1785.6 ng/ml (128.4–13742.5)] than on day 1 (p=0.01) with no difference in the 40mg group. Peak thrombin levels (mean±SD) at baseline were 434.8±29.7 nm (40mg) and 407.8±18.9 nm (80mg). In the 80mg group, peak thrombin levels were 138.7±19.6, 86.6±19.9 and 82.8±19.9 nm after 2, 4 and 6 hours, and were significantly lower than in the 40mg group. Compared with baseline on day 1, peak thrombin levels in the 80mg group were lower on day 4 (317.5±28.3 nm; p=0.005) with no difference in the 40mg group (p=0.5).

Conclusion: In non-surgical cancer patients, enoxaparin 80mg significantly reduces the extent of coagulation activation and thrombin generation as compared with 40mg. Interventional studies are needed to investigate if these patients could benefit from intensified thromboprophylaxis.

PP6.7-14

Biomarkers and coagulation tests for assessing biosimilarity of a generic LMWH: results of a study in healthy subjects with enoxaparin

Kuczka K¹, Blume H², Donath F², Warnke A², Harder S¹
¹Pharmazentrum Frankfurt/ZAFES, Institute of Clinical Pharmacology At The Johann Wolfgang Goethe-University Frankfurt/Main, Germany, ²SocraTec R&D GmbH, Oberursel, Germany

Objectives: LMWHs differ regarding influence on clotting tests and TFPI release. Biosimilarity therefore becomes an issue with generic LMWHs. No bioequivalence study on generic LMWHs has been reported before.

Design and methods: A generic enoxaparin ("test") was compared with the originator ("reference") in 20 volunteers after single subcutaneous administration of 40mg enoxaparin (4000IU/ml anti-FXa). Target variables were anti-FXa and anti-FIIa activity, aPTT, PiCT and TFPI over 24h. Bioequivalence was analyzed by the geometric mean ratios of test/reference and their confidence intervals (CIs).

Results: The anti-FXa activity profile demonstrated bioequivalence of test and reference with CIs of 93–99% (AUC0-tlast) and 88–95% (Amax). Anti-FXa activity (AUC0-tlast) was slightly lower for test compared to reference (2.57 vs. 2.69h*IU/ml), as the maximum anti-FXa activity was slightly lower for the test product (0.38IU/ml) compared to reference (0.42IU/ml). CIs of AUC0-tlast (89–102%) and Amax (90–103%) of anti-FIIa activity also fulfilled bioequivalence criteria. Anti-FIIa activity (AUC0-tlast) was similar after administration of test compared to reference (0.28 vs. 0.30h*IU/ml). 90% CI for cmax of TFPI ranged from 90–113%. cmax of total TFPI (baseline subtracted) was 13.0ng/ml (test) and 12.9ng/ml (reference), respectively. There was only a fair correlation between anti-FXa-activity and TFPI-release (r2 =0.2; p<0.05). aPTT and PiCT profiles also were superimposable.

Conclusions: Bioequivalence with the originator enoxaparin was demonstrated by ex vivo inhibition of FXa and FIIa activity, coagulation tests (aPTT, PiCT) and in vivo TFPI release. Whether such data also prove biosimilarity needs to be consented.

PP6.7-15

External quality control for coaguheck INR monitors: a new concept

Meijer P¹, Klufft C¹
¹ECAT Foundation

Point-of-care testing of the INR is becoming more and more common practice for warfarin anticoagulation monitoring. Proper performance is of major importance because of the direct link between INR measurement and anticoagulation therapy. The European Concerted Action on Anticoagulation (ECAAA) has developed a quality control system using a set of 5 certified plasma samples. In a recent study performed by the ECAT Foundation the usefulness of this control programme was established. On the basis of the results of this study a new concept for an external quality control programme was designed. The quality control is performed by patients themselves by means of „user-friendly“ instructions. After return of the results these are immediately evaluated using a linear regression model for the comparison of the test results with the assigned values. On the basis of the assessed slope, intercept, regression coefficient and number of samples within the acceptance limits, a monitor passes quality control or does not. If a monitor does not pass, it is in addition checked by the Anticoagulation Clinic against a reference monitor using whole blood. A recent pilot study within The Netherlands has shown the feasibility of this approach. An important feature of this quality control system is the ability to monitor the accuracy of the test results within the clinically important INR range from 2.0 – 4.5. This quality control approach may assist in the over-all quality of home-testing of the INR.

PP6.7-16

Reducing complications associated with the therapeutic and prophylactic use of anticoagulants – experiences from an interdisciplinary corporation-wide patient's safety project

Gerdson F¹, Alsen H¹, Arendt U¹, Kersting T¹, Klein-Weigel P¹
¹for the members of the DRK Kliniken Berlin corporation wide working group on safety aspects of anticoagulant therapy, DRK Kliniken Berlin, Berlin, Germany

Errors associated with the use of anticoagulants substantially contribute to patient's morbidity and mortality. Therefore, Joint Commission International in 2008 defined reduction of harm associated with the use of anticoagulants as one of their leading patient's safety goals. Feeling constrained by that idea, we initiated an interdisciplinary corporation-wide patient's safety project to minimize critical incidents. Therefore an interdisciplinary working group was initiated and standards for the safer use of anticoagulants in the therapy and prophylaxis of thromboembolic diseases were defined and distributed. Thereafter, the corporation-wide implementation was



systematically evaluated. We will report about critical issues of a safer use of anticoagulants and the results of a cross-sectional study evaluating the corporation-wide implementation of our standards in the field of thrombosis prophylaxis in more than 400 surgical and medical patients.

PP6.7-17

Therapeutic long term anticoagulation with two different LMWHs in patients of a neurological rehabilitation clinic

Kuczka K¹, Baum K², Harder S¹

¹Pharmazentrum Frankfurt/ZAFES, Institute of Clinical Pharmacology At The Johann Wolfgang Goethe-University Frankfurt/Main, Germany, ²Asklepios Schlossberg Klinik, Bad König, Germany

Objectives: Therapeutic LMWH treatment becomes necessary if oral anticoagulation is not feasible, e.g. due to severe disability. However, data over longer time periods on LMWH-associated coagulation parameters are rare. We investigated anti-FXa activity (aFXa), TFPI and D-dimer levels of patients in early neurological rehabilitation over 2 months in an observational cohort study.

Design and methods: Blood of 29 patients (16 males/13 females, median age 76 years [range 46–84] under therapeutic treatment with enoxaparin or tinzaparin was drawn before and 4h after morning injection on day 7 and 2 months after treatment initiation. Data were analyzed by separation of 2 cohorts (tinzaparin cohort: n=15/enoxaparin cohort: n=14), and t-test statistics was used.

Results: Although median dosage (7.000IU/ml aFXa) was comparable for both cohorts, the 4h values of anti-FXa activity (0,76+/-0,31IU/ml; p<0,05) and TFPI levels (84+/-38ng/ml; n.s.) of enoxaparin cohort exceeded those of tinzaparin cohort (0,43+/-0,22IU/ml and 75+/-34ng/ml) after two months of LMWH therapy, and stronger accumulation of aFXa was seen with enoxaparin (p<0,05). The D-dimer levels exceeded a cut-off value (500ng/ml) in most patients at both timepoints, but decreased significantly in both cohorts, e.g. from 1584+/-579 (day 7) to 792+/-546ng/ml (month 2) in Tinzaparin cohort (p<0,05).

Conclusions: In this observational study, enoxaparin seems to confer higher inhibition of FXa activity, and slight accumulation might occur. TFPI levels however seem comparable. Due to the overall high D-dimer levels, interpretation of D-dimer values seems uncertain in this population of long term immobilized patients.