

## Poster Presentations

## PP1 Coagulation Factors and Inhibitors

## PP1.1 Laboratory Methods and Experimental Models

## PP1.1-1

## Substrate specificity of VKORC1

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**Objectives:** Vitamin K epoxide reductase C1 (VKORC1) acts in vitamin K recycling in order to enable its reutilisation. Vitamin K is a co-factor of  $\gamma$ -carboxylase, an enzyme responsible for functional active blood coagulation factors. Carboxylation requires hydroquinone and results in its conversion to vitamin K epoxide which is rebuilt to quinone and further to hydroquinone by VKORC1. The results of our in vitro experiments present comparative data for VKORC1 enzyme kinetics using both, vitamin K1 and K2 epoxide in competition as substrate.

**Methods:** GC-MS analysis was done to confirm the identity of vitamin K1, K2 and their epoxides. Enzymatic activity of VKORC1 was measured by vitamin K1 and K2 quinone production in transfected HEK cells. Separation and detection of substrates and products was carried out by HPLC at 254 nm.

**Results:** Differences were observed comparing single and concurrent K1 and K2 quinone production rate. Using single epoxide as substrate, the VKORC1 showed a considerably 1.5-fold higher preference for the reduction of K2 epoxide compared to K1 epoxide. Furthermore, the formation rate of K2 quinone was 2.5 to 3.5-fold higher when K1 and K2 epoxide in various ratios were added as substrate in order to explore the competitive conversion by VKORC1. This confirmed the enzyme's ability to reduce K2 epoxide significantly more efficiently than K1 epoxide.

**Conclusions:** The competitive conversion of K1 and K2 epoxides by VKORC1 to the correspondent quinones indicated significantly higher preference of the enzyme for using K2 epoxide as substrate and faster production of K2 quinone.

## PP1.1-2

## Selective measurement of human recombinant von Willebrand Factor (rVWF)

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**Objectives:** rVWF that is expressed in Chinese hamster ovary (CHO) cells can hardly be distinguished from plasma-derived VWF (pdVWF). Minute differences are caused because CHO cells lack an alpha2,6-sialyltransferase. Consequently, N-acetylneuraminic acid (Neu5Ac) occurs on the N-glycans of rVWF only in alpha2,3-linkage. We exploited this difference to develop a lectin-based immunoassay that allows selective measurement of human rVWF in human plasma.

**Method:** rVWF/pdVWF was captured by a polyclonal rabbit anti-VWF antibody. alpha2,6-Bound Neu5Ac was then detected using the biotinylated Sambucus nigra agglutinin (SNA) and streptavidin peroxidase.

**Results:** We obtained linear dose-response curves in human plasma ranging from 0.2 to 2.9 mIU pdVWF:Ag/mL. In contrast, rVWF and pdVWF after desialylation showed no response, even at high concentrations. To validate our assay we spiked normal human plasma with rVWF. We compared the VWF:Ag-normalized levels of alpha2,6-linked Neu5Ac in these samples with those calculated on the assumption that all VWF:Ag had only alpha2,6-linked Neu5Ac. The difference between the calculated and the actual levels found correlated ( $R^2=0.99$ ) with the known amount of rVWF present in the mixtures. Thus, we obtained a calibration curve that allowed measurement of rVWF in a range of 0.2 to 1.5 IU/mL in the presence of 1 IU/mL pdVWF.

**Conclusions:** rVWF and endogenous pdVWF can be differentiated based on minute differences between their N-glycan structures. This assay could be useful in the clinical setting to specifically determine the amount of rVWF derived from CHO cells.

## PP1.1-3

## The PT-derived fibrinogen method should not be used for detection of dysfibrinogenemia

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Two laboratory methods are predominantly used to determine plasma fibrinogen concentration (PFC), the Clauss assay and the prothrombin time (PT) derived method.

**Methods:** We studied the fibrinogen parameters in 24 patients with dysfibrinogenemia (14 F, 10M; age median: 49 years). PFC were measured by PT-derived as well as Clauss method on two different coagulometers utilizing different reagents.

**Results:** The median PFC by Clauss assay was 49.5 mg/dl (34 - 207 mg/dl, normal range: 267-437) and 55 mg/dl (50 - 220 mg/dl, normal range: 150 - 450) respectively. The median PFC by PT-derived method was 251 mg/dl (77 - 487 mg/dl, normal range: 184 - 480) and 272 mg/dl (122 - 439 mg/dl, normal range: 200 - 400) respectively. The PT-derived method "overestimated" the PFC by about 5 times the value measured by the Clauss assay. About a third of the patients with dysfibrinogenemia (8/24 respectively 5/24) had levels above 300mg/dl with the PT-derived method utilizing both reagents. No correlation of the results was found when comparing both methods utilizing two reagents from different manufacturers. The distribution and deviation of the results was extremely high, the mean difference was -186 mg/ml to -191 mg/dl depending on the reagent; the standard deviation was 78 mg/dl - 102 mg/dl.

**Conclusion:** The Clauss assay is the diagnostic tool of choice when diagnosing or treating patients with low fibrinogen levels. Using the PT-derived method may potentially pose a greater risk to the patients, since the plasma concentration might erroneously be reported as normal.

## PP1.1-4

## Biochemical quality of the pharmaceutically licensed plasma Octaplas® after implementation of a novel Prion Protein (PrPSc) Removal Technology

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**Objectives:** A new chromatographic step for the selective binding of PrPSc to an affinity ligand developed and optimised for PrPSc capture by the company PRDT (Pathogen Removal and Diagnostic Technologies Inc., USA), was implemented into the manufacturing process of Octaplas®. The aim of this study was not only to evaluate the technical performance of the incorporated chromatographic step, but to demonstrate that the quality of Octaplas® is not impaired by the introduction of this novel technology at large scale routine manufacturing.

**Methods:** Pilot batches of Octaplas® with the implemented chromatographic step were manufactured by Octapharma PPGmbH, Vienna, Austria. The biochemical quality was compared directly after manufacturing as well as after 18 months stor-



age. All plasma samples were tested on global coagulation parameters, fibrinogen levels, activities of coagulation factors and protease inhibitors, as well as markers of activated coagulation and fibrinolysis. In addition, von Willebrand factor multimeric analyses were performed.

**Results:** The study showed that Octaplas<sup>®</sup> produced with and without the ligand chromatography for selective PrPSc capture demonstrates an identical quality. In addition, extensive stability studies showed no significant changes in all parameters tested after 18 months storage.

**Conclusion:** This biochemical characterisation confirmed that the affinity ligand chromatography under the developed conditions can be introduced into the Octaplas<sup>®</sup> manufacturing process, as a mean to reduce potentially present PrPSc. After implementation of this novel technology the product has the same clinical safety and efficacy profile, except for the increased safety margin in terms of prion disease transmission such as variant Creutzfeldt-Jakob disease.

#### PP1.1-5

##### Prototype immunoassay for the detection of the Marburg I-polymorphism of Factor VII-activating protease

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**Objectives:** The Marburg I (MRI) polymorphism of factor VII-activating protease (FSAP) has been associated with incidence and progression of carotid stenosis and venous thromboembolism. At present, testing is performed by PCR assays based on probes or SYBR Green I. To test large study populations it is beneficial to rely on a cost effective and rapid to perform assay method. It appears advantageous to directly determine antigen levels as they are likely to more closely relate to the phenotype than the genotype.

**Design and Methods:** After the development of a suitable and specific monoclonal antibody (mAb) a fully automated ELISA for the qualitative detection of the MRI FSAP variant in human citrated plasma was established. The prototype MRI FSAP assay\* employs an anti-MRI FSAP capture mAb immobilized on microwell strips. Bound MRI FSAP is detected with a horseradish peroxidase-labeled anti-FSAP mAb.

**Results:** The MRI FSAP prototype ELISA correctly identified the genotypes of 131 samples. Genotypes were determined by an amplification-created restriction site PCR test.

**Conclusion:** The established prototype immunoassay is adequate for testing of large study populations and represents a convenient and cost-efficient alternative to PCR testing for the determination of the MRI FSAP variant. \*Product not available for sale

#### PP1.1-6

##### A homogeneous Immunoassay\* for quantitation of Prothrombin fragment 1+2 using LOCI<sup>®</sup> Technology

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**Objective:** Prothrombin fragment 1+2 (F1+2) is generated during the conversion of prothrombin into active thrombin. The immunochemical quantification of F1+2 allows the determination of thrombin formation. Increased F1+2 levels occur in patients with thrombosis, pulmonary embolism, disseminated intravascular coagulation, polytrauma, sepsis and hereditary thrombophilia. Significantly decreased F1+2 levels can be observed in patients undergoing oral anticoagulation or heparin therapy. The quantification of F1+2 may provide information for new anticoagulant patient assessment, for example direct factor Xa and thrombin inhibitors.

**Methods:** We describe the principle of a preliminary homogeneous, high sensitive Luminescent Oxygen Channeling Immunoassay (LOCI<sup>®</sup>) for measurement of F1+2 on a LOCI<sup>®</sup> in-house instrument system.

**Results:** Initial analytical performance data on the F1+2 LOCI<sup>®</sup> showed that the method determines F1+2 within a 10 minute turnaround time and covers an assay range from 0.56 – 5000 pmol/L F1+2 using 5 µl sample volume. No significant interferences were observed from triglycerides, cholesterol, hemoglobin and bilirubin. Prothrombin and thrombin did not interfere with the F1+2 LOCI<sup>®</sup>, as shown by recovery measurements of F1+2.

**Conclusions:** We conclude that LOCI<sup>®</sup> technology is capable to provide excellent sensitivity, precision, turnaround time, and dynamic range suitable to reliably measure F1+2 in human citrated plasma samples in a broad range without sample dilution. The prototype assay\* demonstrated acceptable assay range, reproducibility and turnaround time. \* Under development – not available for sale

#### PP1.1-7

##### High speed centrifugation reduces turn around time of routine coagulation testing

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Traditionally and according to literature samples for routine coagulation testing are centrifuged for at least 10 minutes with up to 3000g. This extends the average response time in haemostaseology laboratories and supports the need for new point-of-care compatible devices, e.g. thrombelastography (final response time 60 minutes) We postulated that there is no significant difference in "slow – standard" versus "fast – hard" centrifugation, which could help to reduce turn around time in coagulation testing substantially (< 15 minutes)

**Methods:** 42 samples from 22 untreated and 20 treated (LMWH) subjects were centrifuged on a standard centrifuge (15 min, 2880g) Megafuge 1.0R (Haereus) versus the small StatSpin Express 2 (IRIS) centrifuge (3 minutes, 4440g) and routine coagulation parameters tested on a Behring Coagulation System BCS XP (Siemens). Statistical analysis was performed with Student's paired T-Test (SPSS 15.0)

**Results:** Paired results for APTT, PT-INR, Fibrinogen, antithrombin and D-Dimer showed no significant differences and very good correlation from  $r=0.982$  to  $0.999$ . Only antithrombin showed a slightly reduced correlation of  $r=0.928$ .

**Conclusion:** Our data would allow a more rapid coagulation testing in emergency settings, which would reduce the turn around time of samples to less than 15 minutes. Furthermore faster standard coagulation testing would minimize the need for other cost intensive point-of-care coagulation testing.

#### PP1.1-8

##### Markers of blood cell activation and complement activation in Factor VIII or vWF concentrates

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**Objectives:** Preparations of commercially available clotting factor VIII (FVIII) are complex protein mixtures. Most of them contain as stabilizers either von Willebrand factor (vWF) or human serum albumin (HAS). In addition to these, factor VIII concentrates contain other proteins as impurities. Aim of the study was to identify such proteins in twelve different concentrates made by genetically engineered cells or isolated from human plasma respectively.

**Methods:** Proteins were separated by two-dimensional polyacrylamide gel electrophoresis, using isoelectric focussing followed by SDS acrylamide electrophoresis (2D-Page). Proteins were stained with silver. Immunoblotting was used to identify major accompanying proteins. Some proteins were quantified by ELISA.

**Results:** As expected, recombinant clotting factor preparations showed less protein spots in the 2D-Page, than plasma derived clotting factor preparations. Among the proteins that were identified in some of the plasma derived coagulation factor concentrates were up to 0.9 µg/IU of the complement activation protein C3a, up to 40ng/IU of the platelet alpha granule protein thrombospondin-1, up to 0.06 ng/IU of the platelet alpha granule protein platelet factor 4 and up to 3.5ng/IU of the leukocyte secreted protein myeloperoxidase and up to 0.02ng/IU of the leukocyte secreted protein alpha defensin. The amount of by-proteins differed between concentrates from different manufacturers.

**Conclusions:** The origin of the plasma used (plasmapheresis plasma or plasma from whole blood donations) to prepare the factor concentrates might influence the protein impurities in these products. Whether the observed impurities influence on the long run chronic inflammatory conditions is so far unknown.

#### PP1.1-9

##### The 'raw' PiCT assay: an easy to use tool for heparin and other anticoagulants monitoring without need for separate calibration

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**Objectives:** Prothrombinase induced Clotting Time (PiCT) assay results are independent of thrombin-mediated FV activation and mainly dependent on the AT-heparin complexes or direct FXa/FIIa inhibitors present in the sample. So far, the PiCT was used with anticoagulant specific calibrators. We postulated that the use of the calibrators might not be needed for anticoagulation monitoring and that 'raw' results (in seconds) could be used instead. We performed a pilot investigation in patients on unfractionated heparin (UFH) and low molecular weight heparin (LMWH).

**Methods:** In 319 samples of patients receiving UFH, concordance correlation coefficients between two different aPTTs, the PiCT and the respective anti-Xa-activity were evaluated. The resulting correlation coefficients were compared. Also, 81 samples of patients under LMWH were evaluated.

**Results:** For UFH, both aPTT's and 'raw' PiCT were all highly significantly correlated to anti-Xa-activity, but correlation was best for 'raw' PiCT ( $r=0.57$  and  $0.68$  for aPTTs;  $r=0.83$  for PiCT) and improvement of PiCT over aPTTs was highly significant ( $p<0.001$ ). Concordance to anti-Xa-activity for PiCT was also very good for 'raw' PiCT ( $r=0.72$ ,  $p<0.001$ ).

**Conclusions:** The correlation to UFH anti-Xa-activity was significantly higher for the 'raw' PiCT assay as compared to the aPTTs; in addition, the PiCT correlated well with anti-Xa-activity of LMWH. Thus, the PiCT assay results seem more appropriate for UFH monitoring than aPTT; importantly PiCT seems adequate to monitor LMWH therapy. Anticoagulant specific calibration seems NOT necessary for UFH and LMWH monitoring with PiCT. If equally sensitive to the new anticoagulants, 'raw' PiCT might become a 'universal' anticoagulant monitoring tool.

#### PP1.1-10

##### The introduction of a novel Prion Protein (PrPSc) Removal Technology for the pharmaceutically licensed plasma (Octaplas®)

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**Objective:** The aim of our studies was to evaluate the incorporation of a novel prion protein removal technology into the manufacturing process of Octaplas®, a solvent/detergent (S/D) treated human plasma. The PrPSc removal was achieved by a chromatographic step, utilising an affinity ligand selected for prion protein binding that was developed by the company PRDT (Pathogen Removal and Diagnostic Technologies Inc., USA).

**Methods:** A validated downscale model of the adapted manufacturing process was used as basis for all investigational studies. Exogenous spike materials derived from brains of hamsters infected with hamster-adapted scrapie 263K were used to investigate the PrPSc binding capacity of the resin. Standard Western blot assays were used for the detection and determination of PrPSc levels in the various samples.

**Results:** Our studies demonstrate that PrPSc binds rapidly and with a very high affinity to the novel prion protein-affinity-resin. Based on the amount of PrPSc captured, as determined by Western blotting of both the product fractions and resin, a very high and robust binding capacity in the order of  $6 \log_{10}$  ID<sub>50</sub> bound per ml resin was demonstrated in this particular cell-free Octaplas® matrix.

**Conclusion:** The incorporation of this new chromatographic technology to remove pathogenic prions, potentially present in plasma, during Octaplas® manufacturing has been shown to be both technologically possible and feasible. The robust, reproducible PrPSc binding demonstrated by the PRDT affinity resin will further improve the safety margin of Octaplas® in terms of prion diseases such as variant Creutzfeldt-Jakob disease.

#### PP1.1-11

##### Coagulation and platelet aggregation induced by the physiological relevant activator collagen/tissue factor

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**Objectives:** Both routine coagulation and platelet aggregation assays obviously do not reflect the in vivo situation. In routine coagulation assays plasma samples are highly diluted and high amounts of thromboplastin are used to trigger clotting. In routine platelet aggregation assays agonists are added exogenously.

**Design and Methods:** We therefore designed laboratory methods for assessing coagulation and platelet aggregation induced by collagen/tissue factor (TF). The clot formation process is examined by means of thrombelastometry (TEM) in whole blood (WB) triggered by addition of collagen, tissue factor, and calcium chloride. Platelet aggregation is examined by means of the impedance method in WB triggered by addition of collagen, TF, calcium chloride, and the fibrin polymerization inhibitor GPRP.

**Results:** These TEM measurements allow sensitive detection of the anticoagulant action of both unfractionated heparin (UH) and low molecular weight heparins (LMWHs). For example, addition of nadroparin or enoxaparin resulted in the same dose-dependent prolongation of the coagulation time and the clot formation time, and in the same dose-dependent reduction of the maximum clot firmness and alpha angle, indicating the same anticoagulant efficacy of these two LMWHs. Impedance measurements showed that endogenously generated thrombin (due to the addition of TF) is a potent platelet agonist. Moreover, a combination of collagen and endogenous thrombin synergistically shortened the lag time until the onset of platelet aggregation.

**Conclusions:** Our laboratory methods allow sensitive monitoring of antithrombotic and antiplatelet actions of drugs in the physiological environment of WB.

#### PP1.1-12

##### VWF:RCo assay results are influenced by the Ristocetin concentration depending on the VWF multimer composition

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**Objectives:** Von Willebrand Factor (VWF) is composed of a varying number of disulfide-linked homodimers. The ability of VWF to promote platelet aggregation in presence of Ristocetin is evaluated by VWF:RCo assays. Recently, it has been proposed to use additional Ristocetin in the assay reagent. The impact of such a modification was investigated using commercially available FVIII/VWF concentrates and in-house preparations.

**Methods:** A plasma-derived FVIII/VWF concentrate was fractionated either by gel filtration or Heparin-affinity chromatography to obtain fractions of different VWF multimer composition. The VWF:RCo activity of these fractions was analyzed in presence of 1.25 to 2.0 mg/mL Ristocetin, respectively. The VWF:RCo/VWF:Ag ratios (specific activities) determined were normalized to the values obtained at 1.25 mg/mL Ristocetin.

**Results:** We observed that for fractions with a high degree of low molecular weight (LMW) VWF multimers the specific activity increased with increasing Ristocetin concentration. In detail, the normalized specific activity increased up to 2-fold for a LMW multimer fraction when Ristocetin was increased to 2.0 mg/mL. For fractions composed mainly of medium and high molecular weight multimers the normalized specific activity remained constant over the whole range of Ristocetin concentrations or even declined. For commercially available concentrates corresponding pattern of Ristocetin responsiveness were observed.

**Conclusions:** The use of additional Ristocetin in VWF:RCo assays results in a disproportional activity increase of LMW VWF multimers compared to high molecular weight VWF multimers. This effect accentuates the activity of VWF samples with an increased portion of LMW VWF multimers that, however, are considered physiologically less relevant.

#### PP1.1-13

##### First experiences with the measurement of Thrombin Generation (TGA) on the Ceveron® alpha in the routine lab

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**Aims:** Aim of the study was to show the suitability of Ceveron® alpha for determination of TGA in routine laboratories for discrimination of normal samples from haemophilic and thrombophilic patient samples, from patients with a Lupus syndrome and patients with bleeding disorders.

**Methods:** TGA is based on monitoring the formation of thrombin by means of a fluorogenic substrate upon activation of the coagulation cascade by tissue factor and phospholipids using 2 reagents differing in phospholipid concentrations (RClow and RChigh) on Ceveron® alpha. Four laboratories participated in the study. All samples used for TGA measurement were characterized by routine parameters.

**Results:** Peak-thrombin values showed the best discrimination between Hemophilia B patients (41.9 nM), Hemophilia B patient after treatment (188.9 nM) and normal controls (208.5 nM). Samples of pregnant thrombophilic patients showed peak-thrombin values (310.9 nM) above normals. Even patients with heparin treatment had high peak-thrombin values (340.5 nM). TGA determined in patients with Lupus showed normal peak-thrombin ratios of RClow/RChigh 0.6 in patients with only high ACA whereas the ratio in patients with high lupus anticoagulants as measured in coagulation tests was 0.98. Oral anticoagulant treatment lowered peak-thrombin formation to 79.0 nM peak-thrombin in samples with INR 2–2.5) and prolonged the tLag to 7.6 min in samples with INR 2–2.5.

**Conclusions:** TGA can be measured reproducibly under routine laboratory conditions. Peak thrombin is the best parameter for discrimination of patient samples from normals in haemophilia, thrombophilia, patients with Lupus syndrome, pregnancy and bleeding disorders. With automated, standardised TGA measurement on Ceveron® alpha large prospective studies can be conducted to confirm these results.

#### PP1.1-14

##### A new device for diagnostics of blood coagulation disorders based on measuring the spatial dynamics of clot formation

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Blood coagulation in vivo is a spatially localized process that proceeds in the vicinity of the site of injury and leads to precisely localized clot formation. The common disadvantage of the most methods for diagnostics of coagulation is that all measurements are carried out in homogeneous media with full mixing, while clotting in vivo



is spatially heterogeneous, with diffusion and convection processes playing a major role. We have developed a new method and a device for diagnostics of the clotting system status. The main idea of the method is that blood clotting is activated on a surface covered with immobilized tissue factor. The clot then propagates into the bulk of plasma. Image of growing clot is registered with a CCD camera using a time-lapse microscopy mode in scattered light. The following parameters are calculated on the basis of the experimental data: lag time (delay between contact of plasma with activator and beginning of clot growth), spatial velocity of clot growth, intensity of light scattering from the clot, and time of full plasma clotting. The studies have shown that the developed method is sensitive to various disturbances in blood clotting system (hemophilias A, B, and C; deficiencies of factors VII and X; anticoagulant therapy; therapy with recombinant factor VIIa; effects of hemodilution; increased microparticle concentration).

#### PP1.1-15

##### Anti-inflammatory and antinociceptive effects of argatroban in carrageenan-induced rat paw edema

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**Objective:** There are wide evidences that inflammation and coagulation are related processes that may considerably affect each other. The aim of this study was to determine the anti-inflammatory and antinociceptive activities of argatroban in a rat model of acute inflammation.

**Design and Methods:** Male Sprague-Dawley rats were slightly anesthetized with thiopental and edema was induced by injecting carrageenan 2% w/v (100 µl) in the hind paw. Paw volume was measured at time zero and at different time intervals. In non-inflamed animals NaCl 0.9% (100 µl) was injected in the paw. Argatroban was administered 30 min before carrageenan injection. White blood cells (WBC) count was determined using a cell counter. C-Reactive Protein (CRP) levels were measured by a rat specific enzyme-immunoassay. Fibrinogen levels were determined using the method of Ratnoff and Menzie (1954). Hotplate latency was used to quantify antinociception.

**Results:** Paw edema reached its maximum 4 h after carrageenan administration and was almost absent at 96 h. WBC count, CRP and fibrinogen were significantly increased at 12, 8 and 36 h following edema induction, respectively. Argatroban attenuated greatly the edema produced by carrageenan and increased the latency period in the hotplate test. WBC and CRP levels decreased significantly in argatroban treated rats. Fibrinogen concentration was unchanged in inflamed rats after argatroban treatment.

**Conclusions:** The results indicate that the synthetic thrombin inhibitor argatroban possesses anti-inflammatory and antinociceptive activities as evidenced in carrageenan induced paw edema model in rats.

#### PP1.1-16

##### New standardized chromogenic assays for automated measurements of Factor IX or Factor IXa in plasma and therapeutic concentrates.

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Automated measurements of Factor IX and Factor IXa are required for testing Prothrombin Complex Concentrates (PCC), plasma Factor IX (Haemophilia B) or recombinant Factor IX (Benefix), or for performing recovery studies in treated patients. Two chromogenic assays were developed for measuring Factors IX or IXa in plasma or in concentrates. For quantitating Factor IX, diluted specimen is incubated with a constant and in excess amount of Factor X, phospholipids, calcium, and Factor VIII:C. Factor IX activation is initiated by Factor XIa containing human thrombin (necessary for Factor VIII:C activation). There is a direct relationship between Factor IX in the tested sample and Factor Xa generation. Factor Xa is then measured with a specific chromogenic substrate. Finally, the colour development, measured at 405 nm, is a direct relationship of Factor IX concentration. The assay is standardized with the International Standards for plasma Factor IX or Factor IX concentrates (NIBSC). The dynamic range in the assayed dilution is from 0.02 IU/ml (100 ng/ml) to 0.0005 IU/ml (2.5 ng/ml). Plasmas are assayed diluted 1:100. A low range is available for Factor IX in Haemophiliacs, with a detection threshold of 0.005 IU/ml in plasma. The assay presents an excellent correlation with conventional clotting methods in normals, dicoumarol treated patients or B Haemophiliacs ( $r^2=0.93$ ). There is no interference of other factors in the tested specimen. No Factor IX deficient plasma is required for testing Factor IX or Factor IXa, and concentrates can be assayed directly diluted in the assay diluent. Factor IXa is measured with a similar method, omitting the activation by Factor XIa. The dynamic range is from 0.025 IU/ml to 0.0005 IU/ml of Factor IXa (0.001 IU is about 1 ng). This assay is standardized with the International Standard for Factor IXa (NIBSC). It is very helpful for testing the activation grade of PCC and other Factor IX therapeutic concentrates. Automation can be easily performed for both assays

on all the automated coagulation instruments. These methods improve the laboratory practice for monitoring Factor IX preparations.

#### PP1.1-17

##### Thrombin generation and sepsis

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**Objectives:** Sepsis is one of the most frequent causes of death in hospitals. Among others, sepsis induces the release of tissue factor from endothelial cells. This leads to an activation of the coagulation cascade and thrombin generation whereas coagulation inhibition is diminished. Furthermore, thrombin intensifies the immunologic reaction. Aim of our study is to determine the predictive value of thrombin generation (TG) assays in sepsis outcome.

**Subjects and Methods:** Sixteen patients (6 male, 10 female) with sepsis were included, mean age was  $60.5 \pm 16.6$  years. Patients were included with an average APACHE-Score of  $20.6 \pm 6.6$ , mortality was 25% (4 of 16), the patients did not receive anticoagulation. The patients where observed of a period of seven days (four blood drawings at day 0, 1, 3 and 7). TG was measured using different methods, ETP (Siemens Healthcare) and Calibrated Automated Thrombogram (CAT, Thrombinoscope) with and without thrombomodulin.

**Results:** Elevated thrombin generation was observed in patients with sepsis. In particular, if thrombomodulin is added to the assay (only CAT) the results reflect a dysbalance in favor of the procoagulant system whereas the DIC-scores remained inconsistent. An overt-DIC was not observed. Patients with a negative outcome showed higher TG at the beginning of the observation period.

**Conclusions:** TG shows a dysbalance of the coagulation system during sepsis. Thrombin generation assays may be a helpful tool to determine outcome in sepsis. Addition of thrombomodulin increases the sensitivity for the protein C system.

#### PP1.1-18

##### Determination of the Factor XIII activation peptide (FXIII-AP) by an optimized ELISA-assay

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**Objectives:** Factor XIII (FXIII) carries out the final step of blood coagulation by crosslinking fibrin and incorporating antifibrinolytic proteins into the clot. FXIII is activated by cleavage of the FXIII activation peptide (FXIII-AP) by thrombin. Recently, we directly measured for the first time the release of the FXIII-AP into plasma upon cleavage by a new FXIII-AP ELISA-assay. Since FXIII-AP is directly involved in the process of clot formation, it may represent a novel diagnostic marker for acute thrombotic events. The purpose of this study was to optimize both the sensitivity and specificity of this ELISA-assay by using two monoclonal antibodies.

**Design and Methods:** To optimize our FXIII-AP ELISA-assay we generated several monoclonal antibodies against a synthetic FXIII-AP. This time the FXIII-AP was KLH-conjugated at its N-terminus to increase accessibility by the antibodies. All monoclonal antibodies were biotinylated and combinations of unlabeled and biotinylated antibodies were tested for highest specificity and sensitivity using activated serum, control plasma, FXIII-depleted plasma and titrations of the synthetic FXIII-AP.

**Results:** We could detect synthetic FXIII-AP down to 0.5ng/ml (10pM). In fully activated serum, we measured a FXIII-AP level of 25–30nM corresponding to 25 or 50% activation whether one or both FXIII-AP's are released per FXIII-tetramer. No cross-reaction was detected with control plasma and FXIII-depleted plasma confirming high specificity.

**Conclusions:** We have established an optimized FXIII-AP ELISA-assay by using two different monoclonal antibodies. This presents an ideal tool for the determination of FXIII-AP in patients with acute thrombotic events such as stroke and myocardial infarct.

#### PP1.1-19

##### Stability of D-dimer measured by the novel INNOVANCE® D-DIMER assay

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**Objectives:** The in-vitro stability of D-dimer is widely assumed but not documented by systematic studies using clinical samples covering a wide range of D-dimer. This study investigates the short- and long-term stability of D-dimer in such samples.

**Design and Methods:** Citrated blood samples from 80 non-selected patients were collected, centrifuged and subjected to the following storage conditions: 40 samples were stored 4 h at room temperature (RT) and additional 24 h at +2-8 °C (short-term storage); 40 samples were stored for 19, 25 and 36 months at <-60 °C (long-term storage). D-dimer was measured on the BCS® System from Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany using the novel INNOVANCE® D-DIMER assay from the same company. The percentage change between baseline value and subsequent measurements were calculated and averaged for each time point.

**Results:** D-dimer values at baseline ranged from 0.23-22.2 mg/L FEU. The mean percentage change after storage for 4 h at RT and additional 24 h at +2-8 °C was +3.8 % and +2.7 %, respectively. The mean percentage change after frozen storage for 19, 25 and 36 months at <-60 °C was -11.7 %, -4.8 % and -9.3 %, respectively. The small decrease of D-dimer values after frozen storage was not time-dependent.

**Conclusions:** The data demonstrate stability of D-dimer in plasma prior freezing for up to 24 h at +2-8 °C as well as in plasma stored for up to 3 years at <-60 °C.

#### PP1.1-20

**The between-laboratory variation of Antithrombin, Proteins C and S in a large international External Quality Assessment (EQA) programme: Is there improvement over time?**

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**Introduction:** There is substantial between-laboratory variation (CVb) for the coagulation inhibitors: antithrombin, proteins C and S. This hampers the comparability and transferability of laboratory results between hospitals. We evaluated whether or not there is improvement of the CVb over time within an EQA-programme.

**Methods:** In the ECAT EQA programme the CVbs for the coagulation inhibitors mentioned were available for the period 2000 - 2007. In total 260 laboratories participated in the programme. The CVb was evaluated at both normal and pathological levels. The annual change was quantified by linear regression.

**Results:** At the end of the study period the CVb was the lowest for the normal level of antithrombin activity (7.0 %) and the highest for the pathological level of total proteins antigen (22.3 %). A significant decrease of the CVb (% change/year) was observed at pathological levels for antithrombin activity (-0.7), proteins activity (-2.0) and free proteins antigen (-3.3). For the normal levels of antithrombin activity and total proteins antigen as well as the pathological level of protein C antigen changes were random. For the other parameters a non-significant trend for improvement was observed.

**Conclusion:** Within the framework of our EQA programme improvement was observed in the inter-laboratory comparability of some parameters at a pathological level.

#### PP1.1-21

**Measurement of Thrombin-alpha-2-Macroglobulin complexes generated in plasma**

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The aim of this study is to evaluate a new assay (TECHNOZYM® TAM ELISA) for measurement of thrombin-alpha-2-macroglobulin (TAM) complexes formed during thrombin generation assay (TGA). A method for standardizable sample transfer from TGA to TAM assay is evaluated. Furthermore, the difference of TAM formation in normal, anticoagulated or thrombophilic samples was evaluated. Thrombin generation was measured using TECHNOZYM®; TGA assay which does not use any inhibitors of fibrin formation. Thus sample transfer to the TAM assay is hampered by clot formation. For a routine application, convenient methods to remove the clot without influencing the result of the TAM assay are necessary. Clots were removed either by centrifugation or filtration. Directly pipetted samples were tested in parallel. In normal plasma the total amount of thrombin generated was 5110 nM ± 321 nM. Approximately 1 % of total thrombin generated was found in TAM complexes (mean value of TAM/AUC ratio 1.1 % ± 0.1 %, mean TAM concentration 56.3 nM ± 3.8). No significant difference in TAM values could be found for the different methods of clot removal (n=8; p=0.1). Pipetting resulted in significantly lower TAM values (n=8; p=0.003) indicating inefficient sample recovery. Compared to normal plasma, in anticoagulated samples ratio TAM/AUC was less than 1 %, thrombophilic samples had slightly higher TAM/AUC ratios than normals. In conclusion we show that filtration is a routinely applicable method for clot removal from TGA reactions prior to determination of TAM and that TAM measurement might be necessary to correctly evaluate TGA results in different coagulation states.

#### PP1.1-22

**Platelet aggregation and endogenous Thrombin capacity (ETC) are not increased in patients after heart transplantation**

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**Objectives:** Thromboembolic complications are frequent in patients with heart transplantation. We examined platelet function and ETC as global estimates of haemostasis in patients with heart transplantation and healthy control subjects.

**Design and Methods:** We compared 52 unselected, stable outpatients, who had undergone heart transplantation to a control collective of 100 healthy blood donors. Whole blood impedance aggregometry was performed using collagen (1 µmol/L) and ADP (5 µmol/L) as platelet agonists. The 6-min impedance values (ω) were collected as results. ETC was determined using a calibrated automated thrombogram (Thromboscope BV) measuring thrombin generation (nM\*min).

**Results:** In general, ETC and platelet aggregation compared well between controls and patients with heart transplantation if they did not take vitamin K antagonists or acetylsalicylic acid (ASA). Collagen-mediated platelet aggregation was much lower (p=0.0002) in ASA users (6.0 ± 3.4 Ohm) than in ASA naïve patients or controls (11.0 ± 2.8 Ohm). ETC was significantly lower (p = 0.0058) in patients taking a vitamin K antagonist (phenprocoumon), (627 ± 427 nm\*min) than in patients on ASA (1740 ± 332 nm\*min) or controls (1681 ± 405 nm\*min). The type of underlying heart disease and the different immunosuppressive drug regimens did not influence the results. A patient history of thromboembolic events was also independent of the results.

**Conclusions:** Platelet aggregation and ETC could not explain a high thromboembolic risk in patients after heart transplantation. Inhibitors of platelet aggregation or oral anticoagulants were effective excluding drug non-response in the presence of immunosuppressive medication. More sensitive phenotyping of coagulation is needed.

#### PP1.1-23

**Evaluation of a micro method for Thrombin generation**

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**Introduction:** An increasing body of evidence shows that Thrombogram-Thromboscope assay may be a useful tool in the diagnosis of hyper- and hypocoagulability. Until now, the sample volume required is 480 µl citrate plasma which restricts the use of this method particularly for studies with neonates and infants. The present study was designed to validate a micro method to minimize blood requirement. The results obtained with this method are compared with those obtained with the standard macro method.

**Method:** Platelet poor plasma was obtained by centrifugation for 10 minutes at 1600 \*g at room temperature. Supernatant plasma was harvested and centrifuged at 22000 g for 10 min (room temperature) in a micro-centrifuge. For the micro method we use half area flat bottom microplates (Greiner) which allows to halve sample volume (240 µl). The platelet-poor plasma (PPP) reagent containing 5 pM tissue factor, and 4 µM phospholipids and the thrombin calibrator was purchased from Thromboscope BV. Assays were performed by means of Fluoroskan Ascent plate reader and Thromboscope software as described by the manufacturer.

**Results:** In spite of equal concentration ratios small, but significantly different, values for some parameters are obtained: lagtime (p = 0.004), tailing time (0.009) and ETP (0.04). Also after standardization to standard human plasma the differences remain for tailing time and ETP.

**Conclusion:** The micromethod offers an alternative to the macromethod for the performance and is particularly useful with neonates and infants since it minimizes blood requirement. Unfortunately the results are only partial transferable.

#### PP1.1-24

**Validation and application of Thrombin generation to detect a hypercoagulable state in patients after a first acute myocardial infarction**

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**Objective:** To determine pre-analytical variables, plasma factors influencing the various parameters of the thrombin generation (TG) curve, as well as the clinical applicability of the Calibrated Automated Thrombogram (CAT) in patients with arterial thrombosis.



**Design and Methods:** TG was performed by means of the CAT ( $\pm$  thrombomodulin). The pre-analytical variables (blood collection, plasma preparation, incubation and analysis temperature, contact activation) were determined in plasma of healthy volunteers. Plasma levels of coagulation factors and inhibitors and TG were determined in plasma from patients with acute myocardial infarction and matched controls, on admission and during follow-up for six months. Data were analyzed by multiple regression models.

**Results:** A standardized protocol is necessary to limit pre-analytical variables including only a minor effect of contact activation. TG mainly depends on the levels of free tissue factor pathway inhibitor, free protein S, FVII, FIX, FXII, fibrinogen, and antithrombin. On admission, TG in patients after an acute myocardial infarction was increased compared to a gender- and age-matched reference population (ETP: 147.4 vs 102.6%,  $P < 0.05$ ; peak height: 215.3 vs 109.6%,  $P < 0.05$ ) and negatively associated with the occurrence of end points, OR=3.7 (1.2–11.5). During follow-up for six months, peak heights were persistently increased in patients compared to controls.

**Conclusions:** Preclinical studies confirm the reproducibility and stability in time of CAT-analysis and the method is suitable to detect a hypercoagulable state in patients after a first acute myocardial infarction. Surprisingly, TG measured during the acute phase of myocardial infarction is an inverse independent risk predictor of clinical outcome.

#### PP1.1-25

##### Different specificity of the PiCT for Factor Xa-inhibitors for the two-step and a genuine one-step performance.

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The PiCT is a two-step clotting assay in which plasma is first incubated with factor Xa and factor V activating venom (RVV-V). After 180 sec, clotting is initiated by CaCl<sub>2</sub>. The assay is sensitive to reversible and irreversible inhibitors of factor X and thrombin. In this two-step assay the inhibition of the added factor Xa occurs progressively in the first step by irreversible plasma inhibitors. With normal pooled plasma, the clotting time increases between 0 to 200 sec incubation from 10–28 sec. As expected, heparinoids and fondaparinux stimulate this inhibition. Unexpectedly, reversible inhibitors (rivaroxaban), reduce this inhibition at low concentration and increase it at higher, resulting in a biphasic curve. We modified the method to a one-step procedure by preparing one reagent consisting of CaCl<sub>2</sub>, factor Xa and RVV-V. This option was chosen because rapid addition of reagents in most coagulation automates still results in delays of approximately 30 sec between additions, which causes significant, progressive inhibition from 10–12.4 sec. Using this one-step PiCT-variant, monophasic prolongation was demonstrated for rivaroxaban. Fondaparinux at high concentrations resulted in minimal prolongation (11 to 14 sec). Effects of heparinoids were reduced compared to the two-step. We conclude that this one-step variant of the PiCT has to be used for reversible Xa-inhibitors. Inhibition by fondaparinux in the two-step original assay targets purified factor Xa and absence of inhibition in the one-step complies with known minimal effects of antithrombin on phospholipid-calcium-factor VaXa complexes. Inhibition of heparinoids in the one step PiCT is mainly targeted at thrombin.

#### PP1.1-26

##### Endogenous Thrombin potential in patients suffering from liver cirrhosis

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**Background:** In patients suffering from liver cirrhosis, alterations of coagulation factor activities are used to estimate the degree of liver dysfunction. The now established assays of endogenous thrombin potential (ETP) supplement the routine coagulation tests by monitoring dynamic parameters of thrombin generation. Therefore ETP parameters were examined at different degrees of liver dysfunction.

**Patients and Methods:** ETP was observed in citrate anticoagulated blood samples from 25 patients suffering from liver cirrhosis. The stage of the liver disease was classified by MELD score, and patients showed a wide range of the score (7 to 33). Thrombin generation and coagulation tests were determined by a commercially available system (BCS, Siemens, Germany).

**Results:** In patients, ETP was significantly reduced to  $277 \pm 37$  mA in comparison to the values of a control group ( $433 \pm 56$  mA), and a similar behaviour to factor V activities showing respective values of  $41 \pm 5\%$  and  $120 \pm 9\%$  could be observed. In addition, ETP showed a linear correlation ( $r = 0.74$ ) to MELD score.

**Conclusion:** In patients suffering from liver cirrhosis, alterations in the hemostasis system was reflected by ETP parameters. Impairment of ETP parameters was comparable to the reduction of factor activities and was dependent on the degree of liver dysfunction.

#### PP1.1-27

##### A novel FVIII assay strongly influenced by physiological conditions

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Currently two FVIII assays are in use: The clotting test and the chromogenic test. The clotting test employs the activation of the contact pathway and hence massive FIX activation. The chromogenic test employs complete activation of FVIII and addition of huge amounts of FIXa as initiator(s). Both approaches do not mimic physiological conditions according to our current understanding of FVIII function. We have addressed this problem by establishing a novel FVIII assay involving no preactivation of FVIII and using only trace amounts (pM) of trigger molecules. Based on our previous FVIII assay (Eich et al, BC&F, 2003) we have reinvestigated several aspects of this assay. Among these were: Influence of FVIII deficient plasmas, amount and type of trigger molecules (FVIIa/ tissue factor, FIXa), type of reporter molecules (FIIa, FXa). Also, assay time and evaluation algorithm were investigated. The novel test procedure was applied to the comparative analysis of plasmas, plasma derived, and recombinant FVIII molecules. Our experiments show that FIXa is the preferable initiator molecule and FVIIa/ tissue factor is less suited. As reporter molecule FXa was found superior to FIIa. Assay time could be shortened significantly to 15 minutes avoiding complications such as inner filter effect. A comparison of the different FVIII sources (plasma, plasma derived or recombinant FVIII) will be presented. We consider our test a progress in FVIII analysis as this test employs trace amounts of initiator molecule, measures different FVIII concentrations against a constant plasma background and thus mimics very closely the conditions in a hemophilic.

#### PP1.1-28

##### The influence of vWF on the Bethesda assay

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**Introduction:** Inhibitor development is a serious side effect of treatment in hemophilic patients. Eradication of inhibitors by immune tolerance induction (ITI) is cost-intensive and the success is not predictable, especially there is no laboratory test to predict ITI success. An increasing number of recent publications suggest a modified Bethesda assay to be helpful in selecting the most promising factor preparation for ITI in hemophilia A. In particular, the influence of von Willebrand factor (vWF) is discussed controversially. As a first pass to evaluate the Bethesda assay as predictive marker for ITI we determine the influence of vWF on the assay.

**Methods:** Different factor VIII preparations (Haemate<sup>®</sup>, Haemoctin<sup>®</sup>, Kogenate<sup>®</sup> and ReFacto<sup>®</sup>) were used in the modified Bethesda assay. Diverse commercial (Immuno) and kindly provided factor VIII inhibitors were tested. The effect of vWF addition to the assays was determined. Concomitantly, the amount of unbound FVIII antibodies (FVIII inhibitor assay, GTI Diagnostics) was measured.

**Results:** Haemate<sup>®</sup> shows the lowest, ReFacto<sup>®</sup> the highest, Haemoctin<sup>®</sup> and Kogenate<sup>®</sup> show comparable inhibitor titres. Addition of vWF has no effect on the assays.

**Conclusions:** Within the used inhibitors, vWF has no significant effect on the assay or the amount of unbound antibodies. A positive appraisal of the Bethesda assay as a predictive marker in ITI is not possible.

#### PP1.1-29

##### Activation of hemostasis after transfusion of cryopreserved hematopoietic stem cells containing dimethyl-sulfoxide (DMSO)

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**Objectives:** Investigation of the toxicity of DMSO-containing cryopreserved hematopoietic stem cells (HSC) on hemostasis by determination of platelet function and of coagulation and endothelial cell activation in patients receiving autologous HSC transplants (HSCT).

**Patients and Methods:** 40 patients (16 female, 24 male, age  $54 \pm 13$  years) received autologous HSCT containing 7.5% DMSO. Underlying diseases were multiple myeloma (n=21), lymphoid neoplasia (n=10), acute leukemia (n=5), and others (n=4). Blood samples were taken immediately before, and 15 min and 16–18 hours after HSCT. We tested whole-blood platelet aggregation (adenosine-diphosphate [ADP 6.4mcM], collagen [COL 3.2mcg/ml], thrombin receptor activating peptide [TRAP 32mcM], and arachidonate [AA 0.5mM]), fibrinogen (FBG), d-dimers (DD), thrombin-antithrombin complex (TAT), von Willebrand factor antigen (VWF:Ag), and cell-membrane microparticle activity (MPA) measured as thrombin-generation.

**Results:** Mean MPA ( $19 \pm 9$  nM,  $32 \pm 19$  nM,  $16 \pm 8$  nM,  $p < 0.001$ ) and TAT ( $4 \pm 2$ ,  $15 \pm 11$ ,  $5 \pm 5$  mcg/L,  $p < 0.001$ ) increased significantly immediately after HSCT, returning to baseline the day after. No significant changes were seen in FBG, DD, VWF:Ag. Only a trend for reduction of platelet aggregation with ADP ( $449 \pm 263$ ,  $380 \pm 277$  AU,  $p = 0.06$ ) was found immediately after HSCT. Discussion: We observed an activation of hemostasis, measured as increase of MPA and TAT immediately after HSCT. This effect is short-lasting and might be attributed to components of the HSC preparation including DMSO. Additional experiments addressing the influence of DMSO on platelet function in vitro and on direct endothelial toxicity are needed. Whether these findings correlate with known adverse reactions to transfusion of cryopreserved HSC still remains to be investigated.

#### PP1.1-30

##### Evaluation of a capillary blood method for INR measurement using Innovin in an outpatient setting

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**Objectives:** To obtain faster results for ambulant oral anticoagulated patients capillary citrated whole blood methods are often used for INR measurement in outpatient clinics. Commercialised reagents for capillary citrated blood are still manufactured from crude tissue extracts with their well known limitations and are slightly more expensive in this setting than modern recombinant thromboplastin reagents for citrated plasma. Therefore the aim of this study was to compare a homemade capillary method using the recombinant thromboplastin Innovin (Siemens Healthcare Diagnostic, Germany) and the commercialised capillary method Hepato Quick (Roche Diagnostic, Germany) with our routinely used method Innovin with citrated plasma on an automatic analyser as the reference method.

**Methods:** In 69 oral anticoagulated outpatients we analysed the INR in capillary citrated blood using the Hepato Quick method and a homemade Innovin method on the coagulometer according to Schnitger & Gross (Amelung, Germany) and in citrated venous plasma with Innovin on the BCS analyser (Siemens Healthcare Diagnostics, Germany).

**Results:** We found a good correlation between both the reference Innovin method on the BCS analyser and the homemade capillary Innovin method ( $r^2 = 0.90$ ) and the commercialised Hepato Quick method ( $r^2 = 0.85$ ). There was also a close match between the two capillary methods ( $r^2 = 0.91$ ).

**Conclusion:** Innovin can be used smoothly for INR measurement also in capillary citrated whole blood and offers a favourable option especially for outpatient clinics.

#### PP1.1-31

##### Two standard sodium citrate concentrations in modern coagulation tubes show no influence on routine coagulation results

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In haemostaseologic laboratories two different buffered trisodium citrate concentrations, either 3.2% (0.109 mol/l) or 3.8% (0.129 mol/l), are used as anticoagulant. Nearly no specific information about the influence of different concentration levels of sodium citrate (3.2% and 3.8%) on particular coagulation parameters exists. Problems could arise for coagulation results based on anticoagulation with both citrate concentration e.g. once blood is drawn by the general practitioner and later on in the hospital. We tested the possible influence of the two standard anticoagulant concentrations in three sample groups (subcutaneous LMWHs, oral anticoagulation with Coumarines, no anticoagulation medication administered) in new sandwich sample tubes keeping the vacuum and the citrate solution more stable.

**Methods:** From 67 subjects blood was drawn into VACUETTE Coagulation Sandwich Tubes (Greiner Bio-One). APTT, PT-INR, Fibrinogen, Factor V and VII and VIII and APC Resistance were measured in both citrate concentrations one hour and three hours after sampling to detect possible influences due to anticoagulants and time. Samples were centrifuged for 15 min with 2880g (Hereus Megafuge 1.0R) and measured on a Behring Coagulation System BCS-XP (Siemens). Statistical analysis was performed with paired Student's T-Test (SPSS 15.0).

**Results:** APTT, PT-INR, Fibrinogen, Factor V, VII, VIII and APC resistance showed no significant differences in the results for the two citrate concentrations and the two points in time. These parameters showed a very good correlation from  $r = 0.991$  to  $0.997$ , only APC resistance correlation ranged from  $r = 0.960$  to  $0.968$ .

**Conclusion:** These data show that 3.2% and 3.8% citrated modern sample tubes do not influence coagulation analysis.

#### PP1.1-32

##### Comparison of the performance of INNOVANCE PFA P2Y\* in citrated or hirudinized whole blood

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In this study the impact of the blood sample anticoagulants buffered sodium citrate (0.109 M) and hirudin on the performance of INNOVANCE PFA P2Y\* was evaluated. Using blood from 28 healthy donors closure times (CT) of untreated and 150 µm MRS 2395 (P2Y12-receptor antagonist) treated samples anticoagulated with r-hirudin were statistically significantly shorter than those anticoagulated with citrate. The CTs of untreated samples ranged from 33 – 84 sec (Mean: 56 sec) for hirudin and from 43 – 76 sec (Mean: 62 sec) for citrate. The distributions of the untreated samples were used to derive specific cutoffs (97.5th percentiles). Different distribution patterns were identified as the reason for the difference observed in MRS 2395 treated samples. While the CTs of citrated samples showed an 'all or none' pattern, the CTs of hirudinized samples were almost evenly distributed between 83 and 300 sec. Despite these contrasting distribution patterns the cutoff-based sensitivity was almost identical (citrate: 96% vs. hirudin: 100%). The CTs in the presence of 75 µm MRS 2179 (P2Y1-receptor antagonist) were significantly prolonged in blood samples of most volunteers, irrespective of the anticoagulant used. The results of the study suggest the usability of r-hirudin as anticoagulant for measurements with INNOVANCE PFA P2Y\*, although it is not the recommended anticoagulant. The CT of INNOVANCE PFA P2Y can also be abnormalized by P2Y1-receptor inhibition, but this interference is considered non-relevant as no P2Y1-receptor inhibitors are currently under commercialization. \*product under development – not available for sale

#### PP1.1-33

##### Quantitative measurement of Factor V Leiden in heterozygous and homozygous patients for the R506Q Factor V mutation

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A new quantitative clotting assay for measuring Factor V Leiden (R506Q mutation) in citrated plasma is available. It allows to overcome the diagnosis variability linked to clotting methods based on the APC-R ratio (clotting time with or without APC), and to grade the risk in patients with the same genetic profile. This new method was used for quantitating Factor V Leiden in normal population (N=30), and in patients carrying the R506Q Factor V mutation (N=61 heterozygous, including 19 dicoumarol treated, and N=18 homozygous). All normals and patients were classified using molecular biology. Other factor V activities (Factor V clotting activity and Factor V antigen) were also measured. Factor V Leiden was always < 10% in normals (usually non measurable), whilst it was in the normal range for Factor V clotting activity and Factor V antigen. Factor V Leiden clotting activity (resistant to APC) ranged from 25% to 75% in heterozygous patients (Mean of 49%, with no significant difference in the dicoumarol treated patients' group), and from 70% to 190% in homozygous (Mean value of 90%). This clotting activity correlated well with the normal Factor V clotting activity or Factor V antigen in homozygous, and was about half these activities in heterozygous. In patients with a low level of Factor V, diagnosis and classification of Factor V Leiden can be improved by calculating the ratio between Factor V Leiden concentration and Factor V clotting activity or antigen (ratio of about 0.5 for heterozygous, and of 1.00 for homozygous, but < 0.1 for normals). This new clotting method allows an accurate measurement of Factor V Leiden clotting activity (resistant to the action of Activated Protein C) and could be very useful for routine classification of patients carrying the Factor V Leiden mutation. Only one clotting test is necessary, and result is fully quantitative. Furthermore, quantitation of Factor V Leiden could be a helpful tool for grading the thrombotic risk in patients with the R506Q Factor V mutation.

#### PP1.1-34

##### Low tissue factor („dilute“) prothrombin time – a „poor man's“ alternative to thrombin generation assays? A hypothesis.

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**Background:** The two most widely used coagulation tests, prothrombin time (PT/INR) and activated partial thromboplastin time (aPTT), are rather unphysiologic (PT/INR triggered by extremely high amounts of tissue factor (TF); aPTT triggered by contact activation which has no role in bleeding and an unclear role in thrombosis). Their clinical relevance is limited. The modern concept of thrombin generation analysis (TGA) with low TF has shown great potential for prediction of bleeding or thrombosis, and for monitoring procoagulant and anticoagulant therapy. But TGA is relatively expensive and time-consuming and not widely available



in clinical laboratories. Dilute prothrombin time (dPT), a more simple low-TF test, is currently used only for lupus anticoagulant screening.

**Hypothesis:** Although TGA yields more detailed information on thrombin generation in vitro, dPT may, for some purposes, be clinically equivalent to TGA. If true, dPT may be a lower-cost alternative to TGA since it requires less resources and uses standard equipment. A suitable dPT must be insensitive to contact factors and sensitive to factors VIII, IX, and XI, and to various procoagulant and anticoagulant treatments. It must identify patients at risk of bleeding or thrombosis and correlate with TGA. Critical analytical aspects of dPT in this potential new field of application that would have to be thoroughly investigated and standardised include type and amount of TF, type and amount of phospholipids(s), and requirement of pre-analytical inhibition of contact activation.

**Conclusion:** Low tissue factor („dilute“) prothrombin time may be clinically equivalent to thrombin generation analysis for some applications. It should be evaluated for the prediction of bleeding and thrombosis and the monitoring of treatment.

#### PP1.1-35

##### Haemostatic status and central nervous system findings in young patients with Thalassaemia Intermedia

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Patients with  $\alpha$ -thalassaemia are known to have a chronic hypercoagulable state and increased incidence of thromboembolic episodes that may affect various organs. The purpose of the present study was to evaluate the haemostatic status and the possible involvement of the CNS in Greek young patients with  $\alpha$ -thalassaemia intermedia. For this purpose 14 patients aged 8 – 20 years participated in a systematic neurophysiologic and neuroimaging study. None of the patients were on a regular transfusion program. Evaluation consisted of brainstem auditory, visual and somatosensory evoked potential examination, as well as, magnetic resonance imaging and angiography. In all patients the following haemostatic markers were measured: PT, PTT, INR, Fibrinogen, D-dimers, PS, PC, ATIII, APCR, LA, ACL, Anti laGPI-I, F1+2, TAT, as well as, FV Leiden, MTHFR and PrG20210 mutations. None of the patients reported symptoms of overt stroke and none demonstrated abnormal neurological findings on clinical examination. Brain laboratory evaluation was normal in all cases despite the presence of an hypercoagulable state in a significant number of the patients examined: low PC levels in 8 patients, low PS levels in 7 patients, increased F1+2 levels in 7 patients, increased D-dimers in 3 patients, increased heterozygous G20210A prothrombin variant presence in 3 patients, heterozygous MTHFR677T polymorphism presence in 5 patients and homozygous in 2 patients. Although our study confirmed the presence of biological risk factors of thrombosis in  $\alpha$ -thalassaemia intermedia patients, no CNS involvement was established. This could be attributed to the young age of the participating patients.

#### PP1.1-36

##### Effect of freezing method and storage at -20°C and -70°C on kinetic, PT-derived, and immunological fibrinogen levels

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Clinical and epidemiological trials often involve central laboratory analyses of coagulation tests, including fibrinogen. Although rapid freezing by immersion of sample tubes in liquid nitrogen and storage at -70°C is recommended, plasma samples are often transferred directly to the storage compartments, and stored at -20°C. Fibrinogen levels using a kinetic and photometric assay, PT-derived fibrinogen, prothrombin time, and aPTT were measured in plasma samples from 16 healthy blood donors. Four sets of aliquots were prepared. Set A was transferred directly to a -20°C storage compartment, set B was first snapfrozen in liquid nitrogen and then transferred to the -20°C compartment. Set C was transferred directly to a 70°C freezer, set D was first snap-frozen in liquid nitrogen and then stored at -70°C. Aliquots were thawed after one, two, three and four months storage and measured again. Freezing had little effect on fibrinogen levels, differences were below three percent for all variants. Changes were smaller for samples stored at -70°C compared to -20°C, and for snap-frozen compared to not snap frozen samples. Frozen and thawed samples generated slightly higher fibrinogen levels compared to fresh samples. Prothrombin time and aPTT were prolonged, and whereas this effect was quite constant for prothrombin time, the aPTT progressively increased. Apparent fibrinogen levels are slightly higher in frozen and thawed plasma samples, compared to fresh samples, but overall changes are minimal. In contrast to fibrinogen, prothrombin time and aPTT should be measured in fresh since freezing has an inconstant and unpredictable effect on the results.

#### PP1.1-37

##### PC/PS Ratio allows diagnosis of PC and PS deficiency under therapy with Vitamin K antagonists.

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**Objectives:** Testing patients under therapy with vitamin K antagonists (VKA) for a suspected deficiency of vitamin K dependent inhibitors Protein C (PC) and Proteins (PS) is a common problem in the routine coagulation laboratory. We have evaluated the use of a ratio of PC-activity and free PS-antigen, to address this problem.

**Design and Methods:** From 847 patients diagnosed with thrombophilia 1143 independent laboratory reports were obtained. These included 121 reports from 79 patients with known PS deficiency, and 66 reports from 47 patients with known PC deficiency, respectively. Therapy with VKA was defined as an INR>1.5. PC/PS ratio was calculated as PC-activity divided by free PS antigen.

**Results:** The median (25th - 75th pct.) of PC/PS ratio was quite unaffected by the absence / presence of VKA: 1.16(0.93–1.54)/ 1.30(1–1.76). Results for PC activity were 107(91–123)/ 54(45–67), for free PS antigen 90(71–109)/ 42(30–56). The AUC ratio for the diagnosis of PS and PC deficiency were 0.93% and 0.93%, respectively, compared to 0.89 for PC activity for the diagnosis of PC deficiency and 0.89 for free PS antigen for the diagnosis of PS deficiency. A reference interval of 0.76–1.84 showed 80% sensitivity for PC and PS deficiency with a specificity of 94% and 90%, respectively.

**Conclusion:** The ratio of PC activity and free PS antigen allows the diagnosis of PC and PS deficiency using one single reference range for patients with and without VKA therapy.

#### PP1.1-38

##### Evaluating the performances of dilute Russel viper venom tests (DRVVTs): Gradipore LA versus STAclot (Roche Diagnostics).

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**Objectives:** DRVVTs detect lupus anticoagulants (LAC) in the diagnostic work-up of thrombosis. We compared the new automated STAclot to the Gradipore LA assays.

**Design and Methods:** Twenty healthy control subjects and 297 consecutive patients in whom LAC were suspected, were examined. Patients using vitamin K-antagonists were excluded. Clotting times were determined using the Gradipore LA screen and confirm assays (Haemochrom) and the KC10 (Amelung) as well as the STAclot DRVV Screen and confirm assays and the automated STA compact analyzer (Roche Diagnostics). Pathological screen-to-confirm ratios (SCR) and cut-offs were calculated based on the means + 2 SD. Analytical performances were not determined.

**Results:** The 95% reference intervals ranged from 29.1–41.2, 30.0–36.3, 28.3–34.5, and 28.1–33.9 sec for the LA and STAclot screen and confirm assays, respectively. Clotting times obtained with screen and confirm assays, were shorter by 2–4 sec (P<0.0001, paired t-test) with STAclot than with LA assays. Their relationships were linear (Passing-Bablok) throughout the entire range (Cusum test for linearity). LA and STAclot screening tests detected abnormal screening times in 43 and 92 samples, respectively. Pathological SCRs were obtained for 48 and 88 samples using LA and STAclot assays, respectively. Identical results were found using normalized SCRs; the sensitivity and specificity of the STAclot SCRs were 77.1% and 81.5%, respectively, compared to the Gradipore LA SCRs.

**Conclusion:** Although LA and STAclot reagent are comparable, the latter test identified more patients as potential carriers of lupus anticoagulants. The clinical significance of these findings remains to be elucidated.

## PP1.2 Thrombin and Thrombin Receptors

## PP1.2-1

## New low-molecular synthetic Thrombin inhibitors

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**Objectives:** Search, design and synthesis of new thrombin inhibitors, which can be used as anticoagulants.

**Design and Methods:** A virtual screening of some molecular databases was performed using an original docking program (www.keenbase.ru). First the virtual hits were checked experimentally for thrombin inhibition activity in a pure buffer system. Thrombin specific chromogenic substrates hydrolysis was used. Then the inhibitors were screened for anticoagulant activity in blood plasma by thrombin clotting time, the test for thrombin generation and an original method for determination of clot propagation rate in nonstirred plasma. The new class of thrombin inhibitors was designed using the structures of the best anticoagulants which were found. The acute toxicity of new inhibitors was tested on mice model.

**Results:** The new thrombin inhibitors were synthesized and checked experimentally. The minimal concentration needed for the 50% inhibition of thrombin specific chromogenic substrate hydrolysis in the buffer (IC50) appears to be equal to 2.5 nm, whereas the IC50 for the decrease of the thrombin generation in plasma – 0.25 µm. Intraperitoneal injection of a dose 1000-times higher in a comparison with the IC50 necessary for the decrease of thrombin generation in plasma was used to determine acute toxicity of these compounds on mice. Any significant pathological changes in mice were not observed.

**Conclusions:** A new class of low-molecular direct thrombin inhibitors with high anticoagulant activity in plasma was designed. These inhibitors are nontoxic in doses 1000-times higher than IC50 for thrombin generation in plasma.

## PP1.2-2

## Coagulation-active serine proteases as a survival factors for tumor cells in malignant effusions

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Ca. 30 % of cancer patients suffer from malignant effusions during progression and metastasation of their cancer disease. Their treatment is extremely difficult, as tumor cells within these effusions rarely respond to cytostatic drugs. Analysis of malignant effusions revealed all coagulation factors of the 'tissue factor pathway' (e.g. FIIa 35.2 ± 22 %, FVa 8.15 ± 4.04 %, FVIIa 24.67 ± 24.78 %, FXa 7.54 ± 4.19 %, TF 247.9 ± 210.7 µg/ml). Spontaneous formation of fibrin fibers could be observed, which, in addition to very high prothrombin-fragment F1.2-concentrations and D-dimer-concentrations, proves an active coagulation and fibrinolysis system. Only 8% of these effusions contained >12000/µl thrombocytes. Thrombocytes are easily activated, resulting in an uncontrolled initiation of the coagulation system. Thus, the lack of thrombocytes makes malignant effusions an interesting model to study the interaction of the coagulation system with tumor cells. FXa and thrombin remain active for weeks or even months within these effusions ex vivo, which might be due to the significantly reduced AT concentration (36.7 ± 18.58 %, p=0.029). Using FACS and rtPCR, 70–85 % of the isolated tumor cells expose thrombomodulin and PAR1, which interact with thrombin and FXa. In vitro experiments, using a PAR-1 expressing tumor cell line, showed that thrombin and FXa are able to induce proliferation and resistance against platinum and doxorubicin. We presume that coagulation-active serine-proteases (e.g. FXa, FIIa) act as survival factors for tumor cells in malignant effusions and contribute to the poor prognosis of these patients. Supported by DFG

## PP1.2-3

## Dabigatran inhibits both clot-bound and fluid phase Thrombin in vitro: Effects compared to Heparin

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Thrombin bound to fibrin in an existing thrombus is protected from inhibition by UFH/AT. This surface can remain "active" despite UFH / LMWH therapy. At trough levels or after ending therapy with heparin, this active thrombin could initiate further prothrombotic events.

**Objectives:** Dabigatran is a reversible, direct inhibitor of thrombin. This study investigated the ability of dabigatran to inhibit thrombin bound to fibrin, or thrombin in plasma as compared to UFH in vitro.

**Design and Methods:** Clots were generated in platelet rich plasma, extensively washed and transferred to 0.5 ml plasma containing dabigatran, UFH or buffer. For fluid-phase, thrombin (20 pM) was added to plasma containing dabigatran, UFH or buffer and incubated for 1 hr at 37°C. Fibrin formation was measured as FPA release using ELISA. IC50 in fluid phase and clot-bound conditions was calculated.

**Results:** Both compounds inhibited fluid and clot-bound thrombin with increasing concentrations. Dabigatran inhibited both clot-bound and fluid phase thrombin similarly, IC50 of 200 nm and 186 nm, respectively. The IC50 of UFH-induced inhibition of clot-bound thrombin was 98.7 nm and in fluid phase 8.4 nm. When converted into a ratio of clot-bound: fluid phase IIA, 1.07 and 11.76 were obtained for dabigatran and UFH.

**Conclusions:** Dabigatran equally inhibits both clot-bound and fluid phase thrombin, consistent with targeted inhibition of the active site of thrombin by a small molecule. UFH is less effective in inhibiting clot-bound vs fluid phase thrombin. Thus dabigatran could be effective in clinical situations where surface-bound thrombin plays a role.

## PP1.2-4

## Influence of crystalloid and colloid solutions on dynamics of thrombin generation and thrombin activatable fibrinolysis inhibitor during major orthopedic surgery

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**Background:** The aim of this study was to find out whether impaired clot formation and fibrin polymerization observed with colloid fluids are related to changes in thrombin dynamics, activity of thrombin activatable fibrinolysis inhibitor (TAFI) and concentrations of fibrin monomers indicating relative FXIII deficiency.

**Methods:** Thrombin dynamics (TDT), fibrin monomers, and TAFI were measured in 54 orthopaedic patients not receiving fibrinogen concentrate during surgery of the spine and randomized administration of either 4 % gelatin solution (GEL), 6 % hydroxyethyl starch (HES) (130/0.4) or exclusively Ringer's lactated (RL) solution.

**Results:** Measurements of TDT decreased significantly more with both colloids than with Ringer's lactated solution. (GEL vs HES, ns; GEL vs RL, P=0.01; HES vs RL, P=0.03) but remained within the normal range. Activity of TAFI decreased intraoperatively irrespective of the fluid used while concentrations of fibrin monomers increased significantly more with administration of gelatin than with hydroxyethyl starch and Ringer's lactated solution (GEL vs. HES, P=0.02; GEL vs RL, P=0.007; HES vs RL, ns). No fluid specific correlation was found for the change in thrombin dynamics and the change in fibrinogen/fibrin polymerization which was also not correlated to changes in concentrations of fibrin monomers.

**Conclusion:** During orthopaedic surgery and mild dilution using colloid or exclusively crystalloid fluids a moderate change in thrombin dynamics, activity of TAFI and concentrations of fibrin monomers was observed. However, these changes are unlikely related to the observed impairment of clot formation following colloid administration.

## PP1.2-5

## Thrombin-inducibile platelet function and regulation of PAR-1 in patients undergoing percutaneous coronary intervention: Effects of unfractionated Heparin and Bivalirudin

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**Objective:** Heparin and bivalirudin are approved anticoagulants for percutaneous coronary intervention (PCI). Platelet response to thrombin under high shear conditions affects haemostasis. We therefore examined the specific effects of these anticoagulants on PAR-1.

**Methods:** To simulate in vivo conditions that may precipitate a bleeding event, we added thrombin in vitro to blood samples from 89 patients who had been randomly assigned to receive heparin or bivalirudin for elective PCI and examined thrombin-inducibile platelet function under high shear conditions and PAR-1 expression.

**Results:** Compared to baseline, platelet adherence under high shear increased by 10 percent with heparin, but decreased by 20 % with bivalirudin (p=0.0047). Thrombin-inducibile platelet adherence and size of aggregates was equally inhibited by heparin and bivalirudin. Thrombin-inducibile cleavage of PAR-1 was inhibited by heparin, but not affected by bivalirudin (p<0.0001). Further, PAR-1 internalization was more effectively inhibited by heparin than bivalirudin (p=0.002).



**Conclusions:** Heparin and bivalirudin inhibit thrombin-inducible platelet adherence and aggregate formation, but heparin has stronger inhibitory effects on thrombin-dependent PAR-1 cleavage and internalization, thus providing a biological explanation for lower clinical bleeding rates with bivalirudin.

#### PP1.2-6

##### Thrombin generation in morbidly obese patients after weight loss

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**Objectives:** Patients with Morbid Obesity (MO: BMI > 40 kg/m<sup>2</sup>) suffer from an increased risk of coronary heart disease, stroke, venous thromboembolism and all-cause mortality. Since weight loss by gastric bypass improves cardiovascular and all-cause mortality, and improvements in classic risk factors do not explain this phenomenon, we hypothesize that the plasmatic clotting system might be involved.

**Design and Methods:** 36 MO patients (mean age: 42±13 years) were investigated before and two years after gastric bypass. The thrombin generation assay (Technothrombin TGA, Technoclone, Vienna, Austria) examines the overall function of the plasmatic clotting system: lag phase, peak thrombin and area under the curve (AUC) - representing the endogenous thrombin potential. To investigate associations of changing parameters, deltas were calculated. Student's paired t-test, correlation analysis and regression analysis were appropriately used.

**Results:** Metabolic parameters improved with a mean weight loss of 42±19 kg: BMI: 45±5 vs. 31±6 kg/m<sup>2</sup>; p<0.001; fasting insulin: 19±9 vs. 8±5 µU/ml; p<0.001; 2-hour insulin: 75±46 vs. 16±22 µU/ml; p=0.001; 2-hour glucose: 106±24 vs. 72±26 mg/dl; p=0.001; and HOMA-IR: 4.2±2.6 vs. 1.5±0.8; p=0.001. (always pre- vs. post-operative). Weight loss significantly reduced the peak thrombin (351±184 vs. 269±189; p=0.015) as well as the AUC (4089±1989 vs. 3023±1186; p<0.001). Preoperatively, peak thrombin was tightly associated with fasting insulin levels (R=0.632, p=0.005).

**Conclusions:** Gastric bypass surgery has been proven to significantly improve life expectancy and cardiovascular survival in morbidly obese patients. Since thrombin generation is significantly reduced with weight loss, it may contribute to the reduction in cardiovascular risk associated with obesity.

#### PP1.2-7

##### Thrombin upregulates expression of Oncostatin m (OSM) in human macrophages and peripheral blood monocytes

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**Background:** Hemostatic factors like thrombin play a crucial role in generating thrombotic plugs at sites of vascular damage (atherothrombosis). However, whether hemostatic factors contribute directly or indirectly to the pathogenesis of atherosclerosis remains uncertain. Oncostatin m (OSM) as a member of IL-6 family cytokines is a proinflammatory mediator that is primarily known for its effects on cell growth. The aim of the present study was to investigate if thrombin and OSM can act as a link between macrophages, platelets and the development of cardiovascular disease.

**Methods:** Peripheral blood monocytes (PBMC) were isolated using Ficoll-Paque and magnetically labelled CD14 MicroBeads. For macrophage transformation (MDM) cells were cultivated for 8 - 10 days in the presence of human serum. Plaque Macrophages were isolated from atherosclerotic plaques and positive selection of CD14 positive cells was performed employing CD14 antibodies. All Cells were incubated with thrombin at a concentration of 1U/ml. OSM antigen was determined by specific ELISA. OSM specific mRNA was quantitated by RealTime-PCR.

**Results:** Thrombin increased OSM antigen concentration- and time-dependently up to 20-fold in MDM and up to 8-fold in PBMC. These results could be confirmed on specific mRNA level. In human plaque macrophages stimulation with thrombin leads to a 5-fold increase of OSM mRNA level.

**Conclusion:** Thrombin induces the expression of OSM in human macrophages in vitro. If this effect is also present in vivo it may be a new link between platelets, macrophages and the development of cardiovascular disease.

#### PP1.3 Tissue Factor, TFPI, Factor VII

##### PP1.3-1

##### Smooth muscle cells are a potential source of tissue factor on platelets

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**Objectives:** Microparticle-bound circulating tissue factor (TF) contributes to fibrin clot formation. Several cell types including vascular smooth muscle cells (SMC) can release TF-bearing microparticles. The present study investigates the microparticle-dependent transfer of TF from SMC to platelets.

**Methods:** TF was cloned from human SMC and tagged with a FLAG-tag sequence. Microparticles from SMC, expressing endogenous TF or overexpressing TF-FLAG, were co-incubated with platelets, followed by FACS analysis and Western blotting to measure the TF-content. TF activity was quantified by measurement of the endogenous thrombin potential (ETP) in human plasma.

**Results:** TF levels in platelets significantly increased 2-fold following co-incubation with TF-bearing microparticles from SMC (FACS, n=5, p<0.05), accompanied by a significant 2.2-fold increase in ETP (n=5, p<0.05). To further investigate the transfer of TF the functionally active TF-FLAG variant was overexpressed in SMC and their microparticles. TF-transfer was detected with an anti-FLAG antibody by Western blotting. These experiments also demonstrated TF-transfer from SMC to platelets, resulting in an increased platelet-dependent thrombin generation.

**Conclusions:** Microparticles from human SMC can transfer TF to platelets, thus delivering TF into a highly thrombogenic environment. The transfer mechanism is unlikely to involve P-selectin glycoprotein ligand-1 (PSGL-1), which is not expressed in SMC. SMC are exposed to blood following endothelial injury. Hence TF-transfer from SMC to platelets may contribute to thrombotic events in certain clinical conditions, e.g. coronary angioplasty.

##### PP1.3-2

##### Tissue factor and the risk of venous thromboembolism in cancer patients – Cancer and Thrombosis Study

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**Objective:** Increased tissue factor (TF) levels were reported in patients with malignancy. The association with venous thromboembolism (VTE) has been assumed, but has never been studied systematically. We investigated, whether TF was associated with an increased VTE risk in cancer patients.

**Design and Methods:** The Cancer and Thrombosis Study (CATS) is a prospective observational study performed in patients with newly diagnosed cancer or disease progression after former treatment. Study endpoint was the occurrence of symptomatic, objectively confirmed VTE. TF was measured with a commercially available ELISA (® IMUBIND Tissue Factor ELISA, American Diagnostica Inc.). VTE risk was estimated by univariate Cox regression analysis.

**Results:** 630 patients (310 female / 320 male) were followed for a mean observation time of 503 days. Main tumour entities were malignancies of the breast (n=124), lung (n=81), gastrointestinal tract (n=117), pancreas (n=39), kidney (n=20), prostate (n=52) and brain (n=74). 79 patients had hematological malignancies and 44 other tumours. 46 VTEs were observed. High TF levels were defined as those exceeding the 75th percentile of the total study population. High TF levels were not associated with an increased VTE risk in Cox regression analysis (HR=0.99, 95% confidence interval: 0.50 - 1.95, p = 0.98). TF did not differ significantly among tumour entities (p=0.2) nor according to presence of metastases (p=0.7).

**Conclusions:** High TF levels were not associated with the VTE risk in our study. We would not recommend to include determination of TF in a set of variables for risk stratification in cancer patients.

##### PP1.3-3

##### Plasma TF activity predicts cardiovascular mortality in patients with acute myocardial infarction

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**Objectives and Background:** Tissue factor (TF) contributes to thrombosis following plaque disruption in acute coronary syndromes (ACS). Aim of the study was to investigate the impact of plasma TF activity on prognosis in patients with ACS.

**Methods and Results:** 174 patients with unstable Angina pectoris (uAP) and 112 patients with acute myocardial infarction (AMI) were included with a mean fol-

low up time of 3.26 years. On admission, plasma TF activity and TF antigen were assessed. Patients were categorized into 2 groups: a high-TF activity group with TF >24 pmol/L and low TF activity group with TF <24pmol/L. Fifteen cardiovascular deaths occurred in the uAP group and 16 in the AMI group. In AMI TF activity was 24.9±2.78 pmol/l (mean±SEM) in survivors and 40.9±7.96 pmol/l in nonsurvivors (P=0.024). In uAP no differences were observed (25.0±8.04 pmol/L nonsurvivors vs. 25.7±2.14 pmol/L survivors; P=0.586). Kaplan-Meier estimates of mortality regarding TF activity in AMI were 81.3% and 92.2% with an odds ratio of 3.02 (95% CI[1.05–8.79], P =0.03). The Cox proportional hazards model adjusting for correlates of age and risk factors showed that plasma TF activity but not antigen was an independent correlate of mortality (hazard ratio 9.27, 95% CI [1.24–69.12], P =0.03). In an additional group of patients with uAP and AMI, we identified circulating microparticles as the prevailing reservoir of plasma TF activity in acute coronary syndromes.

**Conclusion:** Systemic TF activity in AMI has an unfavorable prognostic value and as a marker for dysregulated coagulation might add to predict the atherothrombotic risk.

#### PP1.3-4

##### Thalidomide and lenalidomide modulate endothelial thromboresistance through increased tissue factor expression and increased generation of TF-bearing cell fragments in vitro

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**Introduction:** The pathophysiology of the hypercoagulable state associated with immunomodulatory drug (IMiD) treatment in patients with multiple myeloma (MM) remains unclear. We hypothesize that in the context of concomitant presence of cytokines IMiDs lead to hypercoagulability through increased endothelial tissue factor (TF) expression.

**Methods:** Endothelial tissue factor mRNA, protein and activity were evaluated using real time PCR and two commercial TF tests. HUVEC were incubated in vitro in the presence of TNF (10ng/ml) and thalidomide (0.005–50µM) or lenalidomide (0.005–50µM) respectively at concentrations that are observed in mm patients in vivo. In supernatant media of cell-culture experiments endothelial cell fragments were analysed using flow cytometry. All experiments were performed in triplicate.

**Results:** TF mRNA was increased at 1hour incubation in THL(0.5µM)/TNF(10ng/ml) and LEN(0.5µM)/TNF(10ng/ml) treated cells by more than 50% compared to TNF controls. At 20h incubation both THL/TNF and LEN/TNF lead to increased endothelial TF activity (+15% - +70% depending upon the IMiD and the concentration) and antigen levels (approximately +30% at 0.5µM). In the supernatant of TNF and IMiD treated cells endothelial cell fragments were observed that expressed TF activity.

**Conclusions:** IMiDs increase TF expression in endothelial cells in vitro. Our results suggest an overall increased TF turnover with increased gene expression, increased whole cell TF antigen and increased surface activity. Furthermore we detected endothelial cell fragments that express TF activity. These in vitro findings may provide insights into the mechanisms underlying IMiD associated hypercoagulability in vivo.

#### PP1.3-5

##### Decryption of Tissue factor (TF) procoagulant activity on (myelo)monocytic cells by antithymocyte globulin (ATG)

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**Background:** ATG is used in hematopoietic stem cell transplantation to prevent acute graft rejection and/or graft-versus-host disease. Severe thrombocytopenia and disseminated intravascular coagulation are potentially hazardous side effects of ATG, but their underlying pathomechanisms remain poorly understood.

**Design and Methods:** TF procoagulant activity (PCA) of isolated monocytes and (myelo)monocytic cell lines (HL60, U937, THP-1) was measured using a single-stage clotting assay in the presence and absence of inhibitory TF monoclonal antibody. ATG-induced apoptosis was assessed by flow cytometric analysis of annexin-V-FITC binding to surface exposed phosphatidylserine (PS). Cellular microparticles (MPs) were isolated by high-speed centrifugation and characterized by calibrated automated thrombography.

**Results:** Preincubation of isolated monocytes and (myelo)monocytic cell lines with 100 µg/ml ATG (but not PBS or control IgG) resulted in a rapid (within 5 min) and significant (up to 10-fold) increase in TF-specific PCA, an effect that was more potent than physical cell disruption by repeated freeze-thawing or calcium ionophore treatment. Whereas ATG had no effect on annexin-V-FITC binding in a buffer system, ATG induced apoptosis and generation of cellular MPs in a plasma

environment, which was accompanied by an increase in C3a formation as measured by ELISA. ATG-induced cellular MPs were PS-positive and promoted thrombin generation in a TF-dependent manner. Interestingly, ATG had no effect on constitutively TF-expressing myeloma, glioblastoma, or epithelial cancer cells.

**Conclusions:** Rapid decryption of latent TF PCA on (myelo)monocytic cells, which is at least partially dependent on complement-mediated cell death with PS exposure and MP generation, may contribute to ATG-induced intravascular coagulation activation.

#### PP1.3-6

##### Relevance of tissue factor for biomaterial associated blood coagulation

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**Objectives:** The initiation of blood coagulation on biomaterial surfaces usually is attributed to the activation of the contact phase. Tissue factor (TF) up to now is not thought to be relevant for material associated blood coagulation. Regarding new insights into the presence of TF in whole blood this should be reconsidered.

**Design and Methods:** Model materials with clearly defined surface groups (Self assembled monolayers of alkylthiols (SAMs) displaying various ratios of -CH<sub>3</sub>, -OH, and -COOH terminations) were used for studying the relevance of surface properties for the initiation of blood coagulation. An in vitro assay using fresh heparinized whole human blood was used to determine blood reactivity and TF expression and release.

**Results:** The transcription of TF mRNA showed clear differences related to surface properties and increased over time for up to 3 hours (relative expression to initial: hydrophobic -CH<sub>3</sub>: 125±1; negatively charged -COOH: 181±11; hydrophilic OH: 590±50) A positive correlation between TF transcription and presence on leukocytes (microscopic analysis using antibody to VIC7), leukocyte activation (CD11b on granulocytes) and complement activation (C5a in plasma) was shown. A correlation between coagulation activation (plasmatic TAT) and TF mRNA was not yet found in our experimental model.

**Conclusions:** Material related differences for TF transcription and release were found. These differences did not relate to the activation of coagulation as studied, which might relate to methodological limitations like the short incubation time (2 to 3 hours). This set up will be further optimised and more TF related parameters will be analyzed.

#### PP1.3-7

##### Successful management of two patients with hereditary factor VII deficiency undergoing tonsillectomy with rFVIIa (NovoSeven) – normalization of thrombin generation

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**Background:** Congenital F VII deficiency is a rare inherited bleeding disorder, with an estimated incidence of about 1:500,000. Mild to moderate FVII deficiency is characterized by increased bleeding after surgery and trauma. The treatment of bleeding complications in FVII deficient patients usually consists of substitution with FVII concentrates. During the last years it was shown that the administration of rFVIIa (NovoSeven) is a very effective and safe alternative.

**Patients:** We report on two patients with FVII deficiency undergoing tonsillectomy: Patient 1 was an 8-year old female with mild factor VII deficiency (FVII activity 29%). To provide a sufficient haemostasis during surgery rFVIIa was administered before surgery in the recommended dose (15–30µg/kg) and on the 1st until the 10th days post-op. Additionally the patient received tranexamic acid on day 6 – 14 post-op (25mg/kg p.o.). Patient 2 was a 16-year old female with mild factor VII deficiency (FVII activity 35%). Before surgery and until 9 days post-op rFVIIa was administered in the recommended dose. Additionally the patient received tranexamic acid on day 6 – 13 post-op. Several laboratory tests (thrombintime, FVII activity, thrombin generation) were performed.

**Results:** The surgical procedures could be performed without bleeding events. Test results showed a significant effect of rFVIIa on haemostasis. rFVIIa-administration caused a normalization of thrombin generation.

**Conclusions:** Elective surgery in FVII deficient patients can be performed quite safely by giving rFVIIa in the recommended dose. The effect of rFVIIa on thrombin generation can be demonstrated very well throughout the treatment period.



## PP1.3-8

**Blood flow controls coagulation onset via the positive feedback of Factor VII activation by Factor Xa**
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Blood coagulation *in vivo* is initiated by activated factor VII (VIIa) binding to a transmembrane extravascular protein tissue factor (TF); their complex subsequently activates factor X into its activated form factor Xa. Plasma factor VIIa constitutes only 1–2% of its inactive precursor factor VII, which can be activated into factor VIIa in a positive feedback manner by factor Xa during the course of coagulation. Here we use mathematical modeling to show that this reaction plays a defining part in the regulation of coagulation by blood flow. Combination of 1) the factor VII-TF complex activation by factor Xa, 2) factor VIIa-TF complex inhibition by tissue factor pathway inhibitor, and 3) factor Xa removal by flow from the site of vascular damage results in a nonlinear behavior of the clotting system in the presence of flow: clotting is not sensitive to flow at low shear rates, but is potently inhibited when flow is increased. It can be speculated that this mechanism is a special switch protecting vascular system from fibrin thrombus formation in vessels with rapidly flowing blood.

## PP1.4 Other Coagulation Factors and Coagulation Factor Inhibitors

## PP1.4-1

**First product characteristics of human cell line recombinant Factor VIII (Human-cl rhFVIII)**
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A recombinant human factor VIII was produced in the human embryonic cell line, HEK 293F. The advantage of using a human cell line for expression is based on the fact that a human pattern of post-translational modifications, including glycosylation, is obtained. This provides the basis for an improved function and a reduced immunogenicity of the product compared to the presently used recombinant factor VIII products that all have murine glycosylation as those are produced in hamster cell lines. The purification process of the product, Human cell line recombinant factor VIII (Human-cl rhFVIII), showed a significant and reproducible removal of host cell- and process-derived impurities. Preclinical animal studies showed no toxic effects for Human-cl rhFVIII and no disparities in results compared to plasma-derived factor VIII. The glycosylation pattern of Human-cl rhFVIII was found to be typically human and differs from recombinant factor VIII derived from hamster cell lines in several respects. Another characteristic of Human-cl rhFVIII was found to be its significantly higher binding affinity for von Willebrand factor (vWF) as compared with other recombinant factor VIII products. In addition, the capacity of binding to vWF (the number of molecules with ability to bind to vWF) was significantly higher for Human-cl rhFVIII compared with other recombinants. Properties such as binding to phospholipids, activation with thrombin, and thrombin generation were shown to be similar to those of plasma-derived factor VIII. The features of Human cell line recombinant factor VIII give very good promises for its use in future treatment of Haemophilia A.

## PP1.4-2

**Coagulation Factor VIII levels predict long term survival – interactions with gender in a large hospital based cohort.**
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**Introduction:** Elevated coagulation factor VIII activity has been associated with increased risk for both venous and arterial thrombosis. The current study evaluated the influence of Factor VIII levels and interactions with gender on all cause mortality in a large Austrian cohort.

**Patients and Methods:** 11203 individuals were included in this study. The median observation period was 5 years covering a total of 46000 person-years. The death rate was 17.1%.

**Results:** Compared to individuals within the reference category (FVIII:C <94%) hazard ratios gradually increased from 1.4 (95% CI 1.1–1.8) in the 152–170% category (5th decile) to finally 4.4 (95% CI 3.5–5.5) in the >313% category (highest decile, all p<0.05). The association between FVIII:C levels and mortality remained essentially unchanged when considering non cancer mortality, all cause vascular mortality or mortality due to ischemic heart disease. Compared to males females with elevated FVIII:C had a worse outcome resulting in higher hazard ratios reach-

ing 6.8 (95%CI: 4.6–9.9) within the highest decile compared to males (HR: 3.4 (95%CI: 2.6–4.5)).

**Conclusions:** In our large patient cohort we are firstly able to demonstrate that FVIII:C plasma activity is strongly associated with all cause mortality. Additionally, FVIII:C appears to interact with gender. Especially in women FVIII:C might help identifying high risk cohorts which might benefit from individualized prevention strategies.

## PP1.4-3

**Pro-oxidant action of activated Factor X (FXa) in human vascular smooth muscle cells**
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**Objective:** Diabetes and atherosclerosis are associated with oxidative stress, vascular remodelling and thrombosis. Activated factor X (FXa) can elicit coagulation-independent effects such as vascular smooth muscle cell (SMC) proliferation and migration via protease-activated receptors PAR-1 and PAR-2. We investigated if FXa also induces oxidative stress in human vascular SMC.

**Methods:** Human saphenous SMC were serum-deprived (48–72h) prior to stimulation with FXa (30nM). Intracellular oxidant stress was determined by dichlorofluorescein fluorescence. Total protein, mRNA and cell-surface expression levels were measured by western blotting, realtime PCR and FACS respectively.

**Results:** FXa stimulated intracellular oxidant stress in a time-dependent manner over 3–24h (n=5, P<0.05). This was associated with a marked induction of the NADPH oxidase subunit NOX1: mRNA was transiently increased by approximately 4-fold at 1h and total protein approximately 3-fold over 6–24h (both n=4). Preliminary studies also showed increased NOX-1 immunofluorescence at 24h. Other NADPH oxidase subunits (NOX4, p47phox) remained unchanged. PAR-2 mRNA (n=6), total protein (n=4) and cell-surface expression (n=2) were also markedly upregulated over 6–24h, possibly indicating a redox-dependent positive feedback regulation. Constitutive PAR-1 expression was not altered over this time. Increases in oxidant stress and PAR-2 were accompanied by time-dependent induction of the inflammatory cytokine interleukin-6 (IL-6).

**Conclusions:** FXa elicits oxidative stress in human vascular SMC by stimulating NADPH oxidase expression. This is accompanied by upregulation of its receptor PAR-2 and of IL-6. Such actions are likely to support proliferative, migratory and inflammatory processes after vascular injury and contribute to vascular remodeling *in vivo*.

## PP1.4-4

**Improvement of the pharmacokinetic profile of dipetarudin by coupling to crown ether molecules**
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**Objectives:** Dipetarudin is a chimeric thrombin inhibitor being composed of the N-terminal head structure of dipetalogastin II and the exosite 1 blocking segment of hirudin. The pharmacokinetics of dipetarudin is described by an open two-compartment model with a short elimination half-life of about 0.5 hours. The aim of this study was to achieve a better pharmacokinetic profile of dipetarudin via coupling to crown ether molecules.

**Methods:** Crown ether molecules (2-aminomethyl-15-crown-5) were converted into active molecules by several chemical reactions and then coupled to dipetarudin. The reaction mixture was loaded onto a C18-column and the purified compounds of dipetarudin and crown ether molecules were determined by MALDI TOF. Pharmacokinetics of these compounds was studied in rats after intravenous administration of 0.5 mg/kg body weight.

**Results:** Three different constructs were prepared: dipetarudin linked with one, two or three crown ether molecules, respectively. In spite of the linkage of crown ether to dipetarudin no or only a slight reduction in thrombin inhibitory activity was measured. Pharmacokinetic analysis revealed an increase in AUC values and prolongation of the distribution and elimination half-lives of all three constructs in comparison with unmodified dipetarudin.

**Conclusions:** These results suggest that a lower dose of dipetarudin coupled to crown ether molecules can produce the same anticoagulant effect as with unmodified dipetarudin.

## PP1.4-5

**Activation of Prekallikrein by contaminated Heparins, isolated contaminant and related hypersulfated Chondroitin sulfate preparations: Pharmacologic implications**

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**Introduction:** Use of heparins contaminated (CH) with oversulfated chondroitin sulfate (OSCS) has been associated with severe adverse events. Studies were designed to determine the effect of OSCS on the generation of kallikrein (K) in plasma and whole blood systems.

**Materials & Methods:** CH, a contaminant free heparin (CFH), three OSCSs isolated from different heparins, two hemisynthetic chondroitin sulfate preparations (HCS), chondroitin sulfate (CS), dermatan sulfate (DS), and mucopolysaccharide polysulfate (MPS) were compared at concentrations of 0–1000 µg/ml following supplementation to human plasma or whole blood (citrate or hirudinized). K activity was measured using a specific synthetic chromogenic substrate (chromozyme PK) following incubations up to 30 minutes.

**Results:** At a concentration of 100 µg/ml, in citrated plasma OSCS preparations produced the strongest activation of prekallikrein (>0.75/15 mins). CH produced a weaker activity (0.32/15 mins) that was comparable to that produced by CFH (0.24/15 mins). MPS also produced strong activation of prekallikrein into K. In citrated whole blood HCS-1, HCS-2, the three HCs and MPS produced a strong generation of K (>1.5/5 mins). CH produced a weaker effect (1.3/5 mins) whereas CFH was much less (0.25/5 mins). DS also produced a measurable generation of K (1.2/5 mins). CS produced weaker effects (0.6/5 mins). In hirudinized whole blood, all agents produced sizable generation of K (>1.5/5 mins). The contaminants and OSCS preparations were relatively stronger whereas CS was much weaker (0.2/5 mins).

**Conclusions:** These studies demonstrate that the generation of K is a nonspecific effect of sulfated glycosaminoglycans.

## PP1.4-6

**Von Willebrand Factor function under physiological flow conditions: Comparison of five VWF/FVIII concentrates in an in vitro flow-chamber model**

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**Objectives:** The von Willebrand factor (VWF) is essential for platelet adhesion on injured vessel walls above a threshold of about 1000 s<sup>-1</sup> shear rate. Plasma VWF binds to exposed collagen via its A3 domain, resulting in a conformational change of VWF with activation of the VWF-A1 domain, leading to platelet binding through the platelet GPIb receptor. In vivo, VWF has to bind to exposed collagen under high arterial flow conditions. Static assays do not comprise the possible influence of flow on VWF collagen binding and do not measure VWF 'activation' by shear stress. We compared the VWF mediated platelet binding of various VWF/FVIII concentrates under physiological flow conditions using an in vitro flow-chamber model.

**Design & Methods:** Experiments were performed using physiological concentrations of VWF, erythrocytes and platelets in a flow-chamber coated with human collagen III. Adhesion of fluorescence labelled platelets was measured after perfusion or by spiking of Wilate<sup>®</sup> and four other VWF/FVIII concentrates at a physiological shear rate of 1700 s<sup>-1</sup>. The area covered by adhered platelets was quantified using time-lapse microscopy and computer based software analysis.

**Results:** VWF-mediated platelet adhesion significantly differed between the concentrates. Interestingly, the area covered by adhered platelets did not correlate with the VWF multimer size distribution of the tested concentrates.

**Conclusion:** Platelet adhesion to collagen under flow was mediated irrespective of the VWF multimeric distribution of the concentrates. Other structural characteristics of VWF concentrates, as sub-band distribution or native folding of the protein, may affect the VWF function under flow conditions.

## PP1.4-7

**Impact of the Solvent/detergent (S/D) treatment time on Protease inhibitor activity in the pharmaceutically licensed Plasma Octaplas<sup>®</sup> LG**

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**Objective:** S/D treatment is used in the manufacturing process of the pharmaceutically licensed plasma Octaplas<sup>®</sup> LG to ensure safety towards enveloped viruses. The aim of this study was to investigate the impact of the S/D treatment time during Octaplas<sup>®</sup> LG production on the activities of protease inhibitors, in particular proteins and plasmin inhibitor (also known as a2-antiplasmin) activities.

**Methods:** Six pilot batches of Octaplas<sup>®</sup> LG were manufactured by Octapharma PP GmbH, Vienna, Austria. Plasma was treated with a combination of the solvent

[1% Tri(n-butyl)phosphate (TNBP)] and detergent [1% Octoxynol-9] at +30.0 ± 1.0°C. The virus inactivation was performed for 4–4.5 hours or 1–1.5 hours. The S/D reagents were subsequently removed by liquid and solid phase extraction, respectively. The final containers were tested for proteins and plasmin inhibitor activities using commercially available test kits.

**Results:** The 1–1.5 hours S/D treatment resulted in a significantly higher activity of plasmin inhibitor (doubled from 0.3 IU/ml to 0.6 IU/ml) and maintained levels of proteins in Octaplas<sup>®</sup> LG.

**Conclusion:** This biochemical study showed that proteins can be properly maintained and plasmin inhibitor levels can be significantly increased by reducing the exposure time to S/D reagents from 4–4.5 to 1–1.5 hours. The excellent virus safety profile of Octaplas<sup>®</sup> LG is not altered despite this change in manufacturing.

## PP1.4-8

**Antithrombin (AT) gene exon IIIa skipping mutation in a familial AT deficiency with established venous thrombosis**

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**Objectives:** AT deficiency (about 50 % activity) was found in three siblings (father, two daughters) in a family in which the father had had an idiopathic deep venous thrombosis (DVT). One daughter (proposita) also had a thrombosis during pregnancy, although she was under therapy with low molecular weight heparin. A mutation search of the AT gene was therefore performed in this high DVT risk family.

**Methods and Results:** PCR/direct sequencing of the entire coding region and splice junctions of the AT gene were analyzed in the proposita. A heterozygous A>T transversion was found at position -4 in the exon 3a donor splice site (5528A>T). This A>T base exchange was also observed in the father and the sister of the proposita by RFLP analysis with HphI, but was not present in 100 randomly selected subjects. Ectopic transcript (exon 2 to exon 4) analysis of lymphocyte mRNA demonstrated beside the normally sized mRNA (486 bp) also an abnormally sized transcript of about 270 bp. Sequencing of the smaller transcript revealed the lack of exon 3a.

**Conclusion:** According to Berg et al. (Genomics 1992,13, 1359), any AT protein produced from 3a- transcripts is incapable of binding heparin and would most likely be non functional. It can further be assumed that the loss of 72 amino acids renders the protein unstable and it would not be presented in the plasma.

## PP1.4-9

**The role of Factor XIII in chronic inflammatory bowel disease in paediatric patients**

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**Objectives:** Factor XIII is an important molecule involved in the stability of blood clots. In routine coagulation tests factor XIII is not measured. There are two assays available to measure factor XIII, one is detecting the level of factor XIIIa antigen by Elisa, the other measures the clot stability depending on the functional factor XIII molecule. It has been reported that the level of factor XIIIa in adults, determined by Elisa technique, to some degree correlates with the activity of chronic inflammatory bowel disease.

**Design and Methods:** In our paediatric group we evaluated 15 patients with Crohns disease, 15 patients with ulcerative colitis and 30 healthy volunteers, age from 5 to 15, using the functional factor XIII assay. The factor XIII levels were correlated to the activity index levels of the patients.

**Results:** In all healthy volunteers the factor XIII levels were above 70 %, in the group of patients with Crohns disease the factor XIII levels were decreased, but without correlation to the activity index. In the group of patients with ulcerative colitis the levels of factor XIII were although decreased but with a high correlation of factor XIII levels to the clinical activity index. All patients with colitis who needed special treatment had factor XIII levels below 50 %.

**Conclusions:** Factor XIII measured by clot stability is easy to measure and shows high correlation to disease activity in patients with ulcerative colitis. It could be a useful marker during routine blood controls

## PP1.4-10

**Prospective observational study to evaluate the Factor XIII activity during allogeneic stem cell transplantation**

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It is known that during an uncomplicated allogeneic peripheral blood stem cell transplantation (PB SCT) the coagulation tests and FXIII value are in the normal ranges. We examined 100 patients who were admitted for an allogeneic stem cell



transplantation in an observational non-interventional study. Compared with usual conventional allogeneic transplantation, they often had no remission of the underlying disease, much longer time in aplasia with more conditioning treatment and a higher risk for GvHD. We would like to demonstrate the result of the first 16 patients. FXIII activity was measured with Berichrom and FXIIIa activity with IL-System/Testkit. Patients: 11 men, 5 women. Middle age at about 45 years. The medial FXIII activity was 77 %. 94% of the patients experienced during the in-hospital treatment a FXII activity under 70 % and 56 % a factor XIII deficiency with values <50 %. The medial time in aplasia with leucocytes <1000/ $\mu$ l was 25 days and for platelets <30.000/ $\mu$ l stood at 26 days. Statistically significant was the development of a aGvHD in association with an FXIII deficiency of <50 % (p = 0.03). Currently not enough patients have been evaluated, so therefore no tendency for a final evaluation can be given. Nevertheless, an interesting point is that more patients showed an FXIII deficiency with values <70 % and <50 % as assumed. Even without a differentiation in organs of manifestation and the severity of the aGvHD contrary to the literature we found a statistically significant correlation between the F-XIII deficiency and the occurrence of a aGvHD.

#### PP1.4-11

##### The association of FXIII VAL34LEU Polymorphism with thrombotic events in patients referring to Iranian blood transfusion organization

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**Background and Objectives:** Replacement of Val34Leu polymorphism in subunit A of coagulation factor XIII results in the replacement of Valine with Leucine in amino acid 34. As a result of this substitution, FXIII Val34Leu polymorphism acts as a factor for individual protection against thrombosis (1). For the first time in Iran, the prevalence of this polymorphism in patients with thrombotic events and in healthy individuals was determined and studied.

**Materials and Methods:** The study was performed as a retrospective case-control one. 200 referral patients with thrombotic complications were admitted to IBTO Thrombosis and hemostasis Laboratory. 100 healthy individuals were studied as control group. Their DNA was extracted using Qiagene kit. Using Polymerase Chain Reaction (PCR) and RFLP methods in the presence of restriction enzyme CfoI, genotypes of FXIII Val34Leu polymorphism were identified. Statistical analysis was performed by SPSS software version 11.5 and confidence coefficient was 95 %.

**Results:** The prevalence of FXIII Val34Leu polymorphism in patients was 23 % in patients and 36 % in healthy individuals. The allele frequencies of leucine in cases and controls were 13 % and 20 % respectively. These results showed significant differences between the two groups.

**Conclusions:** The present study demonstrates the association between FXIII Val-34Leu polymorphism and protection against thrombotic disorders. The higher frequency of Leu allele and Val34Leu genotype in controls than in patients confirmed the results. Key words: Polymorphism, Factor XIII, Valine, Leucine, Thrombosis